Let us now suppose n_2 to be $= 2n_1$; then we get:

$$\ddot{q}'_1 + n_1^2 q'_1 = (4c n_1^2 - b n_1^2 + 2p) q'_1 q_2,$$

$$\ddot{q}_2 + n_2^2 q_2 = (2c n_1^2 - \frac{1}{2} b n_1^2 + p) q'^2_1.$$

So we find:

$$\begin{cases} \ddot{q}'_1 + n_1^2 q'_1 + 2 d_2 q'_1 q_2 = 0, \\ \ddot{q}_2 + n_2^2 q_2 + d_2 q'^2_1 = 0; \end{cases}$$

where

$$d_2 = -2c n_1^2 + \frac{1}{2} b n_1^2 - p$$

The equations determining the first approximation have exactly the same form as those found in § 4. What was formerly deduced for the simple mechanism holds consequently, if $n_2 = 2n_1$, for an arbitrary mechanism with two degrees of freedom in such a sense that the horizontal projection of the point moving over the surface may be regarded as the representative point for the arbitrary mechanism.

We finally observe that any mechanism for which

$$-2cn_1^2 + \tfrac{1}{2}bn_1^2 - p = 0$$

is not sensitive for the relation $n_2 = 2n_1$. So this is the condition requisite to make the mechanism for $n_2 = 2n_1$ a mechanism of exception in the sense indicated by Prof. KORTEWEG (§ 26 of his paper).

Mechanisms of exception therefore are among others the symmetrical mechanisms (§ 31 of that paper); for here c, b, and p are all equal to zero.

Microbiology. — "*Viscosaccharase, an enzyme which produces slime from cane-sugar*". By Prof. Dr. M. W. BEIJERINCK.

The emulsion reaction.

Many spore-producing and a few non spore-producing bacilli, cause, when growing in presence of cane-sugar or raffinose on neutral or feebly alkaline agarplates, a very peculiar "colloidreaction", which is also valuable for the diagnosis of these bacteria. This reaction consists in the formation, in and also on the surface of the agar around the colonies or streaks, of a liquid-"precipitate", i. e. an emulsion, which can best be recognised in transmitted light, and at the same time in a swelling of the agar caused by the increase of volume produced by the emulsion.

The emulsion consists of drops (see plate) of different size, mostly very small, but sometimes growing to 0,2 mm. so that they may

be distinguished with a magnifying glass. At feeble magnification they might be taken for droplets of oil suspended in the agar, but as strong sulphuric acid dissolves these drops immediately, and a feebler acid more slowly, there can be no question of oil or fat.

Characteristic for the reaction is that it can only be distinctly observed in agar but imperfectly in gelatin. In the agar the process is impeded when acid is produced by the microbes. Thus bouillon-agar, yeastwater-agar, and wort-agar with cane-sugar can well be used, but the emulsion is more distinctly formed in agar with mixtures of substances that prevent the acidification, to which cane-sugar is so very apt. For that reason nitrates as nitrogen-food are especially favourable, as the withdrawing of nitrogen then necessarily must produce an alkali, while for example ammonium-salts, used as source of nitrogen, must promote the acid reaction.

A good experiment to produce the emulsion is the following: A plate is prepared of the composition: tapwater, 2 % of agar, 2 % of cane-sugar, 0,02 % KNO_3 and 0,02 % K_2HPO_4. Nitrogen food may also be quite left out, so agar-plates with 10 % of cane-sugar and bikalium-phosphate only, are very well fit to demonstrate the emulsion with *Azotobacter* and the hereafter mentioned *Bacillus emulsionis*. The quantity of cane-sugar can vary between 0.1 % and 50 % without much difference in the result.

After the solidifying of the agar-plate and the removal of the adhering water, soil-bacilli are dispersed, obtained by shaking some garden-soil with water, and heating it a few minutes at 70° to 80° C. in order to kill the not sporulating microbes, Then the water is poured over the plate and allowed to flow off. The adhering germs, for so far they live, are nothing but spores of bacilli, which can germinate at 30° C.

After one or two days the colonies become visible and simultaneously the emulsion around some of them; the majority does not produce the emulsion.

Cane-sugar may be replaced by raffinose, which acts in the same way; but glucose, levulose, mannose, galactose, lactose, maltose, trehalose, melibiose, mannite, inulin, dextrin and xylose, do not give the emulsion.

The emulsion is distinct round the colonies of *Bacillus mesentericus vulgatus* (see plate Fig. 1), *B. megatherium* and a not yet described soil-bacillus, commonly also found in cane-sugar itself, recognisable by its small terminal spores, which may be called *Bacillus emulsionis* and whose transparent colony is likewise given on the plate (Fig. 2). The emulsion is wanting in *B. subtilis, B. mycoides, B.*

polymyxa, *B. nitroxus*, *B. sphaerosporus*, *B. luteus*, besides in the anaërobes *Granulobacter butylicum*, *Gr. saccharobutyricum* and *Gr. pectinovorum*.

The moulds, the various yeast species, even those which invert cane-sugar, besides all species of *Streptothrix*, and most of the non-spore producing bacteria, do not produce the emulsion either.

An exception to the last rule makes the non-spore producing *Azotobacter chroococcum*, which on plates of 2 % of agar, 2 to 10 % cane-sugar, and 0,02 % K_2HPO_4, in water, gives a strong emulsion, which extends to a large distance round the colonies; later, in their vicinity, perhaps by the influence of a specific enzyme or an acid it vanishes, while near the colonies of the soil-bacilli the emulsion is permanent. With the exception of *B. chroococcum* the other forms of *Azotobacter* do not produce the emulsion. From cultures of *Azotobacter*, prepared with garden soil and destined for the absorption of free nitrogen, a species related to *B. radiobacter* can be obtained, which produces no spores, but does also give a strong emulsion.

Ane mulsion, from a physical view analogous but quite different by the manner in which it takes rise, was described by me on another occasion[1]). It appears when a 10% solution of gelatin in water is boiled with a 10% solution of soluble starch, or with a 2% agar-solution. Even by boiling the two watery solutions do not mix, which, of course, is also the case after solidifying. This reposes evidently on the fact that here two colloidal solutions are brought together, which cannot diffuse and whose emulsionated droplets constantly have a positive surface-tension with regard to each other. The same explanation must hold good for the emulsion formed by the viscosaccharase with regard to the agar, and as I may add, to culture-liquids wherein *Bacillus emulsionis* produces the emulsion also.

The emulsion is produced by an enzyme.

If from the emulsion field round a colony a small piece of agar is cut out, without touching the colony, and placed on an other cane-sugar-agar-plate, the emulsion itself does not diffuse out of it, but into the plate, a substance goes over, which produces the emulsion again and with regard to the quantities used rather strongly. This points with certainty to the presence of an enzyme as the cause of the emulsion, an enzyme which must have the property of moving through the agar by diffusion. This agrees perfectly well with the

[1]) Centralbl. f. Bacteriologie 2te Abt. Bd. 2, p. 627, 1896.

origin of the emulsion round the colonies, for a substance which is evidently insoluble in the agarplate, can only be found at the place where it is produced. This substance having in our case the nature of a plant slime, the enzyme may be called *viscosaccharase*.

The enzyme is prepared by filtering a culture of *B. mesentericus vulgatus* and precipitating the filtrate with alcohol, whereby, of course, other enzymes formed by this bacterium such as diastase, and also the slime substance itself, are precipitated. Whether to the enzymes, present in this mixture invertase must be reckoned, which is usually considered as a secretion-product of *B. mesentericus*, has become doubtful by the discovery of the viscosaccharase, at whose action, as will be seen below, together with the slime, the production of a reducing sugar is stated.

Even in presence of chloroform the emulsion reaction takes rise on cane-sugar agar-plates through the enzyme produced from the *mesentericus* cultures, without anything being perceived of the development of the germs of *B. mesentericus* itself, which may be still present after filtering and precipitating.

It is not difficult to prepare plates of any size containing the emulsion everywhere, and fit for experiments to demonstrate by what influences it may disappear.

To this end the required culture-agar is mixed before solidifying with a not too large number of germs, for example of *B. emulsionis*, and then placed one or two days in the thermostat; when the plate becomes quite turbid by the emulsion, the sugar is washed out and it is ready for the experiment. A drop of dilute acid thereon rapidly produces a clear space.

At the action of viscosaccharase, besides the slime a reducing sugar is found.

When small pieces of agar containing the emulsion are introduced into an experiment-tube and cautiously warmed with a little FEHLING'S copper solution, a strong reduction is seen, which does not take rise with the same sugar-agar if the emulsion is wanting.

The question arose whether this reaction should be ascribed to the slime itself, or if at the same time, through the viscosaccharase, or in another way, some other reducing substance is formed. Therefore small pieces of the agar containing the emulsion were washed out with water, whereby the slime, which cannot diffuse from the agar into the water, remains behind, but the reducing power of the agar is lost, whilst the water used for the washing becomes itself strongly

M. W. BEIJERINCK. Viscosaccharase, an enzyme, which produces slime from cane-sugar.

Fig. 1. Bacillus mesentericus

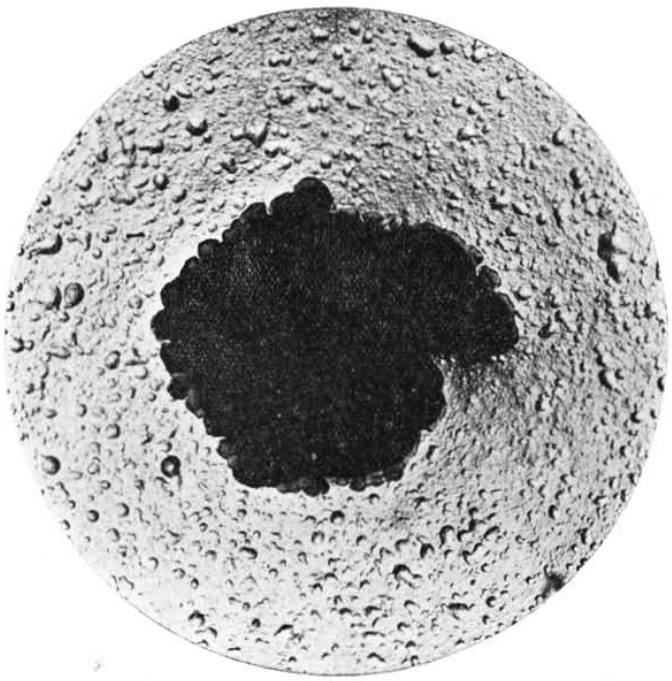

Fig. 2. Bacillus emulsionis.

The emulsion-reaction.

reducing. Hence it is sure that at the "emulsion reaction", together with the non-reducing slime, an easily diffusing and reducing substance (probably a sugar) is formed. The chemical composition of this substance is still unknown, just like that of the slime itself.

The possibility exists that the reducing substance is invert-sugar produced by invertase, which latter enzyme then should always accompany the viscosaccharase. Decisive experiments on this subject in progress.

Viscosaccharase is a synthetically acting enzyme.

As to the nature of the slime it must be accepted that its molecules are much larger than those of cane-sugar, else it would not be clear why the slime cannot diffuse through the agar, which cane-sugar does very easily. Viscosaccharase must therefore be a synthetically acting enzyme. This circumstance suggests a relation between the slime and "dextran" [1]). This is, however, a substance forming the cell-wall of the concerned microbes, which substance may spread in water, and even to some extent diffuse into agarplates, but is not the product of an exo-enzyme, i.e. of an enzyme able to leave the bacterial body and act outside of it like the viscosaccharase. In relation to this it is not astonishing that "dextran" can very well originate from glucose and some other sugars, which do not produce the emulsion.

Very remarkable is the fact that all the hitherto examined bacteria which show the emulsion-phenomenon, are able, at definite culture-conditions, for example on cane-sugar gelatin, when no emulsion is produced, to form non-diffusing "dextran", by which their colonies then become visible on the plates as large transparent drops. This also points to a narrow relation between the two phenomena and leads to the conclusion that the drops of the emulsion must be identic with, or related to dextran.

Perhaps by further research modifications of viscosaccharase will prove to exist, which also act on glucose and other sugars and from these may form "dextran", but which cannot leave the body, or rather the cell-wall of the microbes, and must be considered as endo-enzymes whose product, which itself does not diffuse, cannot be found beyond the limits of the colony.

If in accordance with my expectation, the emulsion is really brought about by "dextran", then light will be thrown on the formation of the wall-substances of plant cells in general; for there is no doubt

[1]) C. Scheibler, Zeitschr. d. Vereins für Rübenzuckerindustrie, Bd. 24, p. 309, 1874. L. Maquenne. Les sucres et leurs principaux dérivés. p. 745, 1900.

that "dextran" is a modification of cellulose, and the till now not explained secondary changes, observed in so many cell-walls, may then freely be ascribed to the action of specific enzymes, related to the viscosaccharase.

Why the emulsion is distinctly observed in agar, and less easily in gelatin-plates, must probably be explained by the dimension of the molecules of viscosaccharase, which are small enough to enter without much trouble the relatively wide canals of the agar, but too large to pass through the much narrower ones of the gelatin.

Many of the experiments here related I owe to Mr. D. C. J. MINKMAN, assistant in my Laboratory.

EXPLANATION OF THE PLATE.

Fig. 1. Colony of *Bacillus mesentericus vulgatus* on: canal water, 2% of agar, 1% of cane-sugar, 0.02% KNO_3 and 0.02% K_2HPO_4, with emulsion around colony. Magnified 8 times.

Fig 2. Colony of *Bacillus emulsionis* n. sp., on canal water, 2% of agar, 0.1% of cane-sugar, 0.02% $ClNH_4$, 0.02% K_2HPO_4, with emulsion around colony, Magnified 9 times.

Microbiology. — *"Variability in Bacillus prodigiosus."* By Prof. M. W. BEIJERINCK.

In a former paper [1]) I showed how easily new constant variants of *Bacillus prodigiosus* and other microbes may be obtained. Here follow some further observations, made with the aid of Mr. H. C. JACOBSEN, assistant in my Laboratory.

The keeping constant of the cultures.

The principle on which the keeping constant of *B. prodigiosus* seems to repose is preventing the cultures from becoming alkaline by their own action. Thus, by re-inoculating in quick succession, for instance every 24 hours, into bouillon or on bouillon-agar at 30° C., each form of *Bacillus prodigiosus*, whether the natural or normal form, or a variant obtained from it, remains unchanged probably for an indefinite time.

For the transplantations only very little material must be used and an abundance of food.

If some lactic acid is added, for instance 0,5 to 1.5 cm^3 normal per 100 cm^3 of bouillon, the culture likewise remains unchanged

[1]) Royal Acad. of Sciences 21 Nov. 1900,.