CHAPTER IV

DISCUSSION

Among various cultural conditions which are the aims of this study, the one which should be found out first is the incubation period, because it is necessary to know the time required to have enough SLO in the cultural fluid to be assayed. The starter used for studying should be in the exponential phase (log phase) which is the most active phase in growth cycle. As shown in Figure 5, the culture of Streptococcus pyogenes (group A) strain C 203 S in Todd Hewitt broth at 37°C was in the lag phase for about four hours after the inoculation. It was then in the acceleration phase three hours later. The exponential phase began approximately from the seventh to the eleventh hour of the cultivation. In this phase the microorganisms have already adapted themselves to their new environment and the cells can grow at maximum rate (87). Thus, the eighth hour culture was chosen to be the starter for the preparation of SLO throughout this study.

1. The Effect of the Incubation Period for SLO Production

Experiments on the effect of the incubation period for SLO production by the strain C 203 S of Streptococcus pyogenes (group A) in Todd Hewitt broth indicated that the maximum level of SLO occurred in the fourth hour of the cultivation (Figure 6, 7).

There was about a 2 hr - acceleration phase after which the strain grew exponentially for 2 hr. Growth ceased within 4 hr after

inoculation. During this period, the pH decreased from 7.5 to 5.6 while SLO increased rapidly until the maximum level was reached, after which it rapidly decreased. On the other hand, it could be said that SLO was released at a constant rate throughout the exponential growth phase but not during the stationary phase. This finding was in accord with the results of other investigators, such as Boszormenyi,et al. (44) and Dassy & Alouf (41).

During the first two hours of the cultivation, the culture was still in the acceleration phase so the level of SLO was not high enough to be detected. The culture then moved to the exponential phase which led to both the rapid decrement of pH and increment of SLO level. Cohen reported that destruction of SLO not only could occur from the acidity of the medium itself (pH below 6.7), but also by proteolysis by the streptococcal proteinase, the production of which was greatly stimulated by glucose and acidity of the medium (45). This can be the reason for the rapid decrement of SLO after its maximum level. Another reason which can be explained is the limiting effect of a glucose concentration of about 0.2 % in Todd Hewitt broth (84). Boszormenyi,et al., summarized from their study about the role of glucose concentration and pH in the formation of SLO that with respect to SLO formation, a glucose concentration of about 1.0 % and neutral pH, maintained during the entire cultivation period, were found to be optimal. They also added that while multiplication was essentially the same at various glucose concentrations, SLO formation was four times greater at 1.0 % than at 0.25 % glucose concentration and twice as great as 3.0 % (44). These two reasons can be supported by the experience of Pappenheimer

and Hottle that the growth and hemolytic titer may be increased by addition of more glucose to the medium and periodic neutralization of the acid formed (46).

Figure 8 is the summary of the two experiments for studying the effect of the incubation period. The interval between the two lines is a sample of variation from different lots of SLO production. Long and Bliss (88) discussed in one of their articles that it had been the experience of most individuals who studied antigenic streptolysin that variations in streptolysin production in various lots of medium constitute a common source of difficulty. Cohen proposed that it was not always clear whether the low levels of SLO (or its absence sometimes) in certain media were due to the inhibition of its synthesis, or release or to : (A) toxin denaturation as this protein is relatively labile and hydrophobic (7) ; (B) inactivation by cholesterol present in complex media ingredients ; (C) degradation by streptococcal proteinase which is released into the medium when pH drops below 6.7 (45). Other explanations about variation in SLO production will be discussed later.

In the case of other strains of this organism, Slade and Knox (42) found that the maximum hemolytic activity of Richards streptococcus (group A, type 3) was obtained in 10.5 hr ; Boszormenyi et al. reported that the maximum cell counts and SLO titers of H 64 A Streptococcus pyogenes strain of the C group were obtained in the sixth to eighth hour ; they also commented that the time of cultivation depended on the length of the lag phase (44) ;

Dassy and Alouf found that the maximum SLO production of group A type 3 Streptococcus pyogenes S 84 strain occurred within 6 hr after inoculation (41).

From the results of these several investigators, it can be assumed that the optimal incubation period for SLO production depends upon the strain of hemolytic streptococci, the cultural medium and the method used to prepare the starter.

2. The Effect of the Age of Starter for SLO Production

It is expected that the culture in the exponential phase is the most active phase in the growth cycle. Therefore, the eighth hour culture, from the growth curve of the strain C 203 S of Streptococcus pyogenes (group A) in Todd Hewitt broth at 37°C, shown in Figure 5, was chosen and is expected to be appropriate as the starter for SLO production throughout this study.

The objective of this section is to indicate the exact age of the starter yielding high SLO production.

It was found from Figure 9 that the starter was in the acceleration phase for about 8.5 hours. Thus quite low growth in the step of SLO preparation, which led to low level of SLO, occurred when the fifth or eighth hour culture was used to be the starters.

Maximum growth in the step of SLO preparation, yielding maximum SLO level, was obtained when the starter was the tenth or twelfth hour cultures which was in the exponential and post-exponential (retardation) phase of growth but not the stationary phase.

When the starter was in the stationary phase, or might even be in the decline phase if total growth was observed by viable cell count, the exhaustion of nutrients and the accumulation of toxic products caused growth to cease completely (86). Therefore, when the culture in this phase of growth was used as the starter, a very low level of growth in the step of SLO preparation occurred and there was no SLO detected in the cultural filtrate, as shown in Figure 9.

Thus, the optimum age of starter for SLO production by Streptococcus pyogenes (group A) C 203 S in Tood Hewitt broth is in the range of 10 - 12 hr.

Several investigators recommended a distinct age of starters, such as 8 hr by Robinson,et al. (89), and 6 hr by Boszormenyi,et al. (44) and Fuvessy,et al. (43) ; but there was no experiment to confirm that these ages of starters were in fact optimum for SLO production.

3. The Effect of the Size of Starter for SLO Production

When the size of starter was varied in the range of 5 - 15 % of fresh medium, it revealed that the quantity of growth obtained in the step of SLO preparation was related to the size of the starter. Namely, the maximum growth occurred when the starter's size was 15 % of fresh medium. As is generally known, the quantity of lysin produced at any period during incubation is closely related to the size of bacterial population (42). As illustrated in Figure 10, the highest level of SLO was obtained at 15 % starter. Therefore, the appropriate size of starter for SLO production by Streptococcus pyogenes (group A) C 203 S in Todd Hewitt broth was 15 % of fresh

medium.

Slade and Knox (42) indicated that a heavier starter required a shorter incubation period to reach maximum growth. However Bernheimer, et al. (39) suggested that if too heavy a starter was used the cultures might become so rapidly acidic that they would no longer continue growing even though neutralized.

Robinson, et al. (88) employed only 1 % starter while Fuvessy, et al. (43) as well as Boszormonyi, et al. (44) employed 10 % starter for SLO production, but no comparative level of SLO from different sizes of starter was made.

4. The Effect of Temperature for SLO Production

Only two points of temperature were used in this study. 37°C to the normal temperature of the incubator in general clinical laboratories and 25°C is assumed to be room temperature. If the result of this study had shown that there was no significant difference between the level of SLO obtained from these two incubation temperatures, it would be both facile and economial to use 25°C as an incubation temperature.

However, it was found that there was no SLO detected when the culture was incubated at 25°C, as shown in Table 7. Therefore, 37°C is still the appropriate incubation temperature for SLO production by the strain C 203 S of Streptococcus pyogenes (group A) in Todd Hewitt broth.

5. The Effect of the Initial pH for SLO Production

Experiments on the effect of the initial pH for SLO production by C 203 S Streptococcus pyogenes strain of the A group in Todd Hewitt broth revealed that the maximum level of SLO was obtained when the initial pH was 7.8. Before and after this point, growth in the step of SLO preparation was low and resulted in a low level of SLO, as illustrated in Figure 11.

There was no SLO detected at the initial pH of 6.6. This may be due to unfavorable initial pH for growth of the C 203 S strain, which led to either a low level or no lysin production. If there were a minute amount of SLO produced, it could be destroyed by both the acidity of the medium (pH below 6.7) and proteolysis by the streptococcal proteinase, as previously mentioned in section 1 of the this chapter.

Growth was lower at the initial pH of 7.2 than at the initial pH of 8.2 while a higher level of SLO was gained.

Thus, the optimal initial pH for SLO production by this strain in Todd Hewitt broth was 7.8 which is the approximate pH of this medium itself. Therefore, there is no need to adjust the initial pH of this medium when a high level of SLO production is required.

6. The Effect of Carbondioxide and Agitation for SLO Production

As generally known, most streptococci are facultative anaerobes (2 - 4) and Jawetz,et al. (4) also commented that growth

and hemolysis of human pathogenic hemolytic streptococci were aided by carbondioxide. Therefore, this section of experiments was set up to prove whether carbondioxide and agitation had any effect to accelerate SLO production.

It was found that growth was much higher when the culture was incubated in atmospheric carbondioxide than in 5 % carbondioxide, where there was no SLO detected as shown in Table 8. In atmospheric carbondioxide total growth and SLO level of the standstill culture were a little higher than of the stirred culture. The observations on the effect of agitation were in harmony with those of Dassy and Alouf (41) who found that a lower yield of SLO obtained might have been due to the slight agitation of medium required for continuous pH correction. Therefore, the optimum SLO production by the strain C 203 S Streptococcus pyogenes (group A) in Todd Hewitt broth requires neither special carbondioxide tension nor agitation during cultivation.