CXXIV. THE RELATION OF THE GROWTH OF CERTAIN MICRO-ORGANISMS TO THE COMPOSITION OF THE MEDIUM.

II. THE EFFECT OF CHANGES OF SURFACE TENSION ON GROWTH.

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DURING the growth of cultures of *Streptothrix corallinus* upon the basal medium [Reader, 1927] with addition of broth or antineuritic concentrates (torulin), it was noticed that the organisms were distributed sometimes at the surface and sometimes throughout the volume of the fluid. This rather haphazard phenomenon suggested the effect of a surface tension factor, and experiments were accordingly devised to determine whether surface tension had an effect upon (1) the amount of growth, and (2) distribution or type of growth.

Previous work upon the subject of surface tension has been summarised by Frobisher [1926]. He draws attention to the significance of surface tension, not only in modifying the growth and morphology of bacteria, but perhaps also in influencing their cell-division, motility, and retention of Gram's stain. He found that differences in ability to grow at low surface tensions provided a simple method for distinguishing species of bacteria. Thus, by comparing the growth of organisms at low surface tensions, he succeeded in distinguishing *B. aerogenes* from *B. typhosus* and *B. morgani*; *B. diphtheriae* from *B. xerosis* or *B. hofmanni*, certain resistant types of streptococci from the more sensitive types. The raising (by the addition of charcoal) of the surface tension of Huntoon's hormone medium for pneumococci was, he claimed, responsible for an improvement of its growth-promoting properties.

Before the publication of Frobisher's results the only direct studies of the effect of surface tension on bacterial growth had been those of Larson and his colleagues. Larson, Cantwell and Hartzell [1919] claimed that the growth of pneumococci and streptococci was, in general, inhibited by surface tensions below about 50 dynes. They had also observed that the growth of *B. anthracis* was checked by a surface tension below 46 dynes, and that the sporulation of *B. subtilis* was similarly affected. It is, however, unfortunate that they did not substantiate their report by a detailed record of experiments. Larson and Larson [1922] studied the rôle of surface tension in pellicle formation. Larson and

Montauk [1923], cultivating tubercle bacilli in a medium containing soap and glycerol, noticed that the organisms grew indeed in this medium, producing a diffuse cloudiness, but appeared to have lost all pathogenicity, so much so that their injection into guinea-pigs was followed by nothing more than local temporary nodules. Tubercle bacilli in sputum seemed likewise to lose pathogenicity when treated with sodium ricinoleate. These results lend plausibility to the suggestion that low surface tension may have been a determining factor in the experiments of Calmette and his colleagues [1921].

Thus previous work shows that there is some evidence of a correlation between the amount of growth and the surface tension of the medium, but there appears to be no reference to any relation to the distribution of the organism throughout the medium.

EXPERIMENTAL.

Surface tension was determined by a drop volume method using a glass tip of approximately 3.8 mm. external diameter. The technique for cultivation and estimation of the growth of the organism, *Streptothrix corallinus* [Reader, 1926, 1, 2], was the same as described in the previous paper [Reader, 1927]. The broth employed was made from fresh ox-heart, trypsinised according to the method of Douglas. The antineuritic concentrates used were provided by Kinnersley and Peters [1925]. Small conical flasks (60 cc.) were used throughout, and the final volume of the medium was always made up to exactly 20 cc. in an attempt to keep the surface area constant.

Two types of measurement of surface tension were employed: (a) the surface tension between the medium and air, and (b) the interfacial tension between the medium and benzene. The latter measurement was undertaken under the belief that this figure might represent more nearly the change at the medium-cell interface. In view of the experiments of Hartridge and Peters [1922] the p_H was kept constant throughout. From Table I it will be seen that the percentage change is much greater in the interfacial surface than in the air surface for any one set of dilutions of medium. In all later experiments it was decided to use the interfacial medium/benzene figure, which seemed to be a more significant value and as it gave larger differences therefore decreased the percentage experimental error.

Table I.

	Pure medium	+ 1/200 broth	+ 1/20 broth
Volume of drop into air (cc.)	0.057	0.056	0.049
Volume of drop into benzene (cc.)	0.202	0.163	0.116

The figures in Table II are measurements of interfacial tension immediately *before* inoculating the flasks with the organism and incubating for 2 days at 31°. It was found that the interfacial tension against benzene did not change even during a prolonged growth period of 4 weeks, suggesting that the factor

lowering interfacial tension is not utilised in growth. α , β and γ represent in descending order the amount of growth in 48 hours gauged by opacity, standard flasks of a definite extract in varying concentrations being used for comparison. In these, growth was arrested after 48 hours by addition of formalin.

Table II.

	Temperature = 16.8°	
	pH = 7.4	
	Time of 1 drop = 40 seconds	
Dilution of broth in salt- sugar medium	Volume of drop into benzene cc.	Growth in 48 hours at 31°
1 in 0	0.057	α Film
1 „ 1	0.058	α „
1 „ 10	0.097	α „
1 „ 20	0.116	α „
1 „ 40	0.127	α „
1 „ 60	0.134	β „
1 „ 80	0.139	β „
1 „ 100	0.144	γ „
1 „ 200	0.163	„
1 „ 300	0.175	„
1 „ 400	0.186	decreasing „
1 „ 500	0.199	„
1 „ 1000	0.202	Deposit
0 „ 1	0.202	↓ „

From Table II it may be observed that a gradual increase in the percentage of broth added to the synthetic medium increased growth up to a certain definite strength (1 in 40) when it was already equal to that obtained in a full broth culture; but whereas the addition of broth lowers the surface tension even when the dilution has reached only one part in 500, this is a figure widely divergent from that for maximum growth (1 in 40) recorded above. Further, when the interfacial tension is lowered even 1.5 % below its initial value (0.199 cc.) the character of growth changes from deposit to film.

Consequently it seemed probable that the amount of growth and the type of growth were not dependent upon the same factor. The film-growth seemed to be due to the lower surface tension of the medium rather than to the desire of the organism for more atmospheric oxygen, nitrogen, or other substance as is so often suggested.

Fig. 1 shows the type of curve obtained with descending dilutions of broth in a synthetic medium. Since the density of the solutions is not appreciably altered until a high concentration is reached, the surface tension can be considered for this experiment as directly proportional to the volume of drop except possibly for a slight error in the first point of the curve.

That the amount and the type of growth are in no way correlative is more definitely shown in Table III. The results there cited are typical examples of a series of fifty such experiments. The letters *A*, *B*, *C*, etc. represent various torulin concentrates and were used merely as sources of bacterial accessory factors. The figures in the second column are relative values only, 0.202 cc. being the value for pure inorganic-glucose medium. It will be noticed that

whenever the surface tension is low a surface film-growth is obtained; but the actual records of growth (α , β , γ , etc.) by no means correspond with the surface tension, or with the presence of films.

Fig. 1.

Table III.

Medium	Volume of drop (cc.)	Growth	Type of growth
A	0.105	α	Dense surface film
B	0.202	α	Rich deposit
C	0.151	α -	Dense surface film
D	0.202	α -	Rich deposit
E	0.127	β	Film
F	0.202	β	Deposit
G	0.184	γ	Film
H	0.199	γ	Deposit

DISCUSSION.

The above experiments show that, when ordinary media are used, the surface tension of the fluid (within the limits recorded) does not affect the amount of growth. These conclusions may seem to be at variance with the work of Frobisher, of Larson, Cantwell and Hartzell [1919], and of Wolf [1923], but it must be remembered that their experiments involved the addition of substances such as soap and alcohol, and are, therefore, beyond the scope of this paper.

As there is no correlation between change in surface tension and amount of growth, it must be concluded that the growth-promoting effect of the medium used was not due to any surface action.

SUMMARY AND CONCLUSIONS.

1. As the amount of growth of *Streptothrix corallinus* in synthetic medium containing additions of broth or antineuritic concentrates (torulin) is not related to the change of surface tension in air or interfacial tension against

benzene, the growth-promoting activity is not due to lowering of tension of the medium.

2. The type of growth (film or deposit) is correlated with change in interfacial tension of the medium against benzene.

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