THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Pu Yung Chang for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

H. Larson
Chairman

E. C. Rosenow

J. F. McClendon

Date May 15, 22

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Pu Yung Chang final oral examination for the degree of

Master of Arts

We recommend that the degree of

Master of Arts

be conferred upon the candidate.

W. O. Larson
Chairman

J. F. McClendon

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Date May 15 - 22

10-20 SM

STUDIES ON THE EFFECT OF THE SURFACE TENSION OF THE CULTURE MEDIUM ON THE BACILLUS TUBERCULOSIS.

A Thesis submitted to
The Faculty of The Graduate School of
The University of Minnesota
By
Pu Yung Chang
In partial fulfillment of the requirements for the
Degree of Master of Arts

June
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The study of methods of cultivation on artificial media of the tubercle bacillus has been a subject of great interest for many years. It was not until the discovery of glycerine media that an accurate study was available. In spite of the fact, there is still a great deal yet to be done in this field, especially on the cause of their characteristic growth on ordinary glycerine media with regard to the physical properties of the media. The work reported in this paper being a continuation of the work conducted by Doctor Larson on(1) his study of the surface tension of the culture media on the growth of bacteria. In his work he found that the surface tension of ordinary liquid media could be reduced by addition of certain soap solutions. With the lowering of the surface tension he was able to change the usual characters of the growth of pellicle bearing organisms, thus bacillus subtilis and bacillus tuberculosis grow down in the body of the media.

The cause of the pellicle formation of such organisms as tubercle bacillus was considered as their vital demand for oxygen. This theory can safely be ruled out inasmuch as tubercle bacilli could grow and grew even better in media or at the bottom of the media when the surface tension is lowered. A more striking fact to disprove our oxygen demand theory is that a lowering of surface tension of the media does not create an increase of oxygen absorption of the media. Green has shown that the ability of a solvent to absorb gases is not directly related to surface tension, the vapour pressure or specific gravity of the solvent. A study of the following table will convince us that the surface tension of aqueous solutions and the amount of oxygen absorbed by 5 cc. of such solutions caused

no variation. The tensions range from 32.0 to 55.0 dynes and the amount of oxygen absorbed is constant. This constant as will be seen to be the same as that for distil water alone.

Solution	Surface Tension	CO_2 absorbed
Water	72.5	.34
Broth	55	.34
Peptone	54	.34
1% Dextrine	52	.34
Broth and Soap	40	.34
Water and Soap	32	.34

Distilled water with a tension of 72.5 dynes absorbs just as much oxygen as a solution of distil water and soap, which has a surface tension of only 32 dynes. Therefore the absorption ability of a solvent is not a function of its surface tension. Skirron found that there was no direct relation between the vapour pressure of the mixture and the ability of the mixture to absorb gases. There is likewise no relation between the specific gravity of a substance and its ability to absorb gases as shown in the following table.

	Sp. Grav.	
Water	1	.02192
Chloroform	1.4878	.207
Alcohol	.7906	.204.4

Thus we see there is no direct relation between the physical properties of a solvent and its capacity for taking up oxygen. This has afforded us an evident proof that the formation of pellicle is not determined by the need of oxygen, hence organisms grow better in media of low surface tension where the supply of oxygen is constant. In this connection we should not overlook the fact, that at the

upper portion of the media there is a selective zone in which the bacteria grow best and has the most tendency to grow. As we see the pellicle forming organisms grow in this zone and not below until they fall by the weight of their growth. On the other hand, those organisms that grow in the media first show signs of development in this zone, i.e., the zone near or at the surface. From this we deduct that there must be certain effects of this zone which favor or are even necessary for the metabolism of bacteria. The reduction of the surface energy is explained here as the probable reason. This is naturally accomplished either by the reduction of surface area of liquid or by reducing surface tension, or both.

The surface tension of the liquid is reduced to the lowest point possible through the concentration of the surface tension depressants at the surface of the liquid. Pellicle bearing organisms probably select this zone because the surface tension reducing substances concentrated there are required for their metabolism. The The pellicle formation is probably a mere coincidence, the surface tension of the fluid being sufficient to support the weight of the organisms just as bodies may be supported on the surface of water due to its high surface tension. The possibility that the lipoids of the cell in ordinary broth, prevent the wetting of the cell and the tension holds the cell up. When the soap is added to the broth the lipoids, being miscible in soap, no longer prevent the wetting of the cell and the water gets on to the cell, and the cell sinks, thus producing a diffuse growth in the medium or at the bottom as sediment without pellicle production. This fact holds true with tubercle bacilli. The tubercle bacillus has a cell membrane which confers upon it its resistance against wetting, hence also against

the entrance of the stains. This membrane contains most of the waxy substances which can be extracted from cultures. Further, this wetting phenomena of cell membranes can also be demonstrated on many spore forming organisms. As it is well understood that in order to bring about the process of sporulation there must be present some unfavorable conditions in which the given organism is grown. Among those the principal one is lack of nutrition. We assume that when the cell membranes are properly wetted they can take up nutrition more readily. Thus we have observed that spore forming organisms lose the property of forming spores when growing in soap broth. Here the loss of spores in low tension may be explained on the ground of proper wetting since the organisms grow. On the other hand, in high surface tension media there is little or insufficient wetting, and because of poor nutritive conditions, spores develop.

When bacteria are grown on liquid media such as broth with a surface tension of 59 dynes the surface tension rises with the growth curve of the organisms until it reaches 68 or 69 dynes depending upon the organism concerned. This observation is important in connection with spore formation. If spore-formation is a function of wetting, it may then readily be understood why spore-formation begins only after the surface tension of the media has risen. The accepted views concerning spore-formation are that this phenomenon occurs as a result of exhaustion of the nutritive elements of the media to the extent where it will no longer support growth. If this were true we would not expect other organisms to grow on the filtrate of a spore culture. Experiment, however, shows that such filtrates usually prove to be excellent culture media for other bacteria, and therefore it cannot be a question of media exhaustion. In fact, the same organism will

grow on such filtrate if its surface tension is again reduced by adding the requisite quantity of soap. This latter experiment disposes of the arguments of those who argue that spore formation is the result of "toxins" being formed in the media which inhibit growth and leads to spore formation. Glycerine, besides acting as a food to tubercle bacilli also probably serves as a wetting agent to the cell membrane so as to enhance the organism to consume more readily nutritive material from the media. Thus we see that the question of the degree of wetting of the cell membrane is one of prime importance.

A detailed investigation has been made on the action of various forms of soaps in culture media for growing tubercle bacilli. We have found that ordinary laboratory broth containing beef extract and peptone has a usual surface tension of fifty-nine dynes per c.m. contrasting with that of ordinary distilled water which has a surface tension of seventy-two dynes. Thus we see that the effect of peptone and the beef extract besides acting as a nutrient food to the bacteria also lowers the surface tension of the broth. Larson found that sodium oleate would prevent the growth of certain organisms and at the same time favoring the growth of the bacillus influenza. He explained the function of sodium oleate as an important surface tension phenomena. This could be proved immediately by the luxuriant growth of well formed colonies. Since the bacillus influenza survives and grows better and more rapidly in the absence of haemoglobin it is self evident that surface tension is the factor concerned since sodium oleate tends to lower the surface tension of the medium.

Thjotta and Avery were able to grow the bacillus influenza

on a blood free media containing the mucoid material from cultures of Friedlander's bacillus and other closely allied organisms. The growth accessory substance or substances which can replace blood and blood derivatives in cultivation of Pfeiffer's bacillus, Thjotta found in both the saline suspension and watery extracts of the heat killed material. They were also able to grow in media containing extracts of yeast cells, green peas and other vegetables. They were not in a position to explain the exact nature of the growth accessory substances, other than presuming that they are substances analogous to vitamines. The above materials probably are merely surface tension depressants. Mucoid substances and extracts of vegetable matters are, no doubt, surface tension depressants. The opposite view to our surface depressant theory seems to hold true with a number of organisms. Many non-pellicle forming organisms attempt to form pellicle or have more abundant growth on the surface in film when the tension of the media is elevated as by charcoal. These characteristics are more or less constant, that is, when they are grown in high surface tension media for then they some times attempt to retain this character.

Still another interesting point brought to our attention is that the potassium salt, especially the saturated soap, viz., the stearate has a specific action on the cell membrane of the tubercle bacillus so that despite the high tension of the medium the organisms still grow throughout the medium. This specific action of the potassium soap could probably be explained by our theory of the miscibility of the fatty material contained in the cell membrane with soap. As a matter of fact, the cell membrane contains a much higher percentage of potassium in their composition although they

grow in a medium which contains more sodium and magnesium. This can easily be brought to view by extracting any cell when we would find that potassium is present in much larger quantities than is sodium.

In considering the various salts utilized by bacteria it is interesting to know that, while sodium seems to be necessary for the growth of nearly all bacteria, in fact as a rule it cannot be replaced by the potassium salts, yet upon analysis of the salt contained in bacteria it has been found that sodium salts are not stored up as are the potassium salts. Sodium salts are therefore probably utilized by the cell as a means of maintaining the osmotic pressure and the proper turgor of the cell. It is a common view that cells in general have a selective action for the various salts in that they allow one salt to pass while preventing passage of other salts. The work of Dornan, however, seems to explain this selective action of salts on a purely physical basis. In growing bacteria in potassium soaps as referred to above, attention is called to the fact that, in doing this it becomes necessary to make a medium free from sodium salts since if sodium were present in any form in the medium it would replace the potassium and we would have sodium soap formed immediately. Whether potassium soap in the absence of any sodium ion is capable of effecting a more perfect wetting of the bacterial cell than is the sodium soap must be set aside for future investigation. If we accept the theory that wetting is a direct function of the surface tension of the media our observations on the effect of potassium soap on the tubercle bacillus will seem to contradict Larson's view that the pellicle forming bacteria grow throughout the medium as the result of

depressing the surface tension.

The object of this work was to develop a method which will enable one to make use of the agglutination test in the diagnosis of tuberculosis. Efforts have been made by many investigators in the past to apply the agglutination test to the diagnosis of tuberculosis. All of these efforts have been unsuccessful owing to the fact that the tubercle bacillus when grown in the laboratory grows in adherent masses from which it seems almost impossible to prepare the homogeneous suspension necessary to carry out the test. The earlier investigators attempted to grow the organism on glycerine broth and by shaking the culture daily hoped to cause the organism to grow evenly throughout the medium, but in no instance were these efforts crowned with success. Besredka reports the preparation of media by treating egg with sodium hydroxide. The peculiarity of this medium lay in the fact that the tubercle bacillus would grow at the bottom of the culture medium rather than on the surface, as is ordinarily the case. Besredka apparently did not realize that by treating the egg, which contains a great deal of fat, with sodium hydroxide he made a sodium soap and that sodium soap thus prepared serves to lower the surface tension of the culture media which caused the tubercle bacillus to grow in the novel manner which he describes.

The French investigators report that antigen prepared from such media serves well in performing the complement fixation test for tuberculosis. Other investigators, however, have failed to substantiate the work of Besredka and his associates and it is therefore probable that these investigators were over enthusiastic

concerning the value of the work.

In reviewing the literature of complement fixation we cannot but be impressed by the fact that the complement fixation method does not lend itself to the diagnosis of tuberculosis. From the literature it is evident that most of the investigators' difficulty lay in the peculiarity of the tubercular antigen since each investigator reports some modification of antigens previously used. In carrying out complement fixation tests on a large number of cases in this laboratory with various antigens it was found that complement fixation is rarely complete with human blood but that excellent fixations may be obtained experimentally by immunizing or infecting laboratory animals with the tubercle bacillus. The conclusion, therefore, must be that the chief difficulty does not lie with the antigen but rather with the serum, since, if the antigen were incapable of fixing complement in the presence of antibody it would not be possible to get such excellent reactions experimentally. We therefore are of the opinion that the human individual is incapable of liberating complement fixing antibodies in contrast to laboratory animals which do so very readily. This being the case it seems hopeless to go further in our efforts to develop the complement fixation reaction for use in the diagnosis of tuberculosis. Our attention was therefore turned to the agglutination test.

The agglutination test is more simple in its technique. It

is perhaps more specific in character than the complement fixation test and therefore we believe the agglutination test should be used in preference to the fixation test wherever possible. As already pointed out in this paper, we found that by growing the tubercle bacillus in a medium of proper surface tension we were able to get a diffuse growth with which there was every reason to hope that it would serve as an antigen in carrying out agglutination experiments. In testing out a large number both of known positive and known negative tuberculosis we found that the agglutination test worked very satisfactorily, using an antigen grown on a low tension medium, the test being apparently specific giving complete precipitation with positive serum and no reaction with known negative serum. The greatest weakness of this method lies in the fact that on low surface tention medium the tubercle bacillus grows slowly, some strains requiring several weeks to develop sufficiently to be used in this work.

Our attention was turned from this to another method which was developed in this laboratory some years ago by Larson and associates. It was the method of emulsifying and disrupting bacteria by treating them with carbon dioxide under high pressure. The tubercle suspension was placed in the apparatus under high pressure. After subjecting the organism to carbon dioxide for some hours it is forced from the pressure chamber through a very fine opening into a second chamber connected with atmospheric pressure. As the bacillary suspension is forced through this capillary opening into the chamber of atmospheric pressure the bacteria are emulsified and partially disrupted resulting in a homogeneous suspension which lends itself admirably as an antigen for the agglutination reaction. The

importance of any test which will aide us in the early recognition of tuberculosis cannot be over-emphasized. While tuberculosis is easily recognized in its more advanced stages, in its incipient form its recognition challenges the ingenuity of the most able medical men. When the disease is so far advanced that its presence is obvious, even with the most careful work it is often so far advanced that only prolonged treatment will restore the patient to health. Such men as Pottenger and other equally well informed in the field of tuberculosis state that if tuberculosis is recognized early cures can be effected in practically all cases. Since agglutinating antibodies are apparently found in the blood stream wherever we have an active infection it would seem therefore that the agglutination test would supply a method of aiding in the early recognition of the disease. Our work, which covers the examination of more than five hundred cases, seems to justify the above statement. The technique in carrying out the agglutination test is as follows.

Tubercle bacilli may be grown on a low surface tension medium; when the growth of the organism has been completed the culture is centrifuged in order to remove the bacteria from the solution containing soap. The sediment is suspended in salt solution, killed by heat, placed in a series of small tubes such as are used in carrying out the Wassermann test and graduated amounts of the serum in question added to each tube. We have worked with dilutions of serums varying from 1 to 20 to 1 to 3000. The material is then placed in the incubator at 37° for two hours, after which it is transferred to the ice-box and left overnight. After the two hours incubation the serum will usually show beginning agglutination which is complete upon standing a few hours in the ice-box. The negative serum, on the other

hand, produces no change in the bacillary suspension. The reactions are so sharp as to leave little or no room for error in reading the results. In using the antigen prepared by the treatment with carbon dioxide as described above, the technique is similar to the one just described. The reactions are equally good, and therefore after trying out both methods we favored the latter method, since the antigen is so much more easily prepared and the test can be carried out on a large scale without difficulty. Results of the agglutination test on five hundred cases is given in the table which follows.

While working with the tests bearing on the diagnosis of tuberculosis we were also interested in the effect of low surface tension medium on the pathogenicity of the tubercle bacillus. It is a matter of common observation that tubercle bacilli, even when killed, cause necrosis of tissues when injected subcutaneously into the tissue structures. In an effort to immunize guinea pigs with the antigen treated by the carbon dioxide method we were struck by the fact that no necrosis was obtained following the injection of such material. Even though antigen was prepared from virulent strains only a temporary enlargement of the regional lymph glands was noted. This enlargement subsided in the course of some days after which the animals presented no changes as a result of experimental inoculation. Protective experiments are now in progress in an effort to determine to what degree this antigen immunizes guinea pigs. Parallel with this work other series of animals were injected with virulent cultures grown on medium of low surface tension. A series of six animals were injected with what were supposed to be virulent cultures but in no instance did these injections result in the development of tuberculosis. While this work is not sufficiently far advanced to

express a final opinion, nevertheless it appears that growing the tubercle bacillus on a low surface tension medium robs it of its pathogenicity. The medium on which the latter antigen was grown was as follows.

Saturated	Unsaturated
Potassium Palmatate	Potassium Oleate
Potassium Stearate	Potassium Rescinoleate
Sodium Palmatate	Sodium Oleate
Sodium Stearate	Sodium Rescinoleate
	Castor Oil Soap.

After trying out various soaps of fatty acid we have been able to confirm Larson's observation that sodium rescinoleate is the most desirable, although not in every respect ideal, as a surface tension depressant. The latter soap is a perfectly clear aqueous solution but does not hydrolyze as readily as other soaps and for that reason has been used extensively in our experiments. Another point which I believe has not been observed heretofore is the fact that soaps of the saturated fatty acids do not depress surface tension of water more than is done by any colloid, namely, to a point of about fifty-nine dynes. Whereas all the soaps of the unsaturated fatty acids are powerful surface tension depressants. As a matter of fact, soaps of the saturated fatty acids such as stearate and palmate not only fail to reduce the surface tension of the ordinary broth but, on the contrary, raise its surface tension. This is due to the fact that these saturated soaps precipitate out readily in the absence of unsaturated soaps and by their surface action adsorb surface tension depressants normally found in broth, thus raising the surface tension of this medium.

4-22-2M

TABLE I.

Potassium Rescinoleate in Pure Potassium Broth

Amount of 5 % Soap added.	Amount of Broth	Surface Tension in dynes	2nd.Day	3rd.Day	4th.Day	5th.Day	8th.Day	10th.Day	12th.Day	14th.Day	16th.Day
0.2 cc.	50	57.3	Pellicul-ar growth	Pelli-cular growth	More surf. growth	Pelli-cular growth	Drop of pellicle				
0.5 cc.	50	54		Growth in med.	More growth	Pelli-cular growth			Drop of pellicle		
1 cc.	50	50		"	Same		More growth		More growth		More growth
1.5 cc.	50	46				Growth in med.	Growth in med.		"		"
2 cc.	50	45				"	"		"		"
2.5 cc.	50	42.5						Growth in med.	"		"
3 cc.	50	42						"	"		"
3.5 cc.	50	40								Growth in med.	"

14.

TABLE II

Sodium Rescinoleate Soap in Pure Sodium Chloride Broth

Amount of 5% Soap added	Amount of Broth	Surface Tension in dynes	2nd.Day	3rd.Day	4th.Day	5th.Day	8th.Day	10th.Day	12th.Day	14th.Day	16th.Day
0.2	50	52.2	Slight growth in media		Growth	Growth	Growth	"	"	Growth in lumps	More growth
0.5	50	50	"	"	"	"	in	"	"	in	"
1 cc.	50	44			Growth in media	"	media	"	"	media	"
1.5	50	40				"	"	"	"		"
2	50	38.5				"	"	"	"		"
2.5	50	38				"	"	"	"		"
3	50	37.9				"	"	"	"		"
3.5	50	36.5					"	"	"		"

TABLE III

Potassium Oleate Soap in Pure Potassium Chloride Broth

Amount of 2% Soap Added	Amount of Broth	Surface Tension in dynes	3rd.Day	5th.Day	7th.Day	10th.Day	12th.Day	14th.Day	16th.Day
0.2	50	49	Slight growth	Growth more in media	Growth in media	Growth in media	No pellicle	No pellicle	More growth in media
0.5	50	42		"	"	"			"
1 cc.	50	37		"	"	"			"
1.5cc.	50	35.5		"	"	"			"
2 cc.	50	35		"	"	"			"
2.5cc.	50	34.5		"	"	"			"
3 cc.	50	34				"			"
3.5cc.	50	33				"			"

TABLE IV.

Sodium Oleate Soap in Pure Sodium Chloride Broth

Amount of 2% Soap added	Amount of Broth	S.T. in dynes	3rd. Day	5th. Day	6th. Day	8th. Day	9th. Day	10th. Day	12th. Day	14th. Day	16th. Day	17th. Day	20th. Day	24th. Day	26th. Day	28th. Day	30th. Day	32nd. Day	34th Day
0.5cc.	50	60	Pellicle slight	Pellicle more	Pellicle	Pellicle	Pellicle												
0.75cc.	50	55					"												
1 cc.	50	48					Grow. in media	Grow.	Grow.	Grow.	More grow.	More grow. in media	No pellicle	No pellicle	No pellicle	No pellicle	No pellicle	No pellicle	No pellicle
1.25cc.	50	64.5					"	"	"	"	"	"	"						
1.50cc.	50	45.5					"	"	"	"	"	"	"						
1.75cc.	50	45							"	"	"	"	"						
2 cc.	50	44							"	"	"	"	"						
2.25cc.	50	42.5							"	"	"	"	"						
2.50cc.	50	39								"	"	"	"						
3 cc.	50	38.5										"	"						

TABLE V.

Data Collected of Sodium Oleate Soap (2%) in Ordinary Broth of Seventy-two Dynes

Amount Soap Added	Amount Broth	S.T. in dynes	2nd. Day	3rd. Day	4th. Day	6th. Day	8th. Day	9th. Day	12th. Day	16th. Day	18th. Day	20th. Day	24th. Day
0.1 cc.	50	60	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth			Pellicle growth	More	
0.5 cc.	50	56	"	"	"	"	"	"			"	growth	
0.75cc.	50	44.9				Grow in media	Grow in media	Grow in media	Abund. growth	Same	Growth in media		More growth
1 cc.	50	44						"	"	Same	"		
1.5 cc.	50	43						White edge	"	Same	"		
2 cc.	50	43.5							"	Same	Slight edge growth		
2.5 cc.	50	43.3							"		Growth		
2.75cc.	50	43							"		"		
3 cc.	50	42.5									"		

TABLE VI.

Potassium Palmatate (2%) in Pure Potassium Chloride Broth

Amount of Soap added	Amount of Broth	S.T. in dynes	3rd.Day	5th.Day	6th.Day	7th.Day	9th.Day	12th.Day	15th.Day	17th.Day	19th.Day	22nd.Day	30th.Day
0.2	cc. 50	70.5	Turpid growth	Pellicle growth	Pellicle growth	Pelli- growth	Pelli- growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pelli- growth	Pellicle growth
0.5	cc. 50	64	"	"	"	"	"	"	"	"	"	"	"
1	cc. 50	62	"	"	"	"	"	"	"	"	"	"	"
1.5	cc. 50	60	"	"	"	"	"	"	"	"	"	"	"
2	cc. 50	58			growth in media	growth in m.	white edge	thin pellicle	pellicle	"	"	"	"
2.25	cc. 50	57.5			"	"	growth in med.	growth in med.	growth in med.	"	"	"	"
2.50	cc. 50	57							"	growth in med.	growth in med.	growth in med.	growth in med.
3	cc. 50	55.5							"	"	"	"	"
3.5	cc. 50	51.8							"	"	"	"	"

TABLE VII.

Sodium Palmatate (2%) in Pure Sodium Chloride Broth

Amount of Soap	Amount of Broth	S.T. in dynes	2nd. Day	4th. Day	6th. Day	7th. Day	14th. Day	15th. Day	16th. Day	18th. Day	20th. Day	22nd. Day
0.1	cc. 50	cc. 64.5	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pellicle growth
0.5	cc. 50	cc. 64.5	"	"	"	"	"	"	"	"	"	"
1	cc. 50	cc. 64.5		"	"	"	"	"	"	"	"	"
1.5	cc. 50	cc. 64.5		"	"	"	"	"	"	"	"	"
2	cc. 50	cc. 64.5		"	"	"	"	"	"	"	"	"
2.25	cc. 50	cc. 64.5		"	"	"	"	"	"	"	"	"
2.50	cc. 50	cc. 64.5		"	"	"	"	"	"	"	"	"
2.75	cc. 50	cc. 64.5				"	"	"	"	"	"	"
3	cc. 50	cc. 61.5				white edge	white edge turpid media	line thicker	thicker still	"	"	"

TABLE VIII.

Potassium Stearate (2%) in Potassium Chloride Broth

Amount of Soap	Amount of Broth	S.T. in dynes	2nd.Day	3rd.Day	4th.Day	8th.Day	10th.Day	12th.Day	14th.Day	16th.Day	20th.Day	30th.Day	40th.Da
0.2	cc.	50cc.	65	Pellicle growth	Pellicle growth								
0.5	cc.	50cc.	64	"									
1	cc.	50cc.	62.8	"									
1.5	cc.	50cc.	61	"									
2	cc.	50cc.	60.8	growth in med.	growth in med.	growth in med.	growth in med.	growth in med.	growth in med.	growth in med.	growth in med.	growth in med.	growth in media
2.25	cc.	50cc.	60.5	"	"	"	"	"	"	"	"	"	"
2.50	cc.	50cc.	60.4	"	"	"	"	"	"	"	"	"	"
3	cc.	50cc.	60	"	"	"	"	"	"	"	"	"	"
3.50	cc.	50cc.	59	"	"	"	"	"	"	"	"	"	"

TABLE IX.

Sodium Stearate Soap (5%)

Amount of Soap	Amount of Broth	S.T. in dynes	2nd.Day	3rd.Day	5th.Day	7th.Day	8th.Day	9th.Day	2 Weeks	17th.Day	3 Weeks	30th.Day
0.1 cc.	100cc.	61.5	Pellicle	Pelli-	Pelli-	Pelli-	Pellicle	Pellicle	Pelli-	Pellicle	Pelli-	Pellicle
0.5 cc.	100cc.	61	"	"	"	"	"	"	"	"	"	"
0.75cc.	100cc.	58	"	"	"	"	"	"	"	"	"	"
1 cc.	100cc.	58.		"	"	"	"	"	"	"	"	"
1.25cc.	100cc.	57.8				"	"	"	"	"	"	"
1.50cc.	100cc.	57.8					"	"	"	"	"	"
1.75cc.	100cc.	57					"	"	"	"	"	"
2 cc.	100cc.	57					growth in media	"	"	"	"	"
2.50cc.	100cc.	57						growth in media	"	"	"	"
3 cc.	100cc.	56.9							growth in media		"	"

TABLE X.

Data Collected of T.B. Growth in Castor Oil Soaps (2 %)

Amount of Soap	Amount of Broth	S.T. in dynes	2nd.Day	4th.Day	6th.Day	9th.Day	11th.Day	13th.Day	15th.Day	16th.Day	18th.Day	20th.	30th. Day	35th Day
0	100cc.	59	Pellicle growth	Pellicle growth	Pellicle growth	Pelli- growth	Pelli- cle growth	Pelli- cle growth	Pellicle growth	Pellicle growth	Pellicle growth	Pelli- cle growth	Pelli- cle growth	Pell cle g.
0.1	cc.	100cc. 58.7	"	"	"	"	"	"	"	"	"	"	"	"
0.6	cc.	100cc. 50.5	"	"	"	"	"	"	"	"	"	"	"	"
1	cc.	100cc. 48.1	"	growth in media	growth in media	growth in media	growth in media	growth in media	growth in media	growth in media	growth in media	growth in media	growth in media	gr. in med.
1.3	cc.	100cc. 46		"	"	"	"	"	"	"	"	"	"	"
3	cc.	100cc. 42		"	"	"	"	"	"	"	"	"	"	"
5	cc.	100cc. 40		"	"	"	"	"	"	"	"	"	"	"
10	cc.	100cc. 37.3		"	"	"	"	"	"	"	"	"	"	"
20	cc.	100cc. 33.7		"	"	"	"	"	"	"	"	"	"	"
30	cc.	100cc. 31.7		"	"	"	"	"	"	"	"	"	"	"

Discussion.

Table I shows that the tubercle bacillus grows down in the medium when the surface tension has been depressed to below fifty dynes by Potassium Rescinoleate.

Table II summarizes the effect of Sodium Rescinoleate on the tubercle bacillus. It is seen to be about the same as when Potassium Rescinoleate was used.

Table III shows that the effect of Potassium oleate was quite similar to that of the Potassium Rescinoleate.

Table IV shows the effect of sodium chloride and sodium oleate. Under these conditions pellicle formation ceases at forty-eight dynes.

In Table V is given the tabulated results obtained by growing tubercle bacilli in broth containing no sodium chloride. Here we observe the interesting fact that pellicle formation does not cease until the surface tension has been depressed to forty-four dynes or about six points lower than that in the sodium and potassium soaps of rescinoleate.

Table VI is one of the most interesting in the series in that here we have a saturated soap, Potassium Palmatate, which causes the organisms to grow throughout the medium at a tension as high as fifty-eight dynes, something which has not been observed in any of the media depressed with an unsaturated soap. Pellicle formation, however, showed itself on the fifteenth day. This is probably due to the fact that the surface tension of the medium was raised owing to the growth of the bacteria, as has been observed with other cultures. It has already been pointed out in this paper that during their growth bacteria raise the surface tension

of the culture media. This is of particular interest inasmuch as this finding was only recorded in the potassium soap media and never in the sodium soaps.

In Table VII we have Sodium Palmatate showing that there is pellicle formation throughout from the very beginning of bacterial development indicating that sodium soap in contrast to potassium soap is not able to wet the bacteria.

Tables VI and VII would then seem to indicate that potassium soaps exert a specific influence and that, as far as bacteria are concerned, we are not ready to conclude that wetting is in every respect a function of the surface tension of the media.

Table VIII shows the effect of Potassium Stearate. Potassium Stearate, like Potassium Palmatate, apparently wets the bacteria at a higher surface tension since pellicle formation ceased at between sixty to sixty-one days.

Table IX showing the effect of Sodium Stearate soap on the pellicle formation shows that we were not able to cause the pellicle cessation with this saturated soap.

Table X shows that where Sodium Rescinoleate has been used the pellicle formation ceases at about forty-eight days. This is in accord with the work of other investigators in this laboratory where they show that pellicle formation with sodium soaps usually ceased at from between forty-five to fifty days.

Summary and Conclusion.

Tubercle bacilli normally form pellicle when grown on liquid media. The tubercle bacillus, like other pellicle forming bacteria, will grow down in the medium when the surface tension is reduced

showing that the tendency of the organism to grow on the surface of the medium is not due to the demand for oxygen but rather to the physical condition of the medium.

The potassium soaps apparently have a specific wetting effect upon the membrane since the potassium soaps cause the organisms to grow down in the medium at a much higher surface tension than do the sodium soaps.

The selective zone at or near the surface of the medium is probably more desirable for the development of bacteria in that in this zone the conditions of wetting are better and hence the possibility of the organisms to get nutritive material more readily. The castor oil soaps have been found to be the best surface tension reducing agents since they more nearly fulfill the requirements than do the other soaps, although they are by no means ideal.

By analogy with other bacteria it is believed that the tubercle bacillus raises the surface tension of the medium as the growth and development of the culture progress. The pathogenicity of our strains could be attenuated, if not completely removed, by prolonged growth in a medium of low surface tension.

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