วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

September 1971
Vol. 4 No. 3

COMPARISON OF MEDIA FOR GROWTH OF THE FASTIDIOUS ORGANISMS *

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Abstracts

The purpose of this study was to determine the effect of different media on the shape of the growth curves of certain fastidious bacteria. Beta streptococci group A, Pneumococci and Haemophilus influenzae were inoculated into Tryptic soy broth, Tryptic Soy broth with 1% Yeast extract, Heart Infusion broth, Tryptose Phosphate broth and Nutrient broth.

The tests were carried out using 0.1 ml. of 18-24 hrs. broth, then incubating at 37 °C on a Burton Kahn Shakes. After 2 hrs., 4 hrs., and 6 hrs. particle density was determined in nephelometer units using a Colemen Nepho-Colorimeter. The growth curves were plotted on semi-log paper with logarithm of Nephelometer units against time. The results showed that Beta streptococci group A grow best in Tryptic Soy broth with 1% Yeast extract, Pneumococci grow best in Tryptose Phosphate broth. Haemophilus influenzae were unable to grow in these broths because of the absence of hematin and DPN.

Introduction.

All biological system, from microorganisms to man, share a set of nutritional requirements with regard to the chemicals necessary for their growth and normal fuctioning. The microorganisms require the following substantiates this and illustrates the great divesity of nutritional types. (7)

1. All living organisms require a source of energy. Some forms of life, namely green plants, are capable of employing ra-

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diant energy. Forms of life incapable of utilizing radiant energy (e.g., animal life) rely upon oxidations of chemical compounds for their energy.

2. All living organisms require carbon in the form of CO_2, and some require additional organic carbon compounds such as sugars and other carbohydrates.

3. All living organisms require nitrogen in some form. Some types are atmospheric nitrogen, some thrive on inorganic nitrogen compounds, and naturally occuring organic nitrogen compound.

4. All living organisms require sulfur and phosphorus. Some types require organic sulfur compounds, some are capable of utilizing inorganic sulfur compounds, and some have the unique capacity of utilizing elementary sulfur. Phosphorus is usally supplied as phosphate.

5. All living organisms require several metallic elements such as sodium, potassium, calcium, magnesium, manganese, iron, zinc, copper, phosphorus, and cobalt for normal growth.

6. All living organisms contain vitamins and vitamin-like compounds.

7. All living organisms require water for growth. For bacteria all nutrients must be in solution before they can enter the organisms.

Considering of the requirements of bacterial growth, media can be grouped into :

1. Solid media. The solid media contain agar, serum albumin, or gelatin.

2. Liquid media. The main composition of the media is water. There is no agar in this type of media.

3. Semi-solid media. This media contains a smaller percentage of agar or gelatin than solid media.

The groups of media as mentioned will be divided into subgroups according to the purposes of assays and special requirements for growth of some organisms. (2, 5, 6).

1. Enriched media. The media is special for growth of fastidious organisms. It contains blood, serum or extract from plants and animals tissues.

2. Selective media. This type of media is used for selective growth of some microorganisms, especially gram negative bacilli. The media contains specific chemical substances, such as crystal violet, which inhibit the growth of gram positive bacteria.

3. Differential media. The media contains some substance that can be used for making a distinction between organisms. For instance blood agar media can differentiate bacteria that produce a hemolytic zone from a bacteria that cannot produce a hemolytic zone.

4. Assay media. The media is usefull for vitamins, amino acid and antibiotic assays.

5. Media for enumeration of bacteria. The media is usefull for the detection of bacteria in milk and water.

6. Media for characterization of bacteria. This type of media contains a specific substance, cystine tellurite for example, that produces characteristic appearance in bacteria grown on the medium. Corynbacterium diphtheriae colonies will be black on the surface of cystine tellurite medium.

7. Transport media. The media contains oxidation and reduction protective agents. It supports life but not growth of bacteria before culture.

8. Maintenance media. The media is used in preservation of bacteria in the living state.

Distilled water is very important for preparing media (7). Tap water may be used (5) if it had low mineral content. Copper will inhibit the growth of bacteria. The pH of media is also important for the growth of bacteria. Fluid media (7) should have pH adjusted before it is transfered to tubes, flasks, or bottles in suitable volume and covered with cotton plugs, plastic caps or metal caps. Generally the media is sterilized by autoclaving.

Growth of bacteria requires optimal temperature for incubation. The optimal temperature is usually between 35°-37°c. Some bacteria require special conditions, including increased CO_2 atmosphere, unusual acid or alkaline pH conditions, etc.

Material and Method.

Three organisms, Beta streptococci group A, Pneumococci, and Haemophilus influenzae were used. They were obtained from stock cultures.

Media 1. Tryptic soy broth. (Difco)

2. Tryptic Soy broth with 1% Yeast extract. (Difco)
3. Bacto Heart infusion broth. (Difco)
4. Tryptose Phosphate broth (Difco)
5. Nutrient broth. (Difco)

These media were prepared according to manufacture's directions.

Test tube. Screw cap tubes, 20 mm.× 150 mm, were used. These tubes were calibrated with a Colemnn Nepho-Colorimeter using barium sulphate solution and reading Nepholometer units or Opacity units. The tubes for use had a variation not more than ±.02. When calibrated, the tubes were washed with distilled water, autoclaved and dried. 10 c.c of broths was added to each tubes.

3-4 colonies of Beta streptococci group A from a blood agar plate were inoculated into 5 ml. of tryptic soy broth or 5 ml. of heart infusion broth and incubated at 37°c, 18-24 hours.

0.1 c.c of 18-24 hours broth culture was transfered into tryptic soy broth, tryptic soy broth with 1% yeast extract, heart infusion broth, tryptose phosphate broth, and nutrient broth respectively. Nephelometer units were read and then

the tubes were incubated at 37°c on a shaker (Kahn Shaker Burton Manufacturing Company. Los Angelis U.S.A. Model No. (430). Set at 180/min.

Assay of Coleman Nepho-Colorimeter. The following method was used.

1. Adjust dials and knobs as follows.

GALV coarse	- fully clockwise.
GALV fine	- fully clockwise.
BAL	- black scale at zero.
STD	- any position.
BLK	- fully clockwise.

2. Insert "Number 35" standard tube (well mixed), (prepare by used barium sulphate solution equivalent to a 1 : 10 dilution of Mc. Number 1). Cover with light sheild.

3. Adjust illuminated pointer to read 35 on the black scale, with coarse and fine adjustment.

4. Insert "blank" tube containing the broth to be used in the test.

5. Adjust illuminated pointer to zero with BLK control.

6. Read unknown sample.

7. When reading exceed 85% on the unknowns, insert Number 35 tube again and adjust to 17.5% or 10% using the coarse and fine adjustment knobs. Multiply all readings thereafter by 2 or 3.5 respectively.

Reading of Broth Cultures.

The 1 st reading was taken after inoculating organisms into broth and mixing.

The 2nd reading was taken after incubation on the Kahn shaker for 2 hrs.

The 3 rd reading was taken after incubation on the Kahn shaker for 4 hrs.

The 4 th reading was taken after incubation on the Kahn shaker for 6 hrs.

The same procedure was repeated for Pneumococci and Haemophillus influenzae.

Results

Table 1 shows the growth of Beta streptococci group A and Pneumococci in tryptic soy broth medium.

Table 2 shows the growth of Beta streptococci group A and Pneumococci in tryptic soy broth with 1% yeast extract.

Table 3 shows the growth of Beta streptococci group A and Pneumococci in bacto heart infusion broth.

Table 4 shows the growth of Beta streptococci group A and Pneumococci in tryptose phosphate broth.

Table 5 shows the growth of Beta streptococci group A and Pneumococci in nutrient broth.

Graphs were plotted for growth of the organisms on semi-log paper using the average nephelometer units against time for incubation.

Figure 1 is the graph for the growth of Beta streptococci group A in the broths.

Figure 2 is the graph for the growth of Pneumococci in the broths.

Haemophillus influenzae is a fastidious organisms, requiring an infusion medium

enriched with blood or hemoglobin (X factor) and DPN or TPN (V factor). So it was unable to growth in these broths.

Discussion

Beta Streptococci, Pneumococci and **Hemophillus influenzae** are among the fastidious organisms commonly isolated from patients. These organisms need enriched media for their growth. Selection of the proper media for cultivation is very important, and the organisms generally grow poorly in liquid media.

Table I - V show that Tryptic soy broth containing 1% yeast extract supported growth of beta Streptococci group A better than other liquid media. Peptones in this medium are derived from soy bean, and the medium very useful in culturing beta - Streptococci, especially for typing, since medium containing peptone from animal sources may induce some beta-Streptococci to produce an active proteolytic enzyme which destroys M substance. Besides the medium used, there are other factors that stimulate growth rate of the organisms. William F. Vincent and Kathleen J. Lisiewski (1) found that beta-Streptococci group A would grow 4-5 times better if broth cultures were incubated with shaking at 37°c. This is confirmed in our experiment; if incubated without shaking, the organisms grew very poorly. Before measurement of growth by Nephelometer could be determined, cultures had to be incubated at least six hours, while only two hours were required in "shaking" incubation.

Pneumococci grow best in Tryptose phosphate broth; however, growth is very slow. **Hemophilus influenzea** do not grow in any liquid medium tested, because these media lack the X and V factors needed for their growth.

The growth was determined by Nephelometer, using a Coleman Nepho-Colorimeter. This method is easy and not time consuming. However, there are some disadvantages. For example, the method can be applied only to bacterial populations of relatively high density. Accurate measurements require suspensions containing ten million or more bacteria per milliliter. Also, turbidity of cultured broth may result from other factors, such as increases only in the size and shape of the organisms, contamination etc.

From our experiment, we can see that media used in cultivation of the organisms is very important. In good laboratories, quality control of media should always be done (3, 5, 8), because some lots of the medium or the same lot prepared at different times may not support the growth of some organisms.

TABLE 1

Shows the growth of Beta streptococci group A and Pneumococci in Tryptic soy broth medium.

Organism	Incubation time in hours			
	0	2	4	6
Beta Strep. group A 1	0	6	55	304.5
,, ,, 2	0	3	29	108
,, ,, 3	0	3	11	48
,, ,, 4	0	2	13	91
Average	0	3,5	27	113
Pneumococci 1	0	00	00	13
,, 2	0	00	00	18
,, 3	0	00	00	24
,, 4	0	00	00	24
Average	0	00	00	20

0 = No reading

00 = Growth insufficient to obtain reading

TABLE 2

Shows the growth of beta streptococci group A and Pneumococci in Tryptic soy broth with 1 % Yeast extract

Organism	Incubation time in hours			
	0	2	4	6
Beta Strep. group A 1	0	3	114	588
,, ,, 2	0	5	33	470
,, ,, 3	0	3	19	59
,, ,, 4	0	2	20	185.5
Average	0	3	47	325.6
Pneumococci 1	0	00	2	39
,, 2	0	00	9	41
,, 3	0	00	7	40
,, 4	0	00	9	45
Average	0	00	7	41

0 = No reading

00 = Growth insufficient to obtain reading

TABLE 3

Shows the growth of Beta streptococci group A and Pneumococci in Bacto Heart infusion

Organism	Incubation time in hours			
	0	2	4	6
Beta strep. group A 1	0	6	84	206.5
,, ,, 2	0	3	12	27
,, ,, 3	0	3	6	13
,, ,, 4	0	4	18	126
Average	0	4	30	93
Pneumococci 1	0	00	1	1
,, 2	0	00	2	3
,, 3	0	00	1	2
,, 4	0	00	1	1
Average	0	00	1.25	1.75

0 = No reading

00 = Growth insufficient to obtain reading

TABLE 4

Shows the growth of Beta streptococci group A and Pneumococci in Tryptose Phosphate broth

Organism		Incubation time in hours			
		0	2	4	6
Beta Strep. group A	1	0	6	102	574
,, ,,	2	0	5	56	250
,, ,,	3	0	4	22	76
,, ,,	4	0	6	25	157.5
Average		0	5	51	264
Pneumococci	1	0	00	35	82
,,	2	0	00	5	45
,,	3	0	00	20	90
,,	4	0	00	40	96
Average		0	00	25	78

0 = No reading

00 = Growth insufficient to obtain reading

TABLE 5

Shows the growth of Beta streptococci group A and Pneumococci in Nutrient broth medium

Organism	Incubation time in hours			
	0	2	4	6
Beta strep. group A 1	0	2	2	2
,, ,, 2	0	2	2	2
,, ,, 3	0	1	2	2
,, ,, 4	0	2	2	2
Average	0	1.75	2	2
Pneumococci 1	0	00	00	00
,, 2	0	00	00	00
,, 3	0	00	00	00
,, 4	0	00	00	00
Average	0	00	00	00

0 = No reading

00 = Growth insufficient to obtain reading

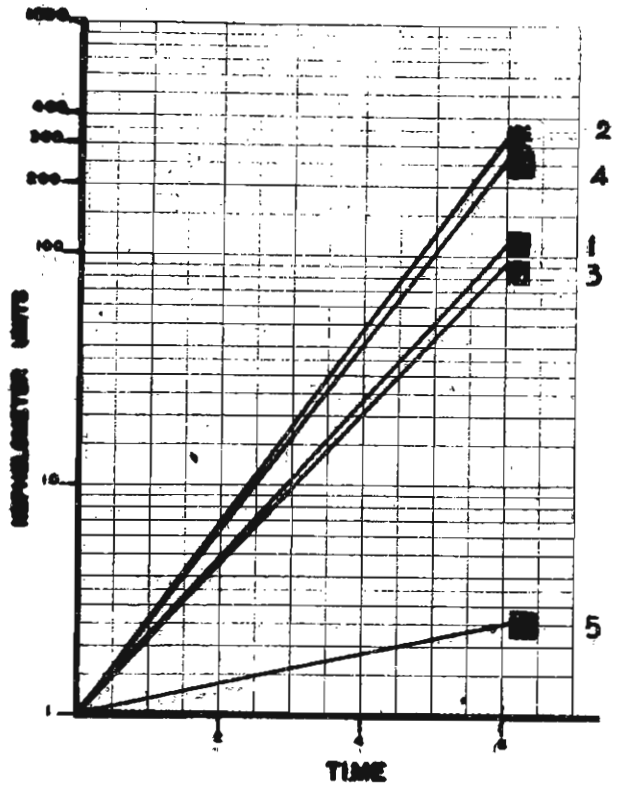

Figure 1

Is the graph for the growth of Beta streptococci group A. in the broths.

1. Tryptic Soy broth
2. Tryptic Soy broth with 1%YE
3. Heart Infusion broth
4. Tryptose Phosphate broth
5. Nutrient broth

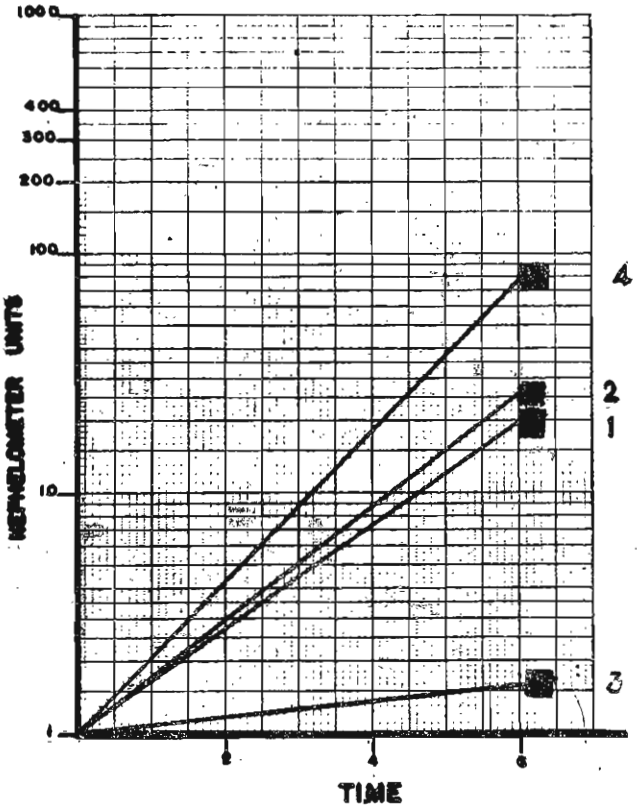

Figure 2

Is the graph for the growth of Pneumococci in the broths.

1. Tryptic Soy broth
2. Tryptic Soy broth with 1%YE
3. Heart Infusion broth
4. Tryptose Phosphate broth

ย่อเรื่อง

จากการเลี้ยงเชื้อ Beta streptococci group A, Pneumococci และ Haemophilus influenzae ใน media 5 ชนิด คือ Tryptic soy broth, Tryptic soy broth with 1% Yeast extract, Heart Infusion broth, Tryptose Phosphate broth และ Nutrient broth โดยใช้ 0.1 c.c. 18–24 hrs. broth culture ของเชื้อดังกล่าวใส่ลงไปใน broth จนครบชุด หลอดละ 0.1 c.c. นำไป incubate 37°C. บน Burton Kahn Shaker ใน incubator แล้วนำมาวัดความขุ่นเป็น Nephelometer units ด้วยเครื่อง Coleman Nepho–Colorimeter หลังจาก incubate ครบ 2 hrs., 4 hrs. และ 6 hrs. ตามลำดับ พบว่าหลังจาก incubate ครบ 6 hrs. Beta streptococci เจริญได้ดีที่สุดใน Tryptic Soy broth with 1% Yeast extract. Pneumococci เจริญได้ดีใน Tryptose Phosphate broth และ Haemophilus influenzae ไม่สามารถจะเจริญใน Broth ชุดนี้ได้.

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