Tuberculous Cervical Adenitis: A Study of the Tubercle Bacilli Cultivated from Fifteen Consecutive Cases.

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The study I wish to report was begun on February 1, 1907. It was thought at that time by Dr. Theobald Smith that there was adequate recognition among workers in this special field of the definite distinction between tubercle bacilli of the human and bovine type, and of the proposition that the bacilli cultivated from a certain small number of cases of human tuberculosis were typically bovine in character; that presumably the human being had become infected from the bovine in these cases and in no others. In the work done up to that time, effort had been directed toward the discovery of instances of this type of infection. It had been shown that these relatively rare cases were never cases of chronic pulmonary tuberculosis; that they were usually cases of infection by way of the intestinal tract in children, particularly cases of mesenteric lymph node disease. Several cultivations from the cervical lymph nodes had likewise given bacilli of bovine type.

It was felt that the time had come when it was possible and advisable to attempt to get definite statistical evidence as to the frequency of the infection of human beings with the bovine type of tubercle bacillus, that the technique had been developed to such a point that the work was feasible, and that the most profitable point of immediate attack would be in the field of cervical lymphatic gland infection—an important, rather frequent disease in any community, one which had been little studied from this point of view, and a field in which a certain percentage of bovine infections might confidently be expected to occur.

Accordingly, at the suggestion of Dr. Smith and under his imme-

1 Preliminary report read at the International Congress on Tuberculosis, Washington, 1908. Received for publication, August 5, 1909.
diate supervision, I undertook to study a consecutive series of cases of tuberculous cervical adenitis with the aim of determining the type of bacillus active in each case. My sincere thanks are offered to Professor Smith for the initial suggestion and for the unceasing advice and attention which alone have made it possible for me, a beginner in this difficult field, to complete the examination of a considerable, consecutive series of cases.

LITERATURE.

There has been so much expert discussion of the relationship between bovine and human tuberculosis that any review of the general literature of the subject may properly be omitted here and I shall confine myself to a summary of those reports of cases of primary cervical adenitis in which the type of the infecting bacillus has been definitely determined.

Smith (1) has described 3 cultures of bovine isolated from tonsils, in which cases the cervical lymph nodes were infected as well. In the general series of cases from the German Imperial Health Office (2), there are reported 4 cases of primary cervical adenitis, 2 of which were due to bacilli of bovine type. At the time these cases were described, the investigators were working with the purpose of discovering bacilli of bovine type in human disease and there was more or less selection of cases. There are a number of other cases reported in which the isolations were made from cervical glands but in which there was generalized tuberculosis. Whether the cervical lymph node disease was primary, secondary, or independent in relation to the general process can not be determined from the reports and they are omitted from the present summary.

The report of the English Commission (3) shows that nine of their cultures were derived from cases of primary cervical adenitis. Of these, 3 were of bovine type.

Oehlecker (4), on the part of the German Imperial Health Office, has reported on isolations from 14 consecutive cases of primary cervical adenitis; 2 infections in adults were both due to bacilli of human type. Of 12 cases in children, 4 were due to bacilli of bovine type.

Duval (5) studied the bacilli isolated from 4 cases of primary cervical adenitis which ran an unusual clinical course. Of these cultures, 2 were of human type. A third was, in most of its features, of human type but gave the reaction of glycerine bouillon characteristic of the bovine type. In regard to the fourth culture (No. 1) the author was in doubt, but finally regarded it as probably an avian bacillus more or less modified.

Park (6) in a preliminary report gave an account of sixteen isolations from primary cervical adenitis. Two of the cultures were of bovine type.
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METHODS.

The material for this study has come to my hand without selection, through the kindness of the staff and house physicians of the Children's Hospital, Boston, and the Boston City Hospital. It has been removed at operation without exception. As a rule, I have received it in moist gauze from the operating room, but in no case have I been able to control its manipulation absolutely, and I am obliged to assume that there has been no contamination with other tuberculous material before it reached my hands.

As soon as possible after receiving the material, guinea-pigs were inoculated. After three to five weeks cultures were made from the guinea-pig. First cultures were always made on Dorset's egg medium. These were uniformly successful. In each case in which the guinea-pigs became tuberculous I have obtained cultures on the egg-medium. Sub-cultures were made at intervals of from three to six weeks to parallel series of egg-medium and glycerine agar. After the glycerine agar culture has become thoroughly established in any case, I have discontinued the series on the egg-medium. The glycerine-agar culture, whenever it has been possible to obtain it, has been used as the stock culture, subsequent transfers and most inoculations having been made from this medium. In two instances, however, no growth was obtainable on glycerine agar and the egg-medium was still in use when the cultures were lost nearly two years after their isolation. Dog serum has not been used as routine, because of the difficulty in obtaining a regular supply. Consequently, the differences in morphology which characterize the two strains when grown on this medium have not been developed.

After an active growth had been established with any culture it was tested for the character of its growth on glycerine bouillon and the changes in reaction which it produced. This was done by inoculating flasks of 5 per cent. glycerine bouillon of determined acidity, usually about 2 per cent., with the culture and removing portions of the fluid at different stages of growth. These portions were titrated. The indicator used was phenolphthalein and the neutralization was done at the boiling point.

The virulence for rabbits was determined and three of the cultures were tested as to their virulence for calves. In the other cases
it has been assumed that the virulence for rabbits was a satisfactory indication of the virulence for cattle. In testing the virulence for rabbits, glycerine-agar cultures were used, as a rule. In the exceptional instances (07.11, 07.93), where no glycerine-agar culture could be obtained, cultures on egg-medium were used. Cultures from two to three weeks old were chosen, a portion of the growth was removed to a sterile test tube and rubbed up thoroughly with a glass rod. Then a little normal salt solution was added and the rubbing continued till a thick emulsion, as smooth as possible, was made. This was then diluted by the further addition of normal salt solution until it had as nearly as possible the density of a twenty-four hour culture of \textit{B. typhosus} in plain bouillon. This suspension is spoken of through the notes and protocols as a standard suspension. This suspension is roughly equivalent to .001 gram of moist culture per cubic centimeter.

In testing the virulence of the cultures for calves, glycerine agar cultures were likewise used. The growth removed was weighed and the suspension prepared on that basis.

**DETAILED STUDY OF CASES.**

I have thus examined 17 cases of primary cervical adenitis. In 2 cases the guinea-pigs failed to develop tuberculosis, although microscopical examination of the tissues showed the pathological picture of tuberculosis, in one case most typically. The 15 cases in which tuberculosis developed in the guinea-pig all gave positive cultures. The protocols of these cases are given in detail in the following pages.

**Case 07.8.—**The original cultures on egg-medium grew well, although not luxuriantly. The first subcultures made to glycerine agar grew, although feebly. The third subculture on glycerine agar grew fairly vigorously and was approximately as strong as a fresh culture of human type. The earliest attempts to transfer from the first and second glycerine-agar cultures to glycerine bouillon failed. A flask inoculated Jan. 30, '08, grew well in this medium. The character of the growth on this flask corresponded to that obtained subsequently on a number of occasions. The inoculation spot grew slowly to a white opaque wrinkled island 1 to 2 cm. in diam. From this, after 2 to 4 weeks, there spread out rather suddenly a thin veil-like membrane which covered the surface of the medium in from 1 to 2 weeks more. At the maximum of growth, the thicker spots had become warty in appearance. The remainder of the membrane was relatively thin and translucent with distinctly heavier white opaque veining. The membranes showed little tendency to fall.
Reaction on Glycerine Bouillon.—3 different lots of bouillon were inoculated. The initial acidity in 2 cases was 2%. In one case, 1.5%. In 4 of the 5 flasks inoculated, the acidity, at the time when the surface was just covered by membrane, had fallen to from .4% to .7%. The first flask of the series (Jan., '08) was not again opened for 3 months. The acidity at this time was 3%. The membrane was macroscopically normal and no microscopic examination was made. A second flask (July, '08) became contaminated with mould. In Nov., '08, 3 flasks of the same lot of bouillon were inoculated. The depth of the medium was varied to give a thin, medium, and thick layer. One of these grew very slowly, the acidity slowly fell from 1.5% to 1% in 3½ months. In 2 others, the growth was normal. Surface covered in 5 to 7 weeks. Reactions 0.4% and 0.7% acid at this time. From this point the acidity gradually increased so that it was finally in one case 1.5% (7 weeks after surface was covered), and in the other case, 1.3% (5 weeks after surface was covered). Cultures macroscopically and microscopically pure. The animal tests gave the following result:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Date</th>
<th>Dose</th>
<th>Injection site</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>338 gm</td>
<td>June 18, '08</td>
<td>½ c.c.</td>
<td>Intravenous.</td>
<td>d. 32 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Calf</td>
<td>201 lb</td>
<td>Aug. 12, '08</td>
<td>5 mg.</td>
<td>Intravenous.</td>
<td>d. 17 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Guinea-pig 147</td>
<td>470 gm</td>
<td>April 3, '08</td>
<td>½ c.c.</td>
<td>Intraperitoneal.</td>
<td>d. 11 days(?) of same.</td>
</tr>
</tbody>
</table>

Bacteriological Diagnosis.—B. tuberculosis, bovine type.

Case 07.9.—The first culture on egg medium grew well but not luxuriantly. The first transfer to glycerine agar grew, although feebly. The third generation on glycerine agar was approximately as vigorous as a fresh culture of human type. The transfer to glycerine bouillon was somewhat more readily accomplished than in Case 07.8, and the character of growth was like that described for 07.8.

Reaction on Glycerine Bouillon.—3 different lots of bouillon were used as follows:

I. Sept. 23, '07, inoc. Glyc. Bou. 273, acidity 2.1% (25 c.c. bouillon in 100 c.c. Ehrlenmeyer flask.) Oct. 13, '07, surface covered with a delicate, smooth, translucent membrane with a thick island at the point of inoculation. Acidity 0.35%. Nov. 13, '07, membrane thicker, even with fine wrinkles, white, glistening. Acidity 2.0%. Jan. 2, '07, membrane same. Acidity 2.6%.

II. Oct. 13, '07, inoc. from Flask I to Glyc. Bou. 211. Acidity 1.1%. 100 c.c.
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III. Dec. 2, '08, inoc. Glyc. Bou. R. I. 4. Acidity 1.5 %. Layer 1 in. deep in 100 c.c. flask with 20th generation on glycerine agar. Jan. 21, '09, thin translucent membrane with thicker opaque white veins and one thick warty island covers surface and has begun to extend up sides of flask. Acidity 0.8 %. Feb. 8, '09, membrane somewhat thicker. Acidity 0.4 %. March 2, '09, membrane same. Acidity 0.7 %.

At the end of the first two tests, the cultures looked pure, but no microscopic examination was made. After the third test the culture was proven pure.

The virulence test with this culture resulted as follows:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Date</th>
<th>Dose</th>
<th>Injection site</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit 276</td>
<td>2550</td>
<td>Apr. 3, '08</td>
<td>½ c.c.</td>
<td>&quot;</td>
<td>d. 23 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Rabbit 341</td>
<td>1800</td>
<td>June 16, '08</td>
<td>¼ c.c.</td>
<td>&quot;</td>
<td>d. 42 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Guinea-pig 43</td>
<td>330</td>
<td>June 7, '07</td>
<td>½ c.c.</td>
<td>&quot;</td>
<td>d. 25 days. Generalized tuberculosis.</td>
</tr>
</tbody>
</table>

**Bacteriological diagnosis.**—*B. tuberculosis*, bovine type.

CASE 07.11.—Cultures were obtained on egg medium. This culture grew fairly well in the first and subsequent generations. In spite of repeated trials, no successful transfers to glycerine agar or glycerine bouillon have been ob-

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
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<th>Injection site</th>
<th>Dose</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit 311</td>
<td>2650</td>
<td>Feb. 14, '08</td>
<td>Intravenous.</td>
<td>½ c.c.</td>
<td>d. 26 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Rabbit 336</td>
<td>3330</td>
<td>May 18, '08</td>
<td>Intravenous.</td>
<td>½ c.c.</td>
<td>d. 27 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Rabbit 339</td>
<td>2315</td>
<td>June 18, '08</td>
<td>Subcutaneous.</td>
<td>5 c.c.</td>
<td>Killed 7 weeks. Generalized active tuberculosis.</td>
</tr>
</tbody>
</table>
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From the third generation on egg medium, a successful transfer was made to dog serum. The third dog serum generation was less strong than the second, perhaps due to a fault in the serum. This medium could not thereafter be obtained. The culture was continued on egg medium, growing moderately well until Aug. and Sept., '08. Then a transfer almost failed to grow and since then growth has been very feeble. The virulence tests were made from egg medium or dog serum during the period of most vigorous growth. The results are shown by the foregoing table.

In those cases where the dosage is indicated as ±, the suspension was too thin for the standard and the dose given is an estimate.

**Bacteriological diagnosis:** *B. tuberculosis*, bovine type.

**Case 07.53.**—Cultures on egg medium grew readily from the first. Transfer from the first generation on egg medium to glycerine agar was successful although the growth was not vigorous. The third glycerine agar generation grew as vigorously as the first generation of a culture of the human type. Put on glycerine bouillon in Sept., '07, it grew slowly at first into a small thick white island at the inoculation site. There gradually spread out a thin, veil-like membrane with thicker veins. This thickened slightly in the following weeks. Later growths on glycerine bouillon were of the same general character except that the membrane thickened in the second year of cultivation.

**Titration Tests.**—I. Sept. 23, '07, inoc. Glyc. Bou. 273 (reaction 2.1 % acid). 2 flasks. Thin layers in small flasks. Nov. 29, '07, a thin membrane with one thick spot quite covers the surface of each flask. Reaction (a) 1.9 % acid, (b) 1.7 % acid. Flask (a) developed a growth of mould after being opened. Flask (b) Jan. 1, '08, membrane somewhat thicker. Reaction, 2.0 % acid. Feb. 12, '08, membrane is slightly thicker. Reaction 3.1 %.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Date</th>
<th>Dose</th>
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<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit 337</td>
<td>2100 gm.</td>
<td>June 18, '08</td>
<td>¼ c.c.</td>
<td>Intravenous,</td>
<td>d. 35 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Calf 205</td>
<td>190 lb.</td>
<td>Aug. 12, '08</td>
<td>5 mg.</td>
<td>Subcutaneous,</td>
<td>Killed 70 days. Generalized tuberculosis.</td>
</tr>
<tr>
<td>Guinea-pig 149</td>
<td>350 gm.</td>
<td>April 3, '08</td>
<td>½ c.c.</td>
<td>Intraperitoneal,</td>
<td>d. 20 days. Generalized tuberculosis.</td>
</tr>
</tbody>
</table>
II. Feb. 1, '08, inoc. Glyc. Bou. 304 (reaction 2.0 % acid) from flask (b) of previous test. March 10, '08, the inoculation point has thickened to a yellowish-white island 1 cm. in diam. From this during the past week a thin translucent membrane has rapidly extended over the surface which is now just covered. Reaction 1.9 % acid. June 3, '08, the membrane is somewhat thicker, rather irregular and dry. Reaction 1.6 % acid. Discarded.


Virulence tests with this culture gave the foregoing results (see table).

Bacteriological Diagnosis.—B. tuberculosis, bovine type.

Case 0754.—Cultures were readily obtained on egg medium. Transfers from the first generation on egg medium to glycerine agar were entirely successful, the culture growing vigorously in the first and subsequent generations. Readily transferred to glycerine bouillon but the culture has never grown rapidly. Starting from the inoculation flask the culture extends very slowly, thickening almost as rapidly as it grows over the surface to a thick irregular curdy membrane. The culture tends to extend by putting out veil-like wings, 5 mm. in width, which thicken before others start. Inoculated on a rather thin layer and a large surface of bouillon, the culture rarely covers the surface after several months.


III. Feb. 1, '08, inoc. 5th glycerine agar culture on Glyc. Bou. 304. Reaction 2.0 % acid. April 2, '08, heavy opaque wrinkled membrane with very thin growing margin, some half cover the surface. Reaction 0.7 % acid. June 3, '08, surface covered with a heavy warty membrane having a few niches filled with very thin membrane. Reaction 0.6 % acid.

IV. June 5, '08, inoc. the 13th glycerine generation to Glyc. Bou. 347, 2 flasks. Reaction 1.9 % acid. Flask (a). July 15, '08, surface one-third covered with white opaque pearly membrane. Reaction 4 % acid. Aug. 11, '08, membrane somewhat larger. Reaction 3 % acid. Flask (b). Aug. 9, '08, surface ¾ covered with a thick curdy membrane with some thin marginal areas. Reaction 1.8 % acid. Sept. 10, '08, membrane almost covers surface. Reaction 0.2 % alkaline.

The culture was tested for virulence from glycerine agar with the following results:


Bacteriological diagnosis.—B. tuberculosis, human type.

Case 07,53.—Cultures on egg medium grew readily. Transfer from the first generation to glycerine agar grew luxuriantly. Good but slow growth on glycerine bouillon. The membrane thickened as it spread, becoming wrinkled, warty and opaque.

Titration Tests.—I. Nov. 11, '07, inoc. Glyc. Bou. 273 (reaction 2.1 % acid) from the fourth glycerine agar generation. There was but slight growth until March 1, '08, when it began and progressed steadily. April 2, '08, moderately thick semi-translucent membrane with irregular margins almost covers the surface. Reaction 5.4 % acid.

II. June 5, '08, the eleventh glycerine agar generation was transferred to Glyc. Bou. 347. Reaction 1.9 % acid. Aug. 11, '08, surface is covered with a very heavy wrinkled membrane. Reaction 3.4 % acid.

Tested for virulence, the culture gave the following results:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Date</th>
<th>Dose</th>
<th>Injection site</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea-pig 78</td>
<td>460</td>
<td>Oct. 4, '07</td>
<td>½ c.c.</td>
<td>Intraperitoneal</td>
<td></td>
</tr>
</tbody>
</table>

Bacteriological diagnosis.—B. tuberculosis, human type.

Case 07,56.—Cultures on egg medium grew well in the first generation. Transferred at this time to glycerine agar, the growth was luxuriant and continued so in all subsequent generations. Grew well although not rapidly on glycerine bouillon. The membrane thickened as it grew, resulting in a wrinkled, opaque, rather yellowish surface with thin translucent narrow growing margin.

Titration Tests.—I. Nov. 11, '07, inoc. Glyc. Bou. 273. Reaction 2.1 % acid. Feb. 12, '08, a heavy wrinkled membrane half covers the surface. Reaction 1.9 % acid. April 2, '08, heavy wrinkled membrane covers the surface and extends up the sides of the flask. Reaction 3.8 % acid.

II. Feb. 1, '08, transfer from Flask No. 1 to Glyc. Bou. 304. Reaction 2.0 %. April 2, '08, heavy wrinkled membrane with a narrow thin margin ¾ covers surface. Reaction 1.9 % acid. June 3, '08, surface is ¾ covered with a thick even wrinkled moist membrane. Reaction 4.3 %.

Virulence tests with this culture gave the following results:

| Rabbit 308, wt. 1790 gm. Received ½ c.c. stand, susp. intravenously on Oct. 4, '07. Killed when in apparently good health after 4½ months. Chronic localized tuberculosis was found in the ear at inoculation site, mammary glands in thoracic region, and both kidneys. |

Bacteriological diagnosis.—B. tuberculosis, human type.

Case 07.61.—Cultures were readily obtained on egg medium. They grew well and from the first generation on this medium transfers were made to glycerine agar. These grew luxuriantly, as did all cultures on this medium, for a number of months. In Feb., '08, through accident the culture failed to grow but was recovered by passing older cultures through another guinea-pig. The titration test recorded was done subsequent to this passage through the second animal. The virulence test was done during the first period when the culture was growing vigorously. Transferred to glycerine bouillon, the culture grew slowly but steadily, thickening as it spread. The character of membrane was as in Cases 07.54, 07.55, 07.56, and need not be described in detail.

Titration Test.—Jan. 7, '09, inoc. Glyc. Bou. 4. Reaction 1.5 % acid. Feb. 19, '09, for 2 or 3 days surface has been covered with a moderately thick, uneven, opaque, white membrane. Reaction .5 % acid. March 20, '09, membrane more evenly thickened. Reaction 1.8 % acid.

Virulence test with the culture gave the following result:

Rabbit 309, wt. 1620 gm. Oct. 4, '07, received ½ c.c. stand. susp. intravenously. 3 months later animal was in good condition (wt. 2370). Killed. Had chronic localized tuberculosis in the kidneys.


Bacteriological diagnosis.—B. tuberculosis, human type.

Case 07.73.—Cultures on egg medium grew well but slowly. The first generation was successfully transferred to glycerine agar growing with moderate vigor. The third glycerine agar generation grew with about the same vigor as a fresh culture of human bacilli. The seventh glycerine agar generation grew readily on glycerine bouillon. The inoculation spot thickened and spread slowly for some weeks, when rather quickly there spread out from it a thin translucent membrane extending quickly to and up the sides of the flask. This peripheral membrane thickened slightly in vein-like lines. Subsequent growths did likewise.


II. June 5, '08, inoc. Glyc. Bou. 347. Reaction 1.9 % acid. July 15, '08, surface has been covered for 2 weeks. Reaction .5 % acid. Aug. 11, '08, reaction .9 % acid.

III. July 10, '08, inoc. Glyc. Bou. 364. Reaction 2.0 % acid, with the 13th glycerine-agar generation. July 30, '08, surface just covered. Reaction .8 % acid.

IV. July 10, '08, inoc. Glyc. Bou. 364. Reaction 2.0 % with the thirteenth glycerine-agar generation. July 30, '08, surface covered. Aug. 9, '08, membrane thicker. Reaction .4 % acid. Aug. 28, '08, reaction .3 % alk. Sept. 21, '08, reaction .3 % alk.
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V. Dec. 2, '08, inoc. Glyc. Bou. 4. Reaction 1.5 % acid. Feb. 8, '09, surface has been covered for four or five days. Reaction 1.0 % acid. March 2, '09, membrane thicker. Reaction .6 %.

The virulence of the culture was determined with the following result:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Date</th>
<th>Dose</th>
<th>Injection Site</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf 202</td>
<td>200 lb</td>
<td>Aug. 12, '08</td>
<td>5 cg.</td>
<td>Subcutaneous,</td>
<td>Killed 70 days. Generalized tuberculosis.</td>
</tr>
</tbody>
</table>

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Case 07.74.—Cultures were readily obtained on egg medium. The first generation from this medium grew luxuriantly on glycerine agar, as did all subsequent subcultures on the agar. On glycerine broth, the growth was slow but constant. The growth on this medium corresponded to that described for Culture 07.55.


II. April 2, '08, inoc. from Flask 1. to Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, membrane just covers surface. Reaction 3.9 % acid.


The virulence of this culture for rabbits was tested as follows:

Rabbit 343.—Wt. 1880 gm. June 16, '08, received ½ c.c. stand. susp. intravenously. 2 months later had gained much weight and was in good condition. Killed. Localized chronic regressive tubercles were found in the lungs. There was localized tuberculosis as well in kidneys, liver, and spleen.

Bacteriological diagnosis.—B. tuberculosis, human type.

Case 07.79.—Cultures were readily obtained on egg medium and subsequent subcultures on this medium grew very well. The transfers of the first and second generations on egg medium to glycerine agar failed entirely. Subcultures from the third generation gave a feeble growth. Subsequent subcul-
tures from glycerine agar to glycerine agar were always successful but the growth was at first feeble. The sixth glycerine agar generation was about as vigorous as that of a first subculture of the human bacillus. Transfer from the third glycerine agar generation to glycerine bouillon grew very little for a month and after that spread quickly over the surface. The resulting membrane was similar to that already described for Case 07.9 and has been so at all subsequent trials.

Titration Tests.—I. April 25, '08, inoc. third glycerine agar culture on Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, thin, even, smooth or finely granular, translucent membrane with one small thick wrinkled spot just covers surface. Reaction .5 % acid. July 2, '08, membrane somewhat thicker. Reaction .8 % acid.

II. July 10, '08, inoc. sixth glycerine agar generation on Glyc. Bou. 364. Reaction 2.0 %. July 27, '08, surface just covered by membrane. Reaction 1.4 % acid. Aug. 9, '08, membrane thicker. Reaction 1.1 % acid. Nov. 10, '08, reaction 0.1 % acid.

Tested for virulence this culture gave the following results:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
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<th>Dose</th>
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<th>Result</th>
</tr>
</thead>
</table>

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Case 07.86.—Cultures on egg medium grew readily. The first generation culture on this medium was successfully transplanted to glycerine agar. The first glycerine agar generation grew as vigorously as subsequent ones. Growth was readily obtained on glycerine bouillon. The membrane formed was thick yellowish-white, spreading slowly and thickening as it spread.

Titration Test.—March 17, '08, inoc. the eighth glycerine agar generation on Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, thick, wrinkled, dry membrane covers the surface and sides of the flask. Shows some tendency to fall. Reaction 4.5 % acid.

Virulence test with this culture gave the following results:

Rabbit No. 298, wt. 2200 gm. Dec. 10, '07, received ½ c.c. stand, susp. intravenously. After 3 months animal had gained 450 gm. in wt. Good condition. Killed. Chronic localized tuberculosis was found in the lungs and kidneys.


Bacteriological diagnosis.—B. tuberculosis, human type.

Case 07.92.—Cultures on egg medium grew well from the beginning. An

*While the disease in this case involved the axillary glands it was so apparently successive to primary disease of the cervical glands that it seems proper to include the case in this series.
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attempt to transfer the first egg-medium generation to glycerine agar failed. The second egg medium generation transferred to glycerine agar gave a feeble growth which was, however, successfully maintained in subcultures on this medium. The sixth glycerine agar generation was the first in which the vigor of growth was approximately that of a fresh culture of human type. The fourth generation on glycerine agar inoculated on glycerine bouillon grew slowly for 3 months to a thick yellow-white island about 1 cm. across. Then it began to spread rapidly and in 2 weeks the surface was covered with a thin translucent membrane. This later extended up the sides of the flask and thickened slightly. In subsequent transfers, the character of growth on glycerine bouillon was the same.

Titration Test.—I. April 9, '08, inoc. Glyc. Bou. 328. Reaction 1.9 % acid. June 3, '08, very slight growth at inoculation point. July 15, '08, surface ¾ covered with a very thin, translucent membrane in which are 2 thicker islands. Reaction 1.7 % acid. Aug. 9, '08, surface covered. Membrane somewhat thicker. Reaction 6 % acid. Sept. 19, '08, membrane about same. Reaction 2 % acid.

II. Nov. 30, '08, inoc. Glyc. Bou. 4 (reaction 1.5 % acid). Feb. 8, '09, surface almost covered. Membrane character as before. Reaction 3 % acid. March 2, '09, surface almost covered. Reaction 6 % acid.

Virulence tests with this culture gave the following results:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Date</th>
<th>Injection site</th>
<th>Dose</th>
<th>Result</th>
</tr>
</thead>
</table>

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Case 07.93.—Cultures were readily obtained on egg medium. The first subcultures grew, although never vigorously. Repeated attempts to get growth on glycerine agar and glycerine bouillon by transfer from the egg medium cultures failed entirely. The culture was maintained on egg medium for 12 generations, when it was lost through accident. In the meantime, its virulence had been tested with the results shown in the following table (page 95).

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Case 07.94.—Cultures on egg medium were readily obtained. They grew well from the beginning. Subcultures from the first generation to glycerine agar gave a very fair growth which was maintained. The fifth glycerine agar generation gave as vigorous a growth as a fresh culture of human type. Transfer from the fifth and subsequent glycerine agar generations to glycerine bouillon gave good growths. The membrane either spreads at once as a thin
translucent film over the whole surface or the inoculation point thickens to a yellowish-white island .5 to 1 cm. across, and from this a thin membrane suddenly spreads over the whole surface. When the cultures became older, the thinner parts of the membrane became thicker in small spots and vein-like lines.

_Titration Test._—I. March 17, '08, inoc. Glyc. Bou. 328. Reaction 1.0 % acid, with the fifth glycerine agar generation (2 flasks). April 8, '08, surface of flasks is just covered. Flask (a) has reaction .9 % acid. June 3, '08, flask (b) membrane has largely fallen and been replaced by a second thinner film. Reaction 1.2 % acid.

II. June 5, '08, inoc. Glyc. Bou. 347. Reaction 1.0 % acid with the eighth glycerine agar generation. July 15, '08, surface ¾ covered. Reaction .7 % acid. Aug. 11, '08, surface covered. Reaction .7 % acid.

III. July 10, '08, inoc. Glyc. Bou. 364. Reaction 2.0 % acid, with the ninth glycerine agar generation. July 30, '08, surface just covered. Reaction 1.1 % acid. Aug. 9, '08, membrane thicker. Reaction .3 % acid. Sept. 10, '08, reaction .1 % acid.

IV. Dec. 2, '08, inoc. Glyc. Bou. 4. Reaction 1.5 % acid. 3 inch layer in a 500 c.c. Ehrlenmeyer flask with the twelfth glycerine agar generation. Jan. 21, '09, surface ½ covered. Reaction .1 % acid. Feb. 8, '09, surface covered. Reaction .4 % acid. March 2, '09, membrane thicker. Reaction .9 % acid.

V. Dec. 2, '08, inoc. same culture as in (4) in same bouillon but 1½ inch...
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Layer in 200 c.c. flask. Jan. 21, '09, surface covered. Reaction .6 % acid. Feb. 8, '09, membrane thicker. Reaction .6 % acid. March 2, '09, membrane same. Reaction .7 % acid.

Virulence tests with this culture gave the results shown in the foregoing table.

Bacteriological diagnosis.—B. tuberculosis, bovine type.

Differential Characteristics.

Before proceeding to collect and analyze the results as set forth in the foregoing protocols, it will be well to review briefly the characteristics which it is necessary to consider in the differentiation of mammalian tubercle bacilli into the two types, human and bovine. Theobald Smith (7) in 1898 first pointed out that the bacilli derived from tuberculous tissue of cattle differed in fundamental particulars from those derived from cases of human pulmonary tuberculosis. The observations recorded at that time have been repeatedly made since by workers in all countries. Somewhat extended and modified by later experience, the differentiation today is reached by developing the following points:

Grown under special conditions, that is on coagulated dog serum, the recent isolations differ in morphology. The bovine bacilli are short, straight, plump, and stain solidly. The human bacilli are longer, frequently curved, thinner, and when stained are often beaded. The initial cultivation of the tubercle bacillus is more difficult from the bovine than from the human source. Grown on glycerine bouillon, there are constant differences in the character of the growth. The bacilli from the human disease grow as a thick wrinkled membrane from the beginning which tends to spread rather slowly, and thickens about as quickly as it spreads. A favorable culture of bovine origin will spread rapidly as a thin translucent membrane over the whole surface. If the culture has been long under cultivation, the membrane may thicken evenly till it is not to be distinguished from the completed human membrane. If recently isolated, either this thickening does not occur or it develops in irregular lines or spots.

Finally, the bacilli differ widely in virulence. The inoculation disease from the bovine bacillus in young cattle tends always to become general. Inoculated intravenously in small doses, the cattle usually die of acute miliary tuberculosis. If human bacilli are inoculated in similar dose intravenously, local chronic disease results at most. If the human bacilli are inoculated intraperitoneally, intrapleurally, or subcutaneously, even in large dose, only a local chronic disease results. The virulence of the two bacilli for rabbits was found to be exactly the same as for cattle.

In 1903, Theobald Smith (8) published the further observation, that corresponding to the qualitative differences between the character of the growth of the two types of bacilli on glycerine bouillon,
there are characteristic differences in the amount of acid and alkali production on this medium. It has proven to be a matter of some difficulty in other hands to develop consistently these changes in chemical reaction, and in such fashion as to aid in the classification of cultures. I have studied the reaction changes produced by all the cultures in this series, but factors unforeseen and probably extraneous, it seems to me, have led to marked irregularity in the results. For this reason I shall, in the following pages, consider the grouping of the cultures according to the characters first given above, virulence for rabbits and cattle, growth character on glycerine bouillon, and adaptability in artificial cultivation. Subsequently, in a separate section, I shall discuss the reaction changes on glycerine bouillon.

Virulence.—In dealing with cultures of the tubercle bacillus recently isolated, the character which must be deemed of first importance in an attempt to classify by type is that of virulence. The animal of choice would be the calf. As stated above, however, it has been abundantly demonstrated that the virulence for rabbits is a perfect index of that for calves. Consequently, rabbits have been selected for most of the tests.

Each culture, as soon as possible after a vigorous growth had been established, was inoculated intravenously into a rabbit in doses of one-half cubic centimeter of the standard suspension (approximately .5 milligram). Nine of the cultures killed the rabbits in from two to three weeks with lesions of generalized tuberculosis. Six cultures did not kill. The animals inoculated with the latter cultures ultimately gained in weight, were chloroformed after two to four months, and showed at autopsy a small or moderate grade of localized chronic tuberculosis. Several of the nine cultures which killed were retested in the same dose to exclude the possibility of accidental, coincidental disease. The result was as before. Finally each of the nine virulent cultures was inoculated intravenously in 1/100th of the standard dose, corresponding to about 1/200 milligram of culture. This dose killed in several instances in less than twenty-five days; eight of the animals were dead by the fortieth day. The ninth rabbit survived two months, failed rapidly, was killed and found to have an active, generalized, progressive tuberculosis.

Nine of the fifteen cultures studied were thus found to be viru-
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lent for rabbits. Six cultures were not virulent for this animal. It will be noticed that the difference in virulence was a positive one. For the non-virulent group, one-half cubic centimeter of the standard suspension was a dose which failed to kill, and judging from the reaction and the lesions in the surviving animals it was considerably below the fatal dose. One one-hundredth of this amount was found to be certainly fatal in the case of the virulent group. The animal which lived two months after inoculation was killed because of extraneous circumstances. It was failing rapidly and the disease at autopsy was found to be general and progressing, and the animal could not have survived much longer (see Case 07.92). The grouping of the cultures in respect to virulence is shown in the following table. The numbers refer to the case number in the corresponding protocol.

<table>
<thead>
<tr>
<th>Virulent for rabbits</th>
<th>Non-virulent for rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.8</td>
<td>07.54</td>
</tr>
<tr>
<td>07.9</td>
<td>07.55</td>
</tr>
<tr>
<td>07.11</td>
<td>07.56</td>
</tr>
<tr>
<td>07.53</td>
<td>07.61</td>
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<tr>
<td>07.73</td>
<td>07.74</td>
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<tr>
<td>07.79</td>
<td>07.86</td>
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<tr>
<td>07.92</td>
<td></td>
</tr>
<tr>
<td>07.93</td>
<td></td>
</tr>
<tr>
<td>07.94</td>
<td></td>
</tr>
</tbody>
</table>

It has been abundantly shown that a corresponding virulence for cattle can be assumed. However, three of these cultures (07.8, 07.53, 07.73) which attracted our attention by reason of apparent abnormalities in reaction on glycerine bouillon were tested on calves. Three calves were inoculated intravenously with five milligrams of each of these cultures. All died in less than three weeks and at autopsy showed an acute, generalized, miliary tuberculosis.

One calf was inoculated in each case with five centigrams of culture subcutaneously in the region of the neck. They were killed seventy days later. There was found at autopsy in each case a large tuberculous lesion at the site of inoculation. The lymphatic gland anterior to the shoulder was much enlarged and tuberculous. Other glands in the same chain were more or less involved. Small tubercles were found in moderate numbers in the internal organs of all the
Paul A. Lewis.

animals. These three cultures, then, were proved virulent for calves; and the experiments afford additional support to the existing evidence that virulence of mammalian tubercle bacilli, for rabbits and cattle go hand in hand.

Cultural Characters.—The six cultures which failed to kill rabbits were, in each instance, transferred at the first subculture to glycerine agar and from the beginning grew luxuriantly on this medium. They were as easily transferred to glycerine bouillon, growing for the most part with a heavy membrane which spread slowly and thickened markedly as it spread. The nine cultures virulent for rabbits have shown a very variable adaptability to culture medium. Subcultured each month to six weeks, I shall give the number of the subculture on glycerine agar at which they have attained practically the luxuriance of growth of the non-virulent cultures as an index of their adaptability. Four cultures grew at once on glycerine agar and at the third subculture were as vigorous as the others. Three cultures failed to grow on the first glycerine agar transfer and required from five to six subcultures to reach maximum vigor. Two cultures, in spite of repeated trials over a period of eighteen months, still refused to grow on glycerine agar or glycerine bouillon. These cultures then failed to grow on two successive lots of egg medium. One of them was entirely lost, the other probably cannot be recovered. The seven cultures which were successfully transferred to glycerine agar also grew well from about the same period on glycerine bouillon.

When they started to grow on this medium soon after the inoculation of the flask, they spread at once as a thin translucent membrane over the whole surface. In the earlier generations, this membrane thickened only irregularly. Some of the later generations have given completed membranes only slightly less thick than those of the non-virulent cultures. When the culture in any flask started more slowly there was always formed a thick wrinkled island one-half to one centimeter in diameter from which there would rather suddenly extend a thin translucent film to cover the surface.

For the reason that dog serum was not regularly obtainable during the earlier period of this study, I have devoted only small attention to the morphological characters of the two groups of bacilli,
and have not recorded in detail the results of such examinations as I made.

In their adaptability to artificial cultivation, and in the character of the growth on glycerine bouillon, the cultures have consistently shown differences entirely in accord with the differences in virulence. The non-virulent strains were readily established in culture, growing with a thick, slowly extending membrane on glycerine bouillon. The virulent cultures were established in culture with more or less difficulty, and in two instances with imperfect success. It seems, therefore, safe to conclude that the nine cultures of lesser adaptability and high virulence are of bovine type, and the six cultures which readily adapt themselves to glycerine agar and which have low virulence for rabbits are of human type.

It should be noted that two of the cultures (07.11-07.93) were more difficult to cultivate than the usual culture of bovine origin being definitely atypical in this respect.

Reaction Curves on Glycerine Bouillon.—In previous paragraphs, I have stated that one of the constant differences between the human and bovine type of mammalian tubercle bacilli, according to Theobald Smith, lies in the change in the chemical reaction of glycerine bouillon, when the bacilli are grown on this medium. Starting with an initial acidity of about 2 per cent., phenolphthalein being the indicator, the first change in either instance is to diminish the amount of acid. In the case of the bovine type, the reaction may even become alkaline. In the case of the human type, the neutral point is more or less closely approached but is seldom passed. The time when the acidity is least usually corresponds to the period of growth when the surface has just been completely covered in the case of a culture growing rapidly. From this point, the human type of culture rapidly returns toward the initial acidity and often goes beyond it. In the case of the bovine type, there may be, and usually is, some increase in acidity but this is most often slight and is sometimes lacking. In a later publication by Smith and Brown, this distinction was maintained, and it was pointed out that there were constant differences between cultures of the human type, some returning to an acidity of about 2 per cent., others tending to return to about 4 per cent. of acid as an end reaction.
In the description of this procedure certain difficulties were pointed out and certain limitations put upon its use as an absolute test for type. The cultures must be maintained free from fungi and other bacteria during the whole period of observation. Certain preparations of bouillon are unfavorable and, consequently, controls of well-known cultures must be carried in every series in which a new lot of medium is used. The cultures must have become perfectly adapted to the medium in order to give regularly progressing growth and typical curves of reaction.

In the hands of other observers, this method as a differential test has given rise to various opinions.

Beitzka (9) thinks it is of no value. One feels, however, that he worked without due regard to the limitations originally laid down.

The English Commission workers (10) thought that the figures of Smith showed no definite line of demarcation, nor did their own. Their results seemed to show that the bovine bacillus when growing to produce as heavy a membrane as the human type would give an acid reaction. Smith’s figures, taken as a whole, do not lend themselves to the interpretation they put on them. Moreover, the most characteristic reaction of the bovine group were obtained by Smith with cultures Bov. I and III which had been very long under cultivation and which grew with the vigor of human cultures.

Bang (11) found that the lines were maintained in the case of certain stock cultures of either type and that the aviari tubercle bacillus gave a reaction curve like that of the bovine type.

Duval (5) used the test in the study of four cultures from human source without presenting fresh evidence as to its validity. His work emphasizes the difficulty of obtaining consistent results with a few tests on freshly isolated cultures. He found one culture (IV) which while having all the other characteristics of a culture of human type, gave an initial fall in reaction to the neutral point or near it with no secondary rise in acidity. He presents some evidence to show that the secondary rise in acidity is due to an autolysis of the human type of bacilli in the presence of glycerine, irrespective of growth.

Work in other hands has obviously done little to establish the
test on a firm footing, and has, on the other hand, raised no serious criticism of it.

In classifying the cultures of my series in one or the other type, I have left to one side the evidence of the reaction curves for several reasons. In the first place, in the case of many of the earlier tests, I am not certain of the purity of the cultures throughout the period of examination. I relied on the macroscopic appearance of the cultures as an evidence of continued purity during the first year of the work. At this time several of the cultures of high virulence gave frankly acid end reactions. Later some flasks were left to be finished as I was leaving the laboratory, and these were kindly taken in charge by Dr. Smith for titration. In one of these he found that while the membrane was macroscopically unaltered, microscopically it contained fungi. This finding, even though it be a single instance, vitiates the value of all of those results in which an acid end reaction was found with bacilli of bovine type except one about to be mentioned. On the other hand, it may be best to consider for the moment the chance that this sort of contamination was exceptional. In that case a number of instances could be cited to show that the cultures of bovine type from human source gave a pronounced acid end reaction in the beginning which gradually became less in subsequent titrations, months later (07.8, 07.9, 07.53). Duval (5) states that his culture (I) suddenly changed after a number of months from the acid to the alkaline type. Keeping in mind this possibility of a change under cultivation, the work can never be repeated with these cultures.

In view of my experience, it may be said that five of the six non-virulent cultures have given the acid end reaction characteristic of the human type. One (07.54) has not done so. The last grows as well as the others and has from the beginning, but after months of growth, the flasks remain at about the neutral point which they gradually attain in the first few weeks. This culture, with Duval's (5) culture (IV), and a culture (M), said to have been isolated by Dr. Ravenel some years ago, are three recorded exceptions to the rule of Smith, that the human type of tubercle bacillus gives a distinctive acid end reaction on glycerine bouillon.

With six of the virulent cultures, working recently, that is, when
the cultures were over eighteen months under cultivation, a perfectly characteristic bovine curve has been obtained. In a seventh culture (o7.8), proved of bovine type, even in respect to its virulence for calves, the lowest end reaction obtained has been 1.3 per cent. acid (initial acidity 1.5 per cent.). This, while a lower reaction than any of the five cultures of human type, is not characteristic. In the case of the two remaining cultures, the reaction curve could not be determined as the cultures could not be established on the medium.

The results of the reaction determinations have been generally in accord with the classification of the cultures on the basis of cultural characters and virulence. Culture o7.54 is an exception. Duval (5), in the case of his similar culture (IV), concluded that he had to deal with an attenuated bovine type. This question may well be reserved to await future results. That cultures of bovine type may, when recently isolated from the human body, present an abnormal reaction curve, is not in any way rendered improbable by the results already reported by others, most of which were obtained with cultures over a year under cultivation; but it is no more than a vague possibility raised by results otherwise unsatisfactory.

Clinical Data.—In closing, it may be noted that a careful analysis of such clinical and pathological data as I have been able to gather in connection with these cases has shown no feature of distinction between the group of cases in which the human and bovine types of bacilli were found, in respect to the lesion, number of bacilli present, duration or recurrence of the disease, family history, previous history or present condition of the patients. There is, however, a striking difference in the age of patients. The average age of eight of the patients giving bovine type of bacillus was 8½ years. The youngest was 18 months and the oldest 18 years. The average age of six patients yielding the human type of bacillus was 17½ years, the oldest was 32 years, and youngest 8 years. Of the cases of cervical gland tuberculosis in persons over 15 years of age, four-fifths were infections with human type. Of the cases in persons below 15 years of age, seven-ninths were of bovine type. Hence the bovine type of infection would seem to be more common in children and the human in young adults.
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SUMMARY.

The examination of fifteen consecutive cases of primary tuberculous cervical adenitis resulted in the isolation of nine cultures of *B. tuberculosis* of bovine type and six cultures of human type, the classification being made on the basis of adaptability to artificial cultivation, character of growth on glycerine bouillon, virulence for rabbits, and, in three instances, virulence for calves.

The results of the determination of the reaction changes in glycerine bouillon due to the growth of the cultures on this medium were in general accord with the classification as based on other characters. One culture otherwise of human type was found to give the slightly acid or even alkaline end reaction characteristic of the bovine type of bacillus. One culture, otherwise of bovine type, has so far given an end reaction more highly acid than is usual with bovine cultures. Two cultures, highly virulent for rabbits, showed even less adaptability to artificial cultivation than the usual true bovine cultures and the reaction change could not, therefore, be determined.

BIBLIOGRAPHY.