of τ_n and σ the curve (π) consists of a curve of order $(2n-1)$ and a right line (§ 3).

On A lie besides the $2n$-fold right line and a $2n$-fold torsal right line $6n$ single right lines more.

The plane σ contains $2(n-1)$ right lines of A and touches A in the points of a curve of order $(n+1)$, which is the locus of the points where the curve of the complex (π) touches its n-fold tangent $(\sigma\pi)$. For, if the ray s_0 resting on l corresponds to the ray t_0 cutting σ in T_0, then one of the points of contact of the curve of the complex of the plane (lT_0) with σ lies in the trace L_σ of l; consequently the indicated points of contact lie on a curve of order $(n+1)$. This curve is generated by the pencils (L_σ) and (S) arranged in a $(1, n)$ correspondence; so it has in S an n-fold point.

The plane τ touches A according to a curve of order $(n+1)$ which is the locus of the points of contact of the curves (π), in planes π through l, with the traces $(\pi\tau)$. This curve has an n-fold point in the trace L_τ of l on τ; the tangents in this multiple point are the traces of the planes π cutting $(\sigma\tau)$ on the n rays s conjugate to the rays t drawn out of L_τ.

The plane τ has farthermore the envelope τ_n in common with A. For, while a point P of the right line $(\pi\tau)$ bears in general n tangents of the curve of the complex (π) determined by the rays s corresponding to the n rays t drawn through P, two of those tangents coincide as soon as P lies on the envelope τ_n; then however P belongs to the curve (π), thus to the surface of the complex A.

Microbiology. — "*An obligative anaerobic fermentation Sarcina.*" By Prof. M. W. BEIJERINCK.

The following simple but yet delicate experiment gives rise to a vigorous fermentation, caused by a sarcine, wherein microscopically no other microbes are perceptible and which, when rightly performed, can produce a real pure culture of this fermentation organism. The simplicity of the experiment is the result of many previous investigations, partly made conjointly with Dr. N. GOSLINGS, which have gradually rendered clear the conditions of life of the examined microbe.

Bouillon with 3 to 10 % glucose, or malt wort, is acidified with phosphoric acid to an acidity of 8 cc. normal per 100 cc. of culture liquid and introduced into a bottle, which is quite filled with it and fitted with a tube to remove the gas. The infection is done

with an *ample quantity* [1]) of garden soil, from which the heaviest and roughest portion has been removed, but in which so much solid substance is left behind that in the nutrient liquid it forms a muddy deposit from 5 to 7 or more millimeters thick. The culture is effected in a thermostat at 37° C. After 12 hours already the liquid is in a strong fermentation, which lasts from 24 to 36 hours, and whereby the surface is covered with a rough scum, produced by gas bubbles mounting up from the depth. Whilst the liquid itself remains wholly free from microbes, the microscopical image of the deposit shows a luxuriant, pure or almost pure culture of a sarcine, of which the elementary cells measure for the greater part about 3.5 μ, so that the species belongs to the largest forms known, and the multicellular sarcine-packages are easily visible to the naked eye. The cells are colorless and transparent and the packages present irregular sides. Here and there, but much less generally, a brownish intransparent form is seen, with more regularly cubical packages of which the cells measure 2 to 2,5 μ.

The scum floating on the fermenting fluid consists of slime in which the evolved gas remains for a time imprisoned. This slime is produced by the outer side of the sarcine cells, whose walls for the rest consist of cellulose, which becomes violet-blue by zinc-chloride and jodine. This reaction was discovered in 1865 in the stomacal sarcine by SURINGAR [2]), who on this account argued the vegetal nature of this organism, which fully corresponds to the small-celled fermentation sarcine. The large-celled form more resembles the figures which LINDNER [3]) gives of his *Sarcina maxima*, found, as he expresses it, in "Buttersäuremaischen", hence, in wort wherein a spontaneous butyric fermentation. I am not, however, convinced that both these forms do really belong to two different species of sarcine, as it is well known that in this genus of microbes great morphological differences may occur in the same species.

The gas is a mixture of about 75 % carbonic acid and 25 % hydrogen; methan is not present. Besides, a moderate quantity of acid is formed, which for example, in a nutrient liquid with an acidity of 6 cc. per 100, may mount to 12 cc., a percentage only found back in the technical lactic fermentations. Furthermore a peculiar odor originates, reminding of the ordinary lactic-acid fermentation, by

[1]) With *little soil for infection*, the experiment becomes doubtful.

[2]) W. F. R. SURINGAR, De sarcine (*Sarcina ventriculi* GOODSIR), pag. 7, Leeuwarden 1865. Here very good figures are to be found.

[3]) Mikroskopische Betriebscontrolle in den Gärungsgewerben, 3e Aufl. p. 432, 1901.

Lactobacillus. If, as is probable, this acid will prove to consist entirely, or for the greater portion, of lactic acid, the fermentation sarcine may be considered as the most differentiated lactic-acid ferment hitherto known.

When using a sufficient quantity of soil for the infection, that is a relatively great number of sarcines, which thereby, in the given circumstances, may compete with advantage with, and conquer all other microbes, the experiment described succeeds within very wide limits. Thus the sarcine fermentation may *in this case be obtained as well in an open flask as in a closed bottle*, whence it follows that the sarcine can suffer a moderate quantity of oxygen; and it will appear below, that a slight quantity is even wanted under all circumstances. Notwithstanding this, the name of obligative anaerobic remains applicable as the cultivation at full atmospheric pressure is impossible. The acid may further be varied between 3 and 11 cc. normal phosphoric acid per 100 cc.. The phosphoric acid may be replaced by lactic and even by hydrochloric acid, if the acidity of the latter is not taken higher than 6 to 7 cc. per 100 cc., but not by nitric acid.

Instead of glucose cane sugar may be used, but with milk sugar and mannite the experiment does not succeed. As source of nitrogen only peptone can be used, such as found in malt-wort or bouillon; simpler nitrogen sources, like asparagin, ureum, ammonia and saltpeter, are unfit for the nitrogen nutrition of the sarcine. The limits of the temperature are wide and may vary between 28° C. and 41° C.

Although the experiment may thus be modified in many respects, the first described arrangement is recommendable, as it is best adapted to the optimum of the different conditions of life of the organism.

A property peculiarly important for this research is the readiness with which the function of fermenting, that is the power of evolving gas, gets lost under the influence of a secretion product, probably the acid, and through which all transports with old material become perfectly useless. Hence it is necessary to transport cultures still in fermentation to insure the success of further experiments.

That some aeration enhances the life-functions of this obligative anaerobic and that access of a little air is even necessary in the long run, is evident from the fact that the most vigorous fermentations are obtained in a closed bottle, with the deposit got in an open flask, whereas renewing of the nutrient liquid formed above the deposit in a closed bottle will after few repetitions give rise to diminuation or cessation of the fermentation.

For the continuation of the culture by inoculating *slight quantities* of material of a rough fermentation into the same nutrient liquid, two precautions should be taken. First, the inoculation should be done into the medium, freed from air by boiling, the bottle being entirely filled with the hot liquid, so that on cooling no air can dissolve. Second, an acidity of less than 7 proves not sufficient, hence this should be 8 or 10 cc., as otherwise the lactic acid ferments might prevail and supplant the sarcine.

From the necessity of expelling the air we see that the fermentation sarcine undoubtedly belongs to the ordinary anaerobics, which, considering the success of the rough accumulation experiment *with aeration*, might perhaps not have been expected; but the fact holds good in the same way for the butyric acid ferment, generally accepted as an obligative anaerobic, so that, also with respect to the fermentation sarcine, there should be spoken of "microaerophily." Further examination shows that in deep test-tubes with maltwort-agar, very easily pure cultures may be obtained, whereby the sarcine is recognisable by the obvious size and the remarkably rapid development of its colonies. On the other hand, on maltwort, or broth-bouillon-glucose-agar-plates with or without acid at 37° C., with access of air, no growth at all of the sarcine takes place, as might be expected. Of course the packages can also be seen on the plates without growing and be removed in a pure condition. When we make use of little acid for the rough accumulation, colonies of lactic acid ferments, belonging to the physiological genus *Lactobacillus*, will develop on the plates at the air, which can grow as well with as without air, but whose other life conditions correspond to those of the sarcine. In this case the experiment shows at the same time that everywhere in garden soil real lactic acid ferments are present, whereof the proof had not been given until now.

When using much acid, for example 10 cc. or more normal acid per 100 cc. of culture fluid, through which the vital functions of the sarcine, such as rapidity of growth and the faculty of assimilating oxygen, are lessened, certain alcohol ferments, proper to garden soil, come to development, but they can, together with some of the other impurifications of the rough accumulations, as moulds, *Mucor* and *Oidium*, be checked and expelled by exclusion of air, hence, by culture in closed bottles. To this end however, it is necessary to render the conditions for the sarcine as favorable as possible and not allow a temperature below 37° C.

The staying out of the butyric acid fermentation (caused by *Granulobacter saccharobutyricum*), which so readily originates with exclusion

of air in glucose-bouillon and maltwort, is due to the acidity of about 8 cc. or more, whereby this fermentation becomes impossible.

Although it is evident from the foregoing, that the growth of the sarcine is less inhibited by the acid than that of the lactobacilli and of the butyric ferment, it may still be easily proved that already 7 cc. acid per 100 cc., are less favorable than 3 or 5 cc., also for the development of the sarcine itself, so that the higher amount of acid in the accumulation only serves to render competition with the said ferments possible. If by timely transports into maltwort with more than 8 cc. phosphoric acid, or by separation in solids, real pure cultures are at disposal, the further transfers, with entire omission of the acid, show that then also vigorous growth and fermentation may occur. We thus see how wide the limits are of the life conditions of the sarcine, as soon as competition with all other microbes is quite out of question.

The discovery of this certainly unexpected fermentation has sprung from the working out of the general question which organisms of the soil can develop in a sugar-containing culture fluid in presence of an acid and with imperfect aeration. At temperatures of about 30° C. and lower, alcoholferments, *Mucor racemosus* and *Oidium* prove to be the strongest, but then already a few sarcines are observed. At about 40° C. most alcoholferments of garden soil, besides *Mucor* and *Oidium* can no more compete with the sarcine and the lacto bacilli, which then become predominant. This being fixed the last steps which led to the culture of the fermentation sarcine alone, were the recognition of the obligative anaerobiosis, and of the superiority of the resistence of the sarcine with respect to anorganic acids compared with that of *Lactobacillus* and the butyric ferments.

Above, already, I pointed to the perfect correspondence of the small-celled form of the fermentation sarcine to the description which SURINGAR gives of the stomacal sarcine, and I suppose that in the cases of non-cultivable *Sarcina ventriculi*, of which, for instance, DE BARY speaks [1]), there should really be thought of the fermentation sarcine. This view is supported by different observations in the older literature, cited by SURINGAR. But still more convincing is my accumulation experiment, which proves that the conditions for the existence of this sarcine are just of a nature to render its life in the stomach possible.

It will be easy to obtain certainty thereabout by a repetition of

[1]) Vorlesungen uber Bacterien, 1e Aufl. pg. 96, 1887.

this experiment, not with garden soil for infection material, but by using the stomacal contents of such a case of stomacal sarcine. The "not cultivability" of DE BARY may mean the same as anaerobiosis, for it is well known how difficult it is, even at the present time, to cultivate anaerobics if the particulars of their life conditions are not exactly known.

For the rest I do not doubt of the precision of FALKENHEIM's [1]) and MIGULA's [2]) observations, who have seen aerobic colonies of micrococci originate from stomacal sarcine. It is true that I for my part have not succeeded in confirming this observation with regard to the fermentation sarcine, but for other species of *Sarcina* I have, with certainty, stated the transition into micrococci, and with various anaerobics, although not belonging to the genus *Sarcina*, I have seen now and then colonies originate of facultative anaerobics, which in all other respects, corresponded to the obligative anaerobics used for the cultures. Therefore this modification also seems possible for some individuals of the fermentation sarcine. Accumulation or transfer experiments with stomacal contents will however only then give positive results, if these are used when still in fermentation; with long kept material nothing can be expected.

Already the older observers [3]) as SCHLOSSBERGER (1847), SIMON (1849) and CRAMER (1858) have tried, although in vain, by a kind of accumulation experiments, to cultivate the stomacal sarcine, wherefore they prepared, as nutrient liquid, artificial gastric juice with different additions. Remarkable, and illustrating the biological views of those days, is the fact, that for the infection they did not use the stomacal contents themselves, but beer yeast, supposing, that the sarcine might originate from the yeast cells, which somewhat resemble it, and are always found in the stomach together with the sarcine itself.

Physics. — "*The motion of electrons in metallic bodies.*" II. By Prof. H. A. LORENTZ.

(Communicated in the meeting of January 28, 1905).

§ 11. By a mode of reasoning similar to that used in the last §, we may deduce a formula for the intensity i of the current in a *closed* thermo-electric circuit. For this purpose we have only to suppose the ends P and Q, which consist, as has been said, of the

[1]) Archiv f. experiment. Pathologie und Pharmacologie. Bd. 10, pg. 339, 1885.
[2]) System der Bacteriën. Bd. 2, pg. 259, 1900.
[3]) Cited from SURINGAR (l. c.).