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Tuberculosis In Nakorn Chiangmai Hospital

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Tubercle bacilli or mycobacteria can be demonastrated readily by microscopic and culture procedures. The only sure method of determining the virulence of these microorganisms has been by use of appropriate animal inoculation. From time to time workers have suggested new techniqes for rapid determination of the virulence of mycobacteria. The most interesting of the newer tests have been the growth pattern (cord formation) the description of microscopic morphology of colonies in certain specific media and reaction of tubercle bacilli with a specific chemical agent (the cytochemical reaction.)

It was the purpose of this investigation to study the correlation between these tests of virulence and the classical determination of Mycobacteria, in Nakorn Chiang Mai Hospital

Materials and Methods.

A. Culture :-

Urine, spinal fluid and other material not contaminated with other bacteria may be concentrated by centrifuging and cultered directly. Sputum and stool are first treated with sodium hydroxide which is toxic contaminating microorganism but less so for tubercle bacilli. The liquefied specimen is then neutralized with hydrochloric acid and centrifuged and sediment inoculated into Lowenstein Medium (Difco). Incubation of the inoculated media is continued for 8 weeks.

B. The virulence and the classification

1. Cord formation (The growthy pattern, 3, 9.). It was noted that virulent strains of human and eugonic bovine tubercle bacilli form microscopic serpentine cords of varying length and thickness, which are made up of individual organisms lying side by side and end to end in parallel alignment. It was also noted that those eugonic variants which failed to form cords were avirulent. The most saprophytic strains are negative.

This cord formation is best observed by making smears of the condensation water from positive TB culture on Lowenstein Media.

2. Auramine test. Erlich (2) observed that when Mycobacterium tuberculosis was rubbed

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on to the filter paper impregnated which auramine, a bright yellow dye, the color of the dye persisted for more than 2 minutes after the addition of 0.5% NaOH. Auramine papers containing nonpathogenic mycobacteria were completely decolorized. The unclassified mycobacteria gave positive reactions, but the color produced varied from yellow to brick-red.

3. Catalase activity test studied by Kubica and others (6,7) demonstrated acid fast bacilli produce the enzyme catalase, which is detected by the breakdown of hydrogen peroxide and the active evolution of gas(O_2) bubbles. Detection of catalase activity has proved useful in two ways; (a) tubercle bacilli that become resistant to isoniazide will lose or show a lessened catalase activity: (b) the catalase activity of human or bovine strains may be selectively inhibited by heat.

Catalase activity may be determined in two ways;

1. At room temperature, 0.5 ml. of a 1:1 mixture of 10 % Tween 80 and 30 % hydrogen peroxide is added directly to a culture slant of Lowenstein medium. A positive catalase test is indicated by an active evolution of grossly visible gas bubbles within 2 minutes.

2. To determine the effect of pH and temperature on catalase activity several spadefuls of growth are scraped from the culture slant and suspended in 0.5 ml. phosphate buffer, pH 7, in a screw-capped tube and place in a 68°C. water bath for 20 minutes. After cooling to room temperature, 0.5 ml of the tween 80-peroxide mixture is added, and the reaction is observed as before.

4. Neutral Red test. (5) Dubos and Middlebrook (1) were the first to show that virulent strains of Mycobacterium tuberculosis were able to bine the dye, neutral red, in an alkaline aqueous medium, while the noncord forming variants of tubercle bacilli and nearly all saprophytes would not take up the dye.

5. Niacin test (1) Strong niacin production by an acid fast bacillus from a clinical specimen is strong evidence of its identity as a human tubercle bacillus. Conversely, an accurately performed test resulting in a negative reaction indicates another species of mycobacterium. The niacin test is done by adding a few drops of water or saline solution to the Lowensteinmedia and placing the tube so that the liquid remains on and around the colonies for over night, one or two drops of extract are then transferred to a white porcelain spot plate, and two drops each of the following reagents are added (1) 4 % aniline in 95 % ethanol, which should be nearly colorless, and (2) 10% aqueoue cyanogenbromide. (Both reagents are stored in the refrigerator in brown dropping bottles, and made up fresh each month.) Positive reaction gives a yellow color.

6. Thioglycollate test. (8,10.) This useful test, depends on observation of growth or its absence in fluid thioglycollate medium when inoculated with a mycobacterium. All virulent eugonic strains of mycobacterium fail to grow in this medium; bovine and avine strains require 4 weeks incubation to appear, Many strains of unclassified mycobacteria will grow in thioglycollate medium, but development is slow, and dysgonic, requiring 2 to 4 weeks for initial growth. All saprophytic strains give positive results; growth is rapid (1 to 2 days) and luxuriant, with pellicle formation.

Results.

During the years 2507 to 2510, the clinical laboratory examined 2516 specimens for tubercle bacilli from patients who were admitted to the hospital for a condition other than tuberculosis. More than 9% of the specimens were poitive. (see table 1)

The ages of the patients with positive cultures ranged from 1 to 79 years. A comparison of the number of cases for different age groups is given in table 2.

The 82 positive cultures which grew more than 10 colonies were tested for virulence by cord formation, auramine test, catalase activity test, neutral red test, niacin test, of these 81 strains were positive by the niacin test, and only one strain grew in the thioglycollate media. (see table 3)

Table 1. The clinical laboratory examination

Year	Cultures	Culture positive for TB.	
2507	456 cases	50 cases	10.98 %
2508	606 ,,	84 ,,	13.86 %
2509	760 ,,	54 ,,	7.10 %
2510	694 ,,	38 ,,	5.48 %

Table 2. Number of positive TB cultures in various age groups

Age between	Cases
1—16 years	1
11—20 ,,	6
21—39 ,,	19
31—40 ,,	14
41—50 ,,	14
51—60 ,,	14
61—70 ,,	12
71—	2

Table 3. Results of the tests

Strains	Cord	Catalase test		neutral red	niacin	auramine	thio-glyco.
		direct	heat				
81 strains	+ ve	+ ve	neg	+ ve	+ ve	+ ve	neg
1 ,,	+ ve	+ ve	neg	+ ve	neg	+ ve	+ ve 1 week
saprophytic strain	neg	+ ve	+ ve	neg	neg	neg	+ ve / 4 days

Discussion.

Of the 82 strains are virulents, 81 gave a positive niacin test, and only one strain grew on thioglycollate media. The saprophytic strain used as a negative control was obtained from Dr. Leon J Le Beau.* We found that only one strain is atypical strain (Unclassified mycobacterium) which gave an orange color colony. In those specimens digested with 4% sodium hydroxide, the atypical tuberculosis organisms may have been killed. More over, although the significance of "atypical mycobacteria" isolates is still unclear, (Ellner and Elbogen), (4) there is an increasing appreciation of disease in man caused by atypical tubercle bacilli.

The clinical laboratory examined 2516 specimens for tubercle bacilli from patients who were admitted to the hospital for a condition other than tuberculosis. More than 9% of specimens were positive. We can assume from the results of the cultures at this hospital that this may represent only a small proportion of the actual TB. carriers among the population of Chiang Mai who enter this hospital for treatment and management of some disease other than tuberculosis.

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ย่อจากต้นฉบับ

วัณโรคในโรงพยาบาลนครเชียงใหม่

โดย

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ในระหว่างปี ๒๕๐๗ ถึง ๒๕๑๐ ห้องปฏิบัติการจุลชีววิทยาคลีนิค ได้เพาะเลี้ยงเชื้อเพื่อหาเชื้อวัณโรคจากตัวอย่างตรวจต่างๆ จากคนไข้จำนวน ๒๕๑๖ ราย ซึ่งคนไข้ทั้งหมดเข้ามารับการรักษาในโรงพยาบาลนครเชียงใหม่ ด้วยโรคอื่นๆ ปรากฏพบเชื้อวัณโรคมากกว่าร้อยละ ๙ และเราได้เอาเชื้อวัณโรคที่เจริญขึ้นบนอาหารเลี้ยงเชื้อที่มีมากกว่า ๑๐ โคโลนี เลือกมา ๘๒ ราย เอามาทดสอบหาไวรูเลนซ์และแยก พวก พบว่าทั้ง ๘๒ ราย เป็นไวรูเลนซ์สเตรน ปรากฏว่าเป็นอทิบีคอลสเตรน ๑ ราย

จากผลการเพาะเลี้ยงเชื้อวัณโรคของห้องปฏิบัติการจุลชีววิทยาคลีนิค เราพอจะสรุปได้ว่าจำนวนของคนไข้ที่ตรวจพบเชื้อวัณโรค ซึ่งเป็นคนไข้ที่ไม่ทราบว่าตนเป็นวัณโรคมาก่อน และคนไข้เหล่านี้ย่อมจะเป็นแหล่งแพร่เชื้อวัณโรคได้เป็นอย่างดี

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