FACTORS INFLUENCING THE GROWTH OF SHIGELLA DYSENTERIAE ON LABORATORY MEDIA

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by

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Introduction

In most laboratories, dysentery bacilli are detected in fecal samples by smearing plates of different kinds of media. In some instances the smear plates are made from relative enrichment media seeded with the fecal sample. In all cases several media are used, because it has been found that frequently only one of the several media used shows the presence of dysentery bacilli. The fact that the use of enrichment media or the use of several plating media increases the number of isolations indicates that many of the dysentery bacilli present in the fecal sample do not survive and reproduce in the culture media. It is assumed in this study that a highly efficient plating medium would support the growth of all dysentery bacilli, and there would be no necessity of using so many different media.

The critical life in the growth of an organism lies in the period following the first cell division. If the environmental conditions are so unfavorable that the cells can not adjust conditions to their needs, growth ceases and the cells die. For an isolated cell to reproduce, it is essential that the medium approach ideal growing conditions as nearly as possible. It appears from a review of the literature on media for the isolation of dysentery organisms that these media may not offer a favorable environment for the reproduction of the primary young cells. It has been demonstrated by Salter (1),

Sherman and Albus (2), Stark and Stark (3), and Sherman and Cameron (4) that young bacterial cells are more susceptible to adverse conditions of their environment than are cells in the logarithmic growth phase. The last two workers showed that young cells of Escherichia coli may be killed by abrupt environmental changes within the natural range of growth of the organism. The factors with which they worked were change of temperature and change of osmotic pressure. Huntington and Winslow (5) studied characteristics of physiological youth and found a definite and orderly relationship of changes; metabolic activity increased first, cell volume second, and cell division rate third. These characteristics are also found in higher plants and animals in their physiological youth. Sherman and Albus (6) demonstrated that mature cells from old cultures assumed the characteristics of young cells before reproduction began. They hold the view that during the lag period the old cells undergo a biologic rejuvenescence which fits them for reproduction. Darby and Mallmann (7) demonstrated by practical tests that a medium designed to shorten the lag phase of coliform organisms would actually increase the percentage of samples found to contain these organisms.

The object of this work was to develop an optimum base medium that would promote the growth of dysentery bacilli during the lag and early logarithmic growth stages, the period of physiological youth. Such an optimum medium could then be used as a standard to which could be compared the

selective enrichment and differential media now used and possibly serve as the base of a new diagnostic medium. There is no available information that would indicate that an ideal medium could supplant the use of several different media, but the writer believes that the use of one such medium alone or in series with other media would materially increase the number of isolations.

Historical Review

Hardy and his associates (8) made a careful study of four differential culture media used in the study of acute diarrheal diseases, the four media being eosin-methylene-blue agar, Endo's agar, desoxycholate agar, and sodium desoxycholate-citrate agar. They found the sodium desoxycholate-citrate agar to be of superior value and the two sodium desoxycholate agars used supplementarily to be of outstanding value. MacConkey's agar was found to compare favorably with eosin-methylene-blue agar and Endo's agar but not with the desoxycholate agars. He found desoxycholate agar to promote the growth of all the usual intestinal pathogens and common Gram-negative intestinal bacilli and the desoxycholate-citrate agar to inhibit most of the nonpathogenic organisms and, to a limited degree, some of the pathogens. In his whole survey only three varieties of Shigella dysenteriae were found--Flexner, Sonne, and Newcastle. No Shiga or Schmitz varieties were found on any of the media. The desoxycholate-citrate agar permitted the use of a heavy inoculation. Plain desoxycholate agar was somewhat superior

to either eosin-methylene-blue or Endo's agar, especially for Sonne and Newcastle.

In their investigation of preserving solutions for the recovery of dysentery bacilli from fecal specimens, Bangxang and Eliot (9) studied the efficiency of various plating media and reported the following:

(1) Desoxycholate-citrate agar inhibits the non-pathogenic fecal bacteria, but it does not grow Schmitz, Sonne, Shiga, and some Flexner strains.

(2) Desoxycholate agar is superior to eosin-methylene-blue agar, Endo's agar, and MacConkey's agar. Though it is slightly inhibitive to some of the strains it eliminates all Gram-positive organisms.

(3) Eosin-methylene-blue agar is slightly inhibitory to some strains.

(4) MacConkey's agar showed no inhibitory effect on any dysentery strain tested.

(5) Endo's agar showed no inhibitory effect on any dysentery strain tested.

(6) Brom-cresol-purple agar exhibited no selectivity. In these studies the amount of inhibition exerted by the medium on the growth of the bacteria was based on the degree of growth on the plates. This method of measuring inhibition is a questionable procedure, particularly when there are no controls used, as was true in this study. The conclusions in these studies should therefore be considered on a comparative basis.

In a study on the differentiation and identification of bacillary incitants of dysentery, Coleman (10) confirmed the work of Hardy and his associates in that desoxycholate-citrate agar was preferable for isolating dysentery bacilli. She found that it restricts the growth of a relatively small number of Flexner strains and an appreciable number of Sonne strains and therefore recommended the use of desoxycholate agar without citrate, MacConkey's agar, eosin-methylene-blue agar, or a modification of Endo's agar for a supplementary medium.

The Michigan State Department of Health uses a medium called the S and S medium for the isolation of Salmonella and Shigella organisms. Though no reference to this medium was found in the literature, it is mentioned here because it was tested in the same manner as were the other differential media studied.

To summarize the criticisms of all of the media now used for the isolation of dysentery bacilli, sodium desoxy-cholate-citrate agar appears to be the medium used most successfully. Because it restricts the growth of a number of dysentery strains, different workers have recommended that another differential plating medium be used in conjunction with it. Sodium desoxycholate agar without citrate, MacConkey's agar, eosin-methylene-blue agar, and a modification of Endo's agar have been named as good supplementary media.

The apparent inefficiency of these differential media is indicated by the haphazard occurrence of positive cultures on only one medium. This is true also of other intestinal pathogens such as Eberthella and Salmonella organisms. The reason for this random occurrence of positive cultures on the various differential media could be that the media do not support the growth of the bacterial cells during the most critical period of their development--the period of physiological youth. Only the less delicate, more resistant cells are able to survive and reproduce, and the other cells die before their presence can be detected. With the object of developing a medium that would reduce this mortality to a minimum the following studies were made.

Experimental

In order to formulate a medium that would give maximum reproduction during the period of physiological youth, growth curves in broth media were determined using the technique of Darby and Mallmann (7). A twenty-four hour tryptose agar slant culture of Shigella dysenteriae, Shiga (No. 1032), was used as the source of organisms. To intensify the effects of the different media, the number of cells introduced into the broths was reduced to a minimum (10 to 50 organisms per milliliter). It has been shown by many workers that it is more difficult for a small number of organisms to establish themselves than it is for large numbers of organisms. Growth rates were determined by taking counts of the number of

organisms at different intervals of the growth curve. In each growth curve effort was made to have only one variable, the factor being tested.

The base medium for the different broths was made up in one lot and portioned out in 200 ml. quantities, to which the variable was then added. Duplicate flasks containing 100 ml. each were prepared of each broth. Except in the growth curves in which the optimum concentration of a constituent was tested, the base medium had the following composition:

Peptone.....	2.0%
Sodium chloride...	0.5%
Dextrose.....	0.2%
K_2HPO_4.....	0.4%
KH_2PO_4.....	0.15%

This base medium was selected because it had worked so well for Darby and Mallmann for growing E. coli, an intestinal organism closely related to Shigella dysenteriae. The tryptose agar plating medium was selected for the same reason.

The numbers of organisms in the broth media were determined at 0, 2, 4, 6, and 24 hours after the seeding. These counts were determined by plating two one-milliliter quantities of the following dilutions:

Hours after seeding	
0	1 ml.
2	1 ml., 1-10
4	1 ml., 1-10, 1-100
6	1-100, 1-1T, and 1-10 or 1-10T
24	1-100T, 1-1M, and 1-10T or 1-10M

The dilutions were made in standard milk dilution bottles equipped with Escher stoppers. Plates were poured with buffered tryptose agar (pH 6.8) and incubated 48 hours before the colonies were counted.

Generation time: The generation time of an organism has been defined as that amount of time which elapses between the fission of a cell into two new cells and the subsequent fission of the newly formed cells. The generation time can be computed by a formula developed by Buchner, Longard and Riedlin (11). This formula is

$$g = \frac{t \log 2}{\log b - \log B}$$

when

t = time
B = number of bacteria at the beginning of time period
b = number of bacteria at the end of the time period
g = generation time

A short generation time is indicative of an environment which enhances reproduction in contrast to a long generation time indicating an unfavorable environment. Since the most critical period in the growth and reproduction of bacteria is the very early part of the lag phase, which occurs after the introduction of those cells into a new environment, it was assumed that the medium which gave the shortest generation time during this period would support the growth of the more delicate cells and would be the best medium. Since all cultures were found to be in the early logarithmic growth phase four hours after the broths were seeded, the generation time from zero to four hours was chosen as being the

index to the efficiency of the media, the broth having the shortest generation time during this period being the most favorable medium.

Peptone: The source of nitrogen is especially important in a nutrient medium, not only as to the kind of peptone but also as to the concentration. Of the twelve different peptones tested, Armour I, Armour's Siccum, Proteose Peptone 3 (Difco), and Tryptose (Difco) were found to yield good results, as shown in the tabulated data. Peptones Armour I and Armour II were submitted by Armour and Company for bacteriological investigation and are not on the market as commercial peptones. Table 1 presents the results of the growth curves run for the purpose of selecting the most satisfactory peptone. Since in the first series of peptones Proteose 1 and Armour's Siccum gave better results than did Witte, Bacto, Stearn, or Neo, these two were selected for further study. In the next series Armour I and Proteose 3 were found to be better than Armour's Siccum, Armour II, and Proteose 1 and 2, so these two peptones were studied again by comparing them with Tryptose and Tryptone. Armour I proved to be the most favorable peptone and Proteose 3 the next most favorable. These peptones were selected on the basis of short generation times from zero to four hours after the seeding. To determine the significance of small differences in the index generation times, a few of the growth curves were repeated several times, and in each instance the results were the same as the first growth curve.

TABLE 1

The Influence of Peptones in a Base Medium* on the Early Growth of *Shigella dysenteriae*

Series	Kind of peptone	Number of bacteria per ml.					Generation time in minutes			
		Time of sampling in hours					Time of sampling in hours			
		0	2	4	6	24	0-4	0-2	2-4	4-6
1	Armour Siccum	42	43	540	3600	304M	65	3600	33	44
	Proteose	45	53	320	1800	512M	85	516	46	48
	Neo (Difco)	40	46	170	230	251M	114	602	64	277
	Stearn's	33	60	130	1220	166M	120	139	37	64
	Witte's	49	50	180	370	216M	130	4100	66	116
	Bacto(Difco)	54	44	170	2500	157M	142		62	31
2	Armour Siccum	24	31	500	7200	470M	55	328	30	30
	Armour I	18	30	530	4700	530M	48	161	29	38
	Armour II	36	36	500	4500	160M	63		31	38
	Proteose (Difco)	25	16	410	6000	280M	59		25	30
	Proteose 2 (Difco)	35	60	900	6800	260M	51	157	30	41
	Proteose 3 (Difco)	23	60	750	4200	410M	48	85	33	48
3	Armour I	57	220	3900	79000	470M	39	61	29	28
	Proteose 3 (Difco)	54	120	2500	33000	270M	43	103	27	32
	Tryptose (Difco)	61	130	1400	20000	350M	53	109	35	31
	Tryptone (Difco)	67	140	1500	14000	280M	53	112	35	37

*Base medium consists of: NaCl 0.5%, dextrose 0.2%, K_2HPO_4 0.4%, KH_2PO_4 0.15%; peptone 2%; pH 6.6 before sterilization.

Table 2

Influence of Armour I Peptone Concentration in a Base Medium* on the Early Growth of *Shigella dysenteriae*

Concentration of Armour I	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
per cent									
0.5	19	18	310	5500	33M	59		29	29
1.0	24	15	670	24000	230M	49		22	23
2.0	18	16	1000	32000	250M	42		20	24
3.0	19	20	820	15000	370M	44	1800	22	28

*Base medium consists of: NaCl 0.5%, dextrose 0.2%, K_2HPO_4 0.4%, KH_2PO_4 0.15%; pH 6.6 before sterilization.

After it was found that Armour I was the most desirable peptone, it was necessary to determine the optimum concentration in which to use it. As can be seen in table 2 the most satisfactory concentration proved to be 2 per cent. This concentration of peptone gave an index generation rate of 42, a rate significantly shorter than those yielded by lower and higher concentrations of peptone.

Hydrogen ion concentration. Earlier workers believed that dysentery organisms could not survive in an acid medium. The failure of certain preserving solutions to permit the isolation of the dysentery organisms substantiated this belief. Bangxang and Eliot (9) reported that, though Dudgeon assumed that pH was the factor of paramount importance in preserving the viability of dysentery organisms in stools, they presented data that indicated that the adjustment of the specimen to an alkaline reaction was not the only factor concerned in preserving the viability of dysentery bacilli.

To determine the optimum pH for the growth of Shigella dysenteriae, flasks of broth were prepared and adjusted to pH values of 6.4, 6.6, 6.8, 7.0, and 7.2. All broths were adjusted to the desired pH by the addition of normal NaOH or HCl until the desired reading was obtained on the Beckman pH meter. All pH values given indicate the pH before the broths were sterilized.

A pH of 6.6 to 6.8 was found to be the optimum value for the test organism. To determine the possibility of the

Table 3

Influence of the Hydrogen Ion Concentration of Armour I Broth* on the Early Growth of Shigella dysenteriae

pH	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
6.4	49	250	10000	220T	198M	31	51	22	27
6.6	38	260	8800	355T	263M	31	43	23	22
6.8	60	276	10000	515T	355M	32	55	23	51
7.0	65	260	8800	260T	410M	34	60	23	77
7.2	65	149	3500	87T	198M	42	100	26	25

*Broth consists of: Armour I peptone 2%, NaCl 0.5%, dextrose 0.2%, K_2HPO_4 0.4%, KH_2PO_4 0.15%.

Table 3a

Influence of the Hydrogen Ion Concentration of Tryptose Broth* on the Early Growth of *Shigella dysenteriae*

pH	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
6.4	70	160	2500	50T	112M	47	100	30	27
6.6	58	175	5000	91T	230M	37	75	25	28
6.8	48	145	4100	75T	240M	37	75	25	28
7.0	69	160	3800	65T	224M	41	99	26	29

*Broth consists of: Tryptose 2%, NaCl 0.5%, dextrose 0.2%, K_2HPO_4 0.4%, KH_2PO_4 0.15%.

optimum pH varying with different peptones, five different peptones were studied, and in each instance the optimum pH remained between 6.6 and 6.8. The influence of pH was less marked with Armour I than with any of the other peptones tested. Tryptose, for instance, gave an index generation time of 37 minutes at pH 6.6 and pH 6.8, while pH 6.4 gave an index of 47 and pH 7.0 gave an index of 41 minutes.

Buffer. Salter (1) found that K_2HPO_4 in a concentration up to 1 per cent accelerates the rate of growth of Bacillus communis (Escherichia coli), causing a production of the maximum number of organisms in a shorter time. Perry and Hajna (12) recommended a mixture of 0.4 per cent K_2HPO_4 and 0.15 per cent KH_2PO_4 to give a constant pH; this mixture was also used and recommended by Darby and Mallmann (7) in their study of media for coliform organisms. That phosphates are important in the nutrition of bacterial cells is indicated by the high phosphorus content of bacterial ashes.

The pH of the broths tested was controlled to some extent by the use of the phosphate mixture recommended by Perry and Hajna. Broths containing this mixture gave shorter generation times during the lag phase of growth and larger total numbers of organisms at the end of 24 hours incubation than did the unbuffered broths. The buffered broth gave an index generation time of 51 minutes, while the unbuffered broth gave an index of 58.

Table 4

The Influence of a Buffer Mixture in the Base Medium* on the Early Growth of *Shigella dysenteriae*

Buffer	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
None	32	35	600	10000	130M	58	900	29	29
Present	39	36	1000	12000	270M	51		25	33

*Base medium: Armour I peptone 2%, dextrose 0.2%, NaCl 0.5%; adjusted to pH 6.6 before sterilization.
Buffer mixture: K_2HPO_4 0.4%; KH_2PO_4 0.15%.

Sodium chloride. Mooney and Winslow (13) found glucose to be inhibitive to Salmonella pullorum in an aerated culture unless NaCl was present. Darby and Mallmann (7) found the addition of NaCl to increase the growth of Esch. coli in broth media. Because of these findings the influence of NaCl on Shigella dysenteriae was determined.

It was thought that the addition of the phosphate buffers might alter the osmotic pressure enough to warrant a change in the usual NaCl concentration, so growth rates were determined in broths containing 0.0, 0.5, 1.0, and 2.0 per cent NaCl. Table 5 shows 0.5 per cent NaCl to give the shortest index generation time.

Dextrose. To supply a readily available source of energy, dextrose was added to the base broth in various concentrations. Broths containing 0.0, 0.2, 0.5, 1.0, and 2.0 per cent dextrose were tested. There was not much difference in the results yielded by 0.2 and 0.5 per cent dextrose, but higher concentrations seemed to lengthen the lag phase.

Beef extract. Salter (1) found that 0.3 per cent beef extract added to a peptone water solution gave much greater growth of B. communis than the peptone water solution without the beef extract. It should be noted that there was no phosphate in the base broth used by Salter.

The effect of beef extract on Shigella dysenteriae was determined by making broth media containing 0.0 and 0.3 per cent beef extract and testing them in the usual

Table 5

Influence of Sodium Chloride Concentration in the Base Medium* on Early Growth of Shigella dysenteriae

Concentration of NaCl	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
per cent									
0.0	21	16	360	34000	200M	59		26	18
0.5	39	36	1000	12000	270M	51		25	33
1.0	33	37	800	6700	240M	52	722	29	39
2.0	31	19	160	1400	230M	101		39	38

*Base medium consists of: Armour I peptone 2%, dextrose 0.2%, K_2HPO_4 0.4%, and KH_2PO_4 0.15%; pH 6.6 before sterilization.

Table 6

Influence of Dextrose Concentration in the Base Medium* on Early Growth of *Shigella dysenteriae*

Concentration of dextrose	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
per cent									
0.0	84	110	860	7100	180M	71	301	41	39
0.2	82	110	920	10000	420M	68	278	39	35
0.5	82	110	980	11000	340M	67	278	38	34
1.0	76	95	690	3300	310M	75	361	42	53
2.0	85	95	440	1800	220M	102	722	54	59

*Base medium consists of: Armour I peptone 2.0%, NaCl 0.5%, K_2HPO_4 0.4%, KH_2PO_4 0.15%; pH 6.6 before sterilization.

manner. Since there was no appreciable difference given by the broths containing 0.0 and 0.3 per cent beef extract, it was thought that its inclusion in the base medium would not be justified. Levine (14) used K_2HPO_4 instead of beef extract in a modified Endo's medium which he found very successful. It appears that it is not necessary to use phosphates and beef extract in the same medium.

The optimum medium. From the data presented it appears that a medium with the following composition would give the optimum conditions for early growth of Shigella dysenteriae:

Armour I peptone.....	2.0 %
K_2HPO_4.....	0.4
KH_2PO_4.....	0.15
NaCl.....	0.5
Dextrose.....	0.5
pH 6.6 before sterilization	

The amounts of phosphate given are those amounts recommended by Perry and Hajna (12); however, it might be more practical in this medium to adjust the phosphates to give a pH of 6.6.

It was believed that this medium would shorten the generation time during the period of physiological youth to a minimum.

The toxicity of differential media now in use. To compare the efficiency of the various differential media used for the isolation of Shigella dysenteriae to this so-called optimum medium, broths were made according to the formula of each medium except that no agar was added. It was assumed that agar is only a solidifying agent and does not

Table 7

Influence of Beef Extract in the Base Medium* on Early Growth of *Shigella dysenteriae*

Beef extract	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
per cent									
0.0	37	350	11000	220T	420M	28	37	24	28
0.3	30	340	8700	34T	280M	29	34	25	61

*Base medium consists of: Armour I peptone 2.0%, NaCl 0.5%, K_2HPO_4 0.4%, KH_2PO_4 0.15%; pH 6.6 before sterilization.

Table 8

Comparison of Various Differential Media with Armour I Broth* for Early Growth of Shigella dysenteriae

Medium	Number of bacteria per ml.					Generation time in minutes			
	Time of sampling in hours					Time of sampling in hours			
	0	2	4	6	24	0-4	0-2	2-4	4-6
Endo	38	40	320	1500	less than 10T	78	1800	40	54
MacConkey	33	37	63	250	200M	258	722	157	60
S and S	36	37	245	1500	270M	87	3600	44	47
Armour I	44	61	640	5600	460M	68	278	39	41

*Armour I broth consists of: Armour I peptone 2.0%, NaCl 0.5%, K_2HPO_4 0.4%, KH_2PO_4 0.15%; pH 6.6 before sterilization.

enter into growth reactions of organisms. The growth rates in these broth media were compared with the growth rates in the Armour I medium. The results of this study are presented in table 8. None of the differential media promoted growth so well as the Armour I medium. Listed in order of increasing toxicity, they are Armour I, Endo, S and S, and MacConkey. The desoxycholate media could not be included in this study because of inability to obtain some of the ingredients.

To confirm the preceding results, a technique simulating actual working conditions of these media was employed. A saline suspension of the organism was diluted so that the estimated number of organisms per milliliter was approximately 50. One milliliter of this suspension was placed in each of 25 sterile petri dishes, and five plates were poured with each agar medium. Again the Armour I medium gave the largest count, 43, thus indicating a high mortality of cells on the differential media. Endo's medium, with a count of 33 organisms per milliliter, was less toxic than any of the differential media tested, though MacConkey's agar was nearly as good, as shown by the count of 29. Desoxycholate-citrate agar and the S and S medium, with respective counts of 25 and 13, were the most toxic media tested.

Since only one strain of Shigella was used, and inasmuch as there are other pathogenic members in the group, it was thought advisable to test the other members' growth on the Armour I medium. Growth rates were determined for six

Table 9

Viability of *Shigella dysenteriae* in Various Differential Media and the Armour I Medium

Medium	Plate Counts					Average Plate Count
Armour I	43	46	39	38	49	43
Endo	36	30	34	35	31	33
MacConkey	35	31	25	26	29	29
Desoxycholate-Citrate	27	16	31	29	24	25
S and S	12	13	14	12	13	13

Table 10

Comparison of Armour I Medium* and Plain Nutrient Broth for Early Growth of Dysentery Organisms

Strain of Organism	Plain Nutrient Broth					Armour I Medium				
	Number of bacteria per ml Time of sampling in hours					Number of bacteria per ml Time of sampling in hours				
	0	2	4	6	24	0	2	4	6	24
Newcastle	84	87	84	690	130M	76	84	320	6700	640M
Shiga	43	105	1600	33T	160M	44	140	2200	42T	410M
Flexner	72	75	0	-	-	88	140	3300	150T	340M
Strong	105	160	2100	17T	230M	105	150	2000	16T	770M
Hiss	83	210	2300	85T	220M	96	280	7300	200T	540M
Sonne	130	600	21T	750T	150M	120	630	17T	760T	380M

*Armour I medium consists of: Armour I peptone 2%, NaCl 0.5%, dextrose 0.2%, K_2HPO_4 0.4%, KH_2PO_4 0.15%; pH 6.6 before sterilization.

Table 10a

Comparison of Armour I Medium* and Plain Nutrient Broth for Early Growth Rates of Dysentery Organisms

Strain of Organism	Plain Nutrient Broth				Armour I Medium			
	Generation time in minutes Time of sampling in hours				Generation time in minutes Time of sampling in hours			
	0-4	0-2	2-4	4-6	0-4	0-2	2-4	4-6
Newcastle	---	2400	---	40	115	830	62	27
Shiga	46	93	31	28	43	---	21	28
Flexner	---	2100	---	---	46	180	26	22
Strong	56	200	32	40	260	226	32	40
Hiss	50	90	35	23	38	79	25	25
Sonne	33	55	23	23	34	50	25	22

*Armour I medium consists of: Armour I peptone 2%, NaCl 0.5%, dextrose 0.2%, K_2HPO_4 0.4%, KH_2PO_4 0.15%; pH 6.6 before sterilization.

other members of the group of dysentery organisms in the new medium and in plain nutrient broth. The growth of the Newcastle, Shiga, Flexner, and Hiss strains was much better in the new medium than it was in plain nutrient broth. The Strong and Sonne strains seemed to grow as well in one medium as in the other. It was interesting to note that Flexner could survive only 4 hours in plain nutrient broth, whereas in the new medium it gave a count of 340,000,000 cells per milliliter at the end of 24 hours incubation.

Discussion of Results

In the data which have been presented, the growth rates of Shigella dysenteriae, Shiga, in media of various compositions have been compared. Those media which promoted the early growth of the organism were selected as being the most desirable media, and by the process of elimination a formula was derived for a medium that was believed to be optimum for the organism.

It was found that the type of peptone used materially influenced growth during the early growth phases. Armour I peptone was found to give the shortest generation time, but Proteose 3 and Tryptose were also good. It was found that the addition of NaCl and dextrose increased the value of the medium. The optimum concentration of NaCl was 0.5 per cent and that of dextrose 0.2 per cent. The phosphate buffer mixture recommended by Perry and Hajna proved to shorten the lag period of growth and make the addition of

beef extract unnecessary.

By studying one variable at a time a base medium was formulated to give the shortest generation time with minimal inoculation. When the base medium was compared with the various differential media now commonly used, it was found that these media did not support growth as well as the base medium did. Endo's, MacConkey's, sodium desoxycholate-citrate, and S and S agars were investigated.

The writer believes that the variations which occur in isolating the organism from 5 or 6 media are due at least to a large extent to the inhibition of the organisms by these media, making isolation improbable unless the cells are present in large numbers.

It is realized that the work presented in this thesis has been based on the assumption that a medium which will promote the growth of dysentery bacilli during the lag and early logarithmic growth phases will also permit the survival of an increased number of organisms on a solid medium. It was thought that a broth medium that would enhance reproduction in the most critical period of the growth curve would permit the survival of a larger percentage of viable cells when used as the base of a solid medium. The higher plate counts obtained with the new medium from the same suspension of organisms than with the differential media indicate that the assumption was justified. It is hoped that in the future some selective agent can be added to this base medium

that will make it a highly efficient diagnostic medium.

Summary

1. Armour I peptone was found to be superior to Armour's Siccum, Armour II, Proteose 1, Proteose 2, Proteose 3, Witte, Bacto, Stearn, Tryptose, and Tryptone peptones in a broth medium for the early growth phases of Shigella dysenteriae.
2. A concentration of 2 per cent Armour I was found to be the most desirable concentration of peptone, closely followed by Proteose 3.
3. The pH at which optimum growth was obtained was 6.6 to 6.8.
4. The addition of potassium phosphate buffers produced shorter generation times during the lag phase of growth and larger total numbers of organisms at the end of 24 hours incubation.
5. Sodium chloride in the concentration of 0.5 per cent gave more desirable results than 0.0, 1.0, and 2.0 per cent.
6. It was found that the concentration of dextrose could be varied from 0.2 to 0.5 per cent without causing any significant difference in generation times, but higher concentrations increased the generation times appreciably.
7. The addition of beef extract appeared to have no influence on the rate of growth during the lag and early logarithmic phases.
8. The medium having the following formula was selected as being the most favorable medium for the growth of Shigella

dysenteriae:

Armour I.....	2.0 per cent
K_2HPO_4.....	0.4
KH_2PO_4.....	0.15
NaCl.....	0.5
Dextrose.....	0.2
pH before sterilization:	6.6

9. Generation periods in the new medium were found to be shorter than in Endo's, MacConkey's, and S and S agars.

10. Plate counts obtained from a saline suspension of Shigella dysenteriae were higher on the Armour I medium than on Endo's, S and S, MacConkey's, and sodium desoxycholate-citrate agars.

11. The growth of the Newcastle, Shiga, Flexner, and Hiss strains was much greater in the Armour I medium than in plain nutrient broth; however, the Strong and Sonne strains showed as good or better growth in plain nutrient broth as in the Armour peptone broth.

Literature Cited

1. Salter, R. C. Observations on the Rate of Growth of Bacillus Coli. Jour. Infect. Dis. 24: 260, 1919.
2. Sherman, J. M., and Albus, W. R. Physiological Youth in Bacteria. J. Bact. 8: 127, 1923.
3. Stark, C. N., and Stark, P. The Relative Therman Death Rates of Young and Mature Bacterial Cells. J. Bact. 18: 333, 1929.
4. Sherman, J. M., and Cameron, G. M. Lethal Environmental Factors within Natural Range of Growth. J. Bact. 27: 341, 1934.
5. Huntington, Evelyn, and Winslow, C.-E. A. Cell Size and Metabolic Activity of Various Phases of the Bacteria Culture Cycle. J. Bact. 33: 123, 1927.
6. Sherman, J. M., and Albus, W. R. Function of Lag in Bacterial Cultures. J. Bact. 9: 303, 1924.
7. Darby, C. W., and Mallmann, W. L. Studies on Media for Coliform Organisms. Jour. Am. W. W. Assoc. 31: 689, 1939.
8. Hardy, A. V. Differential Culture Media in the Study of Acute Diarrheal Diseases. U. S. Pub. Health Repts. 54: 287, 1939.
9. na Bangxang, E., and Eliot, C. P. An Investigation of Preserving Solutions for the Recovery of Dysentery Bacilli from Fecal Specimens. Am. J. Hyg. Sect. B. 31: 16, 1940.

10. Coleman, M. B. The Differentiation and Identification of Bacillary Incitants of Dysentery. A. J. P. H. 30: 39, 1940.

11. Buchner, Longard, and Riedlin. Centralbl. f. Bakteriol., 1887, 11, p.1.

12. Perry, C. A., and Hajna, A. A. A Modified Eiijkmann Medium. J. Bact. 26: 419, 1933.

13. Mooney, G., and Winslow, C.-E. A. The Metabolic Activity of Various Colon Group Organisms at Different Phases of the Culture Cycle. J. Bact. 30: 427, 1935.

14. Levine, Max. J. A. P. H. A. 8: 886, 1918.

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