Some Observations on the Method of Preparing Old Tuberculin and on the Titration of Its Potency

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Introduction

In 1890 tuberculin was first produced by R. Koch as a cure for tuberculosis without recognition of the nature of its activity. Many other investigators also produced dozens of preparations and their various usages led to confusion. To that extent various determinable conditions and factors are present in preparing high specific tuberculin. Moreover, the differences in potencies of different preparations of tuberculin are partly caused by the type of method used for testing potency. In this paper the manual technique of preparing tuberculin and the factors involved in titrating potencies are described.

1. Method of Preparing Old Tuberculin

On the subject of producing tuberculin from tubercle bacilli, there are various opinions relating to bacillary strains, bacillary types and virulence of bacilli. Considering the specificity of tuberculin, it should be manufactured from cultures of the homologous type of tubercle bacilli, e.g. human strain tuberculin for human subjects. In this experiment three human Aoyama B-, Asakura-, Frankfurt-strains and a BCG strain were cultivated on Sauton medium. No appreciable difference was noticed between the potencies of tuberculins manufactured from 9-week-old cultures of human Aoyama B and Asakura strains, and from 3-month-old cultures of human Frankfurt and BCG strains. As the potency of tuberculin prepared from the older culture increases gradually, there may be still some difference

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between those strains. Because different strains show optimum production of tuberculin on media of different constituents and at different pH etc, such a problem is full of complexity.

In the second experiment tuberculins were prepared after modification of pH to each of 7.0, 8.5 and 1.0 from 4.2 and after sterilization by heat for thirty minutes at 100°C. Skin reactions of tuberculous animals to 1: 50, 1: 100 and 1: 500 dilutions of the former three tuberculins were measured with tuberculin of pH 4.2 as control. The potencies of the former three tuberculins of Aoyama B and Asakura showed no significantly different intensity from that of the control tuberculin. As the nucleoprotein was removed from the culture filtrate of pHs below 4.2, high potent tuberculin may be prepared by adjusting pH of culture filtrates over 4.2.

We used three methods of condensation: evaporation at 70°–80°C, evaporation under reduced pressure (at 45°–55°C) and ultrafiltration through collodion membrane. The first method was the most suitable of the three, without any loss of activity. Ratio of potency: 1.00: 0.45: 0.56. Condensation by the latter two methods was accompanied by removal of active substances, such as volatile substances, carbohydrates or amino acids. Condensation under mild heating retained high activity, especially in the 48-hour reaction. This is believed to be due to greater viscosity and to change in the colloidal quality of the tuberculin (table 1). Condensation at 100°C is not suitable, since much more heat-coagulable nucleoprotein is removed than when condensation is performed at 70°–80°C.

Table 1. Skin Reactions of Tuberculins Condensed hy Various Methods

Tuberculin		Tuberculin Condensed at 70°–80°C		Taberculin Condensed under Reduced Pressure at 45°–55°C		Tuberculin Condensed by Ultrafiltration	
		1:50	1:500	1:50	1:500	1:50	1:500
		mm	mm	mm	mm	mm	mm
Aoyama B Strain T.	24°	17×17	11×10	13×13	7×7		
		14×13	6× 6	12×12	4×4		
	48°	15×15	7× 7	8× 8	4×4		
		11×11	3× 3	8× 7	3×3		
	24°	18×17	11×11			17×17	12×12
		20×20	13×13			18×17	12×12
	48°	14×13	10× 9			11×10	8× 7
		17×17	11×11			15×15	8× 8
Asakura-Strain T.	24°	16×15	9× 9	15×14	6× 6	12×12	7× 7
		18×18	10× 6	16×14	8× 8	12×12	7× 7
	48°	13×13	4× 4	9× 9	4× 4	8× 8	4× 4
		16×16	8× 8	12×12	4× 4	10×10	4× 4
Ratio of Potency		1.00		0.45		0.56	

2. Titration of Potency of Tuberculin

Most of the commercial tuberculins manufactured in many laboratories may coincide in potency with the International Standard Tuberculin of the National Serum Institute at Copenhagen, when tested by the method recommended by the Health Committee of the late League of Nations. However it has been reported that they show much discrepancy in potency, when applied to human subjects. Among various factors in the titration of potency, the following two items have been criticized:

a) Time of Testing Potencies During Tuberculous Infection of Guinea Pigs

The time of testing potencies, during the course of tuberculous infection of guinea pigs (2, 3 and 5 weeks after start of infection) seems to have no significant difference, in the reactions of 1: 10 and 1: 100 dilutions, between 104°C tuberculin and the commercial tuberculins tested. However, between 70°–80°C tuberculin and three commercial tuberculins, significant differences were noted when tested in the earlier period of infection, but were not noted when tested in the later period of infection. Consequently it is advisable that the potencies of tuberculins should be compared during both periods, especially during the earlier period.

b) Dilution of Tuberculin Tested

In human skin tests positive skin reactions are the erythemata of 10–30 mm in diameter, so that comparisons of potencies of tuberculins should be made in the range of dilutions showing such grades of reactions in tuberculous guinea pigs. From this view point, the concentrations of tuberculins tested are not always suitable in the testing method of the late League of Nations. The use of stronger dilutions of 1: 10 to 1: 1000 in testing the skin reactions of tuberculous guinea pigs is recommended (table 2).

Table 2. Skin Reaction of Dilutions in the Testing Method of the Late League of Nations

Tuberculin		Dilutions 1:500	1:1000	1:2000	1:4000
		mm	mm	mm	mm
Frankfurt-Strain T.	24°	14×14	13×13	13×13	6×6
	48°	17×12	13×10	—	—
Aoyama B-Strain T.	24°	10× 9	6× 6	4× 4	3×3
	48°	6× 6	4× 4	—	—
Asakura-Strain T.	24°	11×10	7× 6	4× 4	3×3
	48°	10×10	6× 6	4× 4	2×2

Summary

1. On the abilities of producing tuberculin from tubercle bacilli, four strains showed no remarkable difference in regard to the period of cultivation. But there may be some difference in the time chosen for the optimum production of tuberculin.

2. Tuberculins prepared after modifying the pH of culture filtrates over 4.2 showed no variation in potencies.

3. Among various condensation methods, such as evaporation at 70°–80°C and at 100°C, evaporation under reduced pressure at 45°–55°C and ultrafiltration through a collodion membrane, condensation by mild evaporation at 70°–80°C led to retention of the highest activity.

4. For measuring potencies of tuberculin, skin reactions should be tested on tuberculous guinea pigs during the earlier period of infection as well as during the later period.

5. The range of dilutions of tuberculin in testing potencies is more suitable in lower dilutions (1: 10 to 1: 1000) than in higher dilutions (1: 2000 to 1: 4000) of the late League of Nations.

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