

Acta Leidensia
[Inst. trop. geneesk.]

vol 12-13 : 132-140 1937-38

IX.

Experiments on the result of sterilizing
suspensions of microbes after Dawson
and Sia, in connection with the
supposed transformation of pneu-
mococci of a certian type into
another type.

BY

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In 1931 DAWSON and SIA¹⁾ published a method of transform-
ing, in vitro, pneumococci of a certain type into pneu-
mococci of a heterological type. In 1928 already GRIFFITH had
found a way to get the same result by injecting them under
certain conditions into white mice.

The method of DAWSON and SIA comes in short to the follo-
wing: A, what is called by them „vaccin”, is prepared by inocu-
lating pneumococci of a certain type into 1500 cc „plain broth”
of AVERY and CHICKERING. Herefore is used the „S” form of
this pneumococcus strain. After putting this broth culture in
the incubator at 37°C for 10 hours, it is kept at 60°C during 10
minutes to avoid, as indicated by the authors, further autolysis
in the culture. After this the whole mass of culture is centri-
fuged by high speed. The centrifugate is suspended in 15 cc
broth. This suspension is sealed in glas ampulles and incubated

at 60°C during 15 minutes. In the opinion of the authors the suspension is sterile by now. The sterility of the suspension is controlled by spreading 1 cc, equivalent to 100 cc of the original culture, of it out on bloodagarplates and by inoculating it into bloodbroth. Further they inject a relatively large amount of it into white mice.

Even if they controlled for 72 hours, the bloodagar and the bloodbroth remained sterile, and the mice survived.

The „R” cultures are set up from a heterological type of pneumococcus. A small part of R culture is inoculated in broth, together with rather a great deal of „vaccin” and a little „anti R serum”. As anti R serum, they use an immune serum from a heterological type of pneumococcus as used for the preparation of the R culture.

This mixture is kept at 37°C and on consecutive days subcultures are laid out therefrom on bloodagarplates.

S colonies from the type of pneumococcus, used for the „vaccin”, appear on the bloodagar.

When imitating this experiment, it is striking, that the suspension which arises after centrifugating and suspending the centrifugate in 15 cc broth, is extremely thick and it is known how difficult it is to sterilise such suspensions.

C. EYKMAN (4) in 1908 inquired the influence of heating on suspensions of microbes. This investigator then pointed to the fact that, when making the suspensions more concentrated, it becomes more difficult to kill the organisms therein by heating. Further he stated, that bacteria suspended in broth are more difficult to kill than those suspended in 0.9% NaCl. It also struck him, that if he did put suspensions, heated from 10 minutes to some hours at 50—90°C, on agarplates, there sometimes appeared growth only after heating.

He could not give an explanation of this phenomenon.

In his publication he refers to SCHUDER, who had already pointed to the necessity of using large quantities of liquids, when inquiring about their sterility.

An explanation of the overdue appearance of the colonies can perhaps be found in the theorie of the „dormant germs”

which has been elaborated by GEORGINA S. BURKE. She worked with *B. Botulinus*.

ELISABETH ECKELMANN (6) also inquired in this direction.

Those „dormant germs” will give sometimes, even after months lasting incubation, cultures.

G. S. BURKE (5) explained these dormant germs by supposing that heating brings about an impermeability of the spore-membrane. This should be the cause, that fluid from the surroundings is with more difficulty absorbed, and because of that the germination is overdue.

So the inquiries of EYKMAN (4) referred to the possibility, that the vaccin, prepared as indicated by DAWSON and SIA, was not sterile. The subject of my investigation was to trace this.

Experiments.

Started was with strains of pneumococci, which had been examined before as to their properties. Strains, which possessed a very high virulence against white mice, were used.

The strain was injected into white mice and transmitted from animal to animal so long, till one drop of peritoneal liquid from a mouse, died by the infection and injected into another mouse, killed the latter within 24 hours. The strain, isolated from this animal was examined by means of agglutination, checking of growth in optochine, solubility in bile and Gram-staining.

The pneumococci were kept virulent by drying the spleens of the deceased mice in vacuo and keeping them in vacuo afterwards, as indicated by OTTEN (3).

Before each experiment the strain was tested again to its virulence.

First experiment.

Herefore served a pneumococcus „type III”. The whole moderately thick suspension, prepared as indicated by DAWSON and SIA, was inoculated from the ampulles, on 5% bloodagar, 5% bloodbroth, 10% serumbroth and Löfflerserum. This was done to be able to find the optimal medium of growth. Agar and

broth were prepared as indicated by AVERY and CHIKERING. The agar contained 1.5% agar. For the serum was taken normal horse serum. Of every culture medium 14 tubes were taken. For the results of the experiment see table I.

TABLE I.

Number of days in incubator at 37°C.	Number of tubes in which growth appeared			
	bloodagar	bloodbroth	serumbroth	Löffler serum
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	1	—	—	—
5	—	—	—	—
6	1	1	—	—
7	2	—	1	—
8	4	—	—	—
Total	8	1	1	0

On the bloodagar appeared colonies which consisted mainly of pneumococci and also in the serumbroth. The bloodbroth was not fit for Gram-staining.

The tubes were kept at 37°C for another 14 days, but an increase of the number of positive cultures was not established. Out of all these tubes, immediately as growth appeared, a transmission was made to fresh bloodagar. When after 2 days no growth had been found on this new bloodagar, a new transmission was made from the original culture on fresh bloodagar.

The remarkable fact showed itself, that, after transmitting the colonies from the bloodagar, in 7 out of 8 tubes no new growth appeared.

So there were left 1 culture from bloodagar (called *a*), 1 of bloodbroth (*b*) and 1 of serumbroth (*c*), which contained morphologically pneumococci.

Those were inoculated in optochine broth 1 : 500.000 and in ox bile and also injected into white mice. *b* was checked in optochine, *a* and *c* not distinctly. *a* and *b* were dissolved in bile. *c* was not. No mice died within 14 days.

The complementfixation reaction was performed with *a* and *b*.

As a serum was used the same serum as used for the identification of type by agglutination; for the complement a guinea-

pig serum in a dilution of 1 : 10. The bacterial suspensions were titrated before. I did work with whole quantities, so that the total volume of the liquid was 5 cc. The serumdoses used were 0.2, 0.1, 0.05, 0.025 and 0.01.

The complementfixation reaction was positive with quantities of 0.2 and 0.1 serum for *a* as well as for *b*.

Second experiment.

For this experiment another strain of type III was used. This time I did not inoculate on Löffler serum, but only on 21 tubes of bloodagar, 21 tubes of bloodbroth and 21 tubes of serumbroth.

This time the suspension in the ampulles was a very thick one. For the results see table II.

Number of days in incubator at 37° C.	Number of tubes in which growth appeared.		
	Bloodagar	Bloodbroth	Serumbroth
1	—	1	1
2	—	8	1
3	—	—	—
4	4	1	3
5	1	1	—
Total	5	11	5

All cultures now grew after transmitting them to new bloodagar. All of them consisted of bacteria which were morphologically pneumococci. 2 cultures of bloodagar (*a* and *b*), 1 of serumbroth (*c*) and 1 of bloodbroth (*d*) were checked in optochine as well as dissolved by ox bile. Not one mouse died within 14 days.

With these four cultures complement fixation reactions were made. The reaction was positive:

- a* with quantities of serum from 0.2 and 0.1.
- b* " " " " " 0.2 and 0.1.
- c* " " " " " 0.2.
- d* " " " " " 0.2, 0.1 and 0.05.

So *d* showed a rather strong complementfixation, *a* and *b* a little less strong positive and *c* a very weak one.

Third experiment.

For this experiment a pneumococcus of type II was used. The strain did grow very badly in the broth, so there appeared a much less thick suspension than the first two times. For the control of the sterility again served 21 tubes of each of the three culture media used before. For the results see table III.

TABLE III.

Number of days in incubator at 37°C.	Number of tubes in which growth appeared.		
	Bloodagar	Bloodbroth	Serumbroth
1	—	—	—
2	—	—	—
3	—	—	—
4	—	—	—
5	2	2	—
6	—	—	1
7	—	1	—
Total:	2	3	1

The tubes were kept in the incubator for ten more days, but further growth did not appear.

Again the phenomenon occurred that after a transmission to new bloodagar, the three cultures from bloodbroth and one from bloodagar did not grow.

Morphologically this bloodagar culture consisted of pneumococci. The bloodbroth could not be controlled.

The two remaining cultures, one of serumbroth (*a*) and one of bloodagar (*b*), consisted morphologically of pneumococci and were checked by optochine as well as dissolved by ox bile. The mice did not die.

When doing the complementfixation reaction with them: *a* was positive with serum quantities of 0.2, 0.1, 0.05 and 0.025. *b* was positive with serum quantities of 0.2, 0.1 and 0.05.

So *a* was very strongly positive, *b* a little less, but still strongly positive.

To investigate if other microbes would show the same results as pneumococci, two more experiments were tried out.

Fourth experiment.

I took a strain of cholera vibrio from the collection of the laboratory. The culture consisted of lively moving vibrios, which were well agglutinated. The suspension was moderately thick. It was inoculated on 10 tubes of blood agar, 10 tubes of ordinary agar, 20 tubes of bloodbroth and 50 tubes of Cholerapeptonwater. The tubes were kept at 37°C for 10 days, but no growth appeared at all.

So this time the suspension was apparently sterile.

Fifth experiment.

I used a strain of typhus bacteria of the laboratory's collection. The strain possessed all the properties of typhus bacteria and was agglutinated to a serumdilution of 1 : 3200. 30 tubes of 10% serum agar, 30 tubes 10% serum broth and 10 tubes 5% bloodbroth were inoculated. The suspension was moderately thick.

For the results see table IV.

TABLE IV.

Number of days in the incubator at 37°C.	Number of tubes in which growth appeared.		
	Serum agar	Serum broth	Bloodbroth.
1	—	—	—
2	—	—	—
3	—	1	—
4	—	—	—
5	2	—	—
6	—	1	—
7	—	—	—
8	1	—	3
Total:	3	2	3

After transmission to fresh serum agar, one culture from serum broth did not grow. All tubes contained moving, Gram-negative bacilli. They did not convert saccharose, but did so to maltose and mannite. They did not form gas in lactose and glucose, did not form indol, caused acid in litmus whey and did not coagulate milk. The agglutination titre varied from 1 : 800 to 1 : 3200.

Discussion.

In all heated suspensions, prepared as indicated by DAWSON and SIA, except in experiment 4, the original bacteria could be denoted. When inoculating the suspension on media, possessing optimal qualities for the growth of the bacteria, then there appeared after a longer time than normal a growth. Exceptions hereon are experiment No. 2 and No. 4. In experiment No. 2 this could be explained by the fact, that the suspension was extremely thick. As EYKMAN indicates, the bacteria therein are less damaged than those in a less thick suspension.

It is a pity I did not count the organisms in the suspensions; therefore, as concerning to the thickness of the suspensions, I was dependent on the cloudiness of them.

In experiment No. 4 no growth at all could be denoted. Apparently cholera vibriones are very much susceptible to heating. To draw the conclusion that they are more susceptible than pneumococci, would however be very premature.

It is a typical fact, that in my experiments the pneumococci absolutely lost their virulence. DAWSON and SIA got virulent forms, after bringing their „vaccin”, R cultures and anti R serum together.

Conclusion.

In suspensions, prepared strictly following the methods of DAWSON and SIA, for getting sterile „vaccin”, which they needed for their experiments, live germs could be indicated in most cases. The fact that these investigators thought their suspensions sterile, can perhaps be explained by their mistake of taking too little of the used suspensions for the controlling of the sterility of it and because they did not leave their controlling cultures long enough at 37°C.

LITERATURE.

1. DAWSON and SIA: Journal of experimental medicine. 1931, 54, 681 and 701.
2. DAWSON: Journal of experimental medicine. 1930, 51, 123.
3. OTTEN: Zentrallblatt für Bakteriologie. 1ste Abt. 1930, 116, 199.

140 S. J. C. DUNLOP, Steriliz. microb. i. c. w. transform. pneumococci.

4. EYKMAN: Biochemische Zeitschr. 1908, 11, 12.
5. BURKE: Journ. of Infect. diseases. 1923, 33, 274.
6. ECKELMAN: Centralblatt für Bakteriologie. 2te Abt. 1917, 48, 140.