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Microbiology. — "*An experiment with Sarcina ventriculi*". By
Professor Dr. M. W. BEIJERINCK.

Some years ago I presented a paper concerning a method to obtain and cultivate an anaërobic fermentation *Sarcina* from garden soil.¹⁾ As the microscopic image and the dimensions of the thus obtained organism corresponded in all respects with the *Sarcina* of the stomach,²⁾ of which SURINGAR³⁾ has given so exact a description, I already then tried to prove their identity by experiments, similar to those with garden soil, with material containing stomach sarcina, which I owed to Professor VAN LEERSUM at Leiden. These experiments, however, failed. A later one, made at Leiden after my indications, proved likewise unsuccessful.

My supposition that the cause of the failure might have been a too strong aëration of the infection material by which the anaërobic stomach sarcina had lost all its vegetative power, induced me to pay special attention to this point at a renewed experiment for which Professor VAN LEERSUM again afforded me an opportunity in the Academic Hospital at Leiden.

It was proved that my supposition had been right: when transferring the contents of the stomach with the sarcina to a fit culture liquid, so quickly that contact with the air might be considered as excluded, it was possible to make the growth and fermentation proceed vigorously.

The experiment was managed as follows.

Some bottles of about 130 c.m.³ were filled with boiling malt extract quite freed from air by previous boiling. The malt extract was prepared by soaking about 20 g. of grist of kiln-dried malt in 80 g. of water, saccharifying one hour at 63° C., boiling and filtering. Some bottles were acidified with phosphoric acid to 5 cm³ N per 100 cm³, some others to 10 cm³ N, and others were not acidified at all. The acidification was applied as the experience with the sarcina of the soil had taught that this organism endures a high

¹⁾ Proceedings of the Meeting of 25 February 1905 p. 580. Archives Néerlandaises Sér 2, T. 9. page 199, 1905.

²⁾ Discovered by GOODSIR, History of a case in which a fluid, periodically ejected from the stomach, contained vegetable organisms of an undescribed form. With a chemical analysis of the fluid by WILSON. Edinburgh Medical and Surgical Journal, T. 57, p. 430, 1842. WILSON asserts he has found acetic acid in the gastric juice, but does not speak of lactic acid, which is in fact produced by *Sarcina ventriculi*.

³⁾ De Sarcine (*Sarcina ventriculi* GOODSIR), Leeuwarden 1865.

degree of acidity much better than all other microbes occurring in the soil, so that the same might be expected with regard to the stomach sarcina if this were indeed identical with it. The further course of the experiment confirmed the correctness of this expectation too.

The bottles destined for the experiment were cooled after closing to about 40° C. and only opened at the moment the infection material was at hand, which consisted in the contents of the stomach of a person suffering of stenosis oesophagi. About 5 cm³ of it was introduced into each bottle and that so quickly after the pumping out of the stomach, that the material had no time, neither to be saturated with air nor to be cooled considerably below the temperature of the body. Microscopically a great many sarcines were to be recognised, other microbes being hardly to be found. It is true that many yeast-cells occurred, but they proved dead and originated evidently from the yeast used for the preparation of the bread-porridge which the patient had eaten. Rests of potatoes and rice were also recognised in the contents.

Before proceeding the following observation may be mentioned here.

Directly after the pumping out of the stomach a little bottle was also quite filled with the thus obtained contents only, closed with a cork and placed in a thermostat at 37° C. The result was that in this bottle, already after a few minutes, so vigorous a fermentation set in that the cork was thrown off. As microscopic examination proved that in this way a very pure *Sarcina* fermentation was obtained, this simple experiment had for the first time demonstrated that the stomach *Sarcina* can be nothing else but an anaërobic fermentation sarcina.

The acid titer of the clear filtrate of the contents was, according to Professor VAN LEERSUM, 3.8 cm³ N per 100 cm³, with phenolphthalein as indicator, whilst free hydrochloric acid seemed quite absent, so that the acid must chiefly have been the lactic acid secreted by the sarcina itself, which is in fact very well possible, as at laboratory experiments the sarcina of the soil grows readily in somewhat saccharified meal-mashes and can form therein about 4 cm³ N lactic acid per 100 cm³. The striking purity of the sarcina fermentation in so heterogeneous a mass as the stomach contents, in which neither lactic acid ferments nor alcohol yeasts were to be found, might have been explained by the presence of free hydrochloric acid, this acid being much better tolerated by the sarcina than by the other microbes. But as this acid seemed to be quite absent, the said pure development of the sarcina in the stomach,

all other organisms being excluded, is not yet quite clear. But we return to our chief experiment.

The bottles prepared as described, arrived at Delft at a temperature of about 25° C. and were directly placed in a thermostat at 35° C. The result was that in all without exception, so as well in absence of acid as with 5 and 12 cm³ N phosphoric acid, already after some hours a distinct fermentation was visible. By and by it increased in vigour and after about 18 hours the sarcina had so much multiplied, that at the bottom of the bottles a thick layer of the so characteristic microbe had deposited, from which an abundant current of fermentation gases, consisting of carbonic acid and hydrogen, mounted upwards. This state continued about 24 hours before the fermentation fell considerably.

My supposition that the earlier experiments had only failed because the stomach contents had been too strongly cooled and aërated during the transit from Leiden to Delft was thus proved to be well founded, and now all doubt is excluded about the identity of the soilsarcina of the hydrogen fermentations and the sarcina of the stomach.

It is of interest still to note here that in this experiment the addition of acid to the nutrient liquid had proved superfluous, as the fermentation had gone on also in the bottles without acid. In these latter bottles, however, many lactic-acid streptococci and lacto-bacilli were visible already after 18 hours' cultivation, which was not at all the case in the bottles with phosphoric acid. Only the latter could thus be used for the continuation of the fermentation by inoculation into a new quantity of culture liquid, without the chance that the sarcina might be overgrown and expelled by the lactic-acid ferments. Likewise as with the sarcina of the soil, by some repeated transfers into the described medium, acidified with phosphoric acid to 13 cm³ N per 100 cm³, it was possible within the course of three days to obtain so pure a culture of the sarcina, that inoculation into the malt extract without acid was successful, not any other microbes coming to development.

The thus obtained fermentations have become very vigorous and are not to be distinguished from the best fermentations with the soil sarcina.

Now that the identity of the latter and that of the stomach is ascertained, still the question exists how it feeds and multiplies at the low temperature and under the other conditions of life of the relatively cold soil, which must evidently be quite different as well from those of the stomach contents as from those of the

described nutrient liquids, so rich in carbohydrates and various nitrogen compounds, and at temperatures between 35° and 40° C.

The answer to this question I hope to give later. That the sarcina should only accidentally occur in the soil and the mud of ditches and not multiply there, cannot be admitted on account of the very common occurrence of this organism; near the Laboratory at Delft, for example, the sarcina could easily be found to a depth of 70 cm in all earth-layers, even in so small quantities of soil as 0.1 to 0.5 g.

Why the sarcina develops so easily in the diseased stomach is in my opinion connected with the readiness with which this organism grows in meal-mashes, supported by the absence of hydrochloric acid which under normal circumstances inhibits all microbic growth in the stomach. The general occurrence of the sarcina is perhaps best shown by the following experiment. If some coarsely ground rye is mixed with water and placed in a thermostat at 30° to 35° C. it will the next day be found in a strong coli-aërogenes fermentation. If then this mass is carefully examined with the microscope many packets will be found of the sarcina in a state of very active multiplication. They clearly originate from the dust deposited on the surface of the corn at the reaping, the sarcina being quite well adapted to endure severe drying.

Although the sarcina of the stomach, in itself harmless, can at most be troublesome by the evolution of hydrogen,¹⁾ it should still be observed that development of this microbe is impossible in absence of carbohydrates, so that at a flesh diet, if there were no reasons to avoid such a regimen, it would soon disappear. A milk diet, too, would have such a result, as well if the milk were acidified by lactic-acid ferments, as without previous acidification. So it was not possible in laboratory experiments to cultivate the sarcina in butter-milk, and even fresh milk, acidified with various quantities of lactic or phosphoric acid, gave only in few instances a feeble growth. In absence of acid the growth of the sarcina in crude milk is quite impossible, this organism being overgrown by the other microbes.

¹⁾ The periodical vomitting observed in some cases of stomach sarcina may be connected with the accumulation of hydrogen, formed in the stomach.