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,.

after a prolonged series of transports, if these are always carried out before the acid is neutralised by the alkali produced from the bouillon by the bacteria themselves [1]).

Addition of 1 to 2 pCt. of glucose acts in the same manner as free acid, *B. prodigiosus* therefrom producing acid which may rise, if sufficient glucose is added, to 3 to 4 cm^3 normal per 100 cm^3 of bouillon. As the titre of alkali, originating in the bouillon alone, can amount to 2.5 cm^3 N per 100 cm^3 of bouillon, and as from 1 pCt. of glucose there results no more than 1.5 to 2 cm^3 N of acid, addition of 1 pCt. of glucose is sufficient to prevent variation, if the re-inoculations take place quickly; but not if effected with long intervals, for in the latter case more alkali may result from the bouillon than acid from the glucose.

If to the bouillon so much ammoniumcarbonate or natriumcarbonate is added that the titre of alkali amounts to about 3 cm^3 N per 100 cm^3 of the medium, *B. prodigiosus* likewise remains constant after repeated inoculations at 30° C., whilst the control culture, without carbonate but for the rest under the same conditions, strongly varies. The same result may be obtained with magnesiumhydrophosphate ($Mg\,H\,PO_4 . 2\,H_2O$) to excess; this, however, quickly precipitates, and in order to be active should be used in a bouillon-agarplate or in a thin layer of liquid. In ordinary bouillon-agarplates 1 pCt. of this salt changes entirely into crystals of ammoniummagnesiumphosphate ($Mg\,NH_4\,PO_4 . 6\,H_2O$) the plate becoming quite transparent; a plate with 3 to 4 pCt. on the other hand, remains white and turbid.

Although it may be admitted that by these various means the formation of secretion products by the bacteria is prevented, on whose stimulating action the variability probably reposes, yet it, is not clear how this preventing takes place. Evidently substances should be thought of here which, once produced, cannot or only with difficulty leave the bacterial body.

Of the said means quick transplantation is the simplest for always disposing of constant stocks for the experiments.

The origin of the variants in general.

When cultures, placed under favourable nutritive conditions, but for the rest prepared without special precautions, are growing older between 10° and 30° C., they exhibit a certain variability at which, as formerly described (l. c.), variants are thrown off, while beside

[1]) At 4 cm^3 of acid per 100 cm^3 of culture liquid the growth of *B. prodigiosus* is slackened, at 9 cm^3 it is quite stopped.

these the original form is found unchanged. As by transplantations in rapid succession (and under constant and favourable conditions) no change occurs during thousands of cell-partitions, this variability cannot repose on some law governed by internal causes only, but a particular agency is wanted, which may have its seat within the cells, but which must yet be enacted on by external circumstances.

Although the variability can reveal itself already in an ordinary same well arranged culture, e.g. in bouillon or in maltwort, allowed to stand for a few weeks, yet this process may considerably be accelerated by repeated transplantations, *not* after a very short time, but with longer intervals, for example two days, with cultures kept at 30° C., a not too small quantity of the material for the inoculation being used, e. g. two loops of the platinum thread. After three or four repetitions, so after about a week, the variation can then be in full course, the first culture, left to itself, not yet showing any perceptible change.

This evidently reposes on the following circumstance. The influence which causes the variability in the culture when it gets older, acts in the chosen conditions already after two days. If now a re-inoculation is performed, the germs affected by that influence can increase as well as those that remained normal, whilst by not re-inoculating, thus in the first culture, the non-affected germs are by far more numerous and remain so as the cell-division slackens after the second day, because of want of food. At inoculation after two days there result at each time new modified germs, and those which are modified already, are enabled to augment without losing their modification.

In this explanation it must further be accepted, that a transplantation after two days gives no cause for atavism; for if this were the case, the reverse ought to take place of what is observed: after a week's growth the first culture should be more varied than that which has repeatedly been transplanted, but this is not so. This shows how carefully the variation experiments must be carried out in order not to become obscure.

Particularly the cultures on solid media must very accurately be observed. If these are allowed to stand for some days or weeks without further precautions, then in many cases, even with magnifying glass or microscope no variation at all can be detected, although it is actually going on, commonly to "rose" or "white".

Colony culture then shows that here and there varied germs or groups of such germs must be present, for from the seemingly homogeneous matter large numbers of white and rose variants are

obtained, which prove as constant as the normal form itself. However unchanged colonies, representing the pure stock and producing a material as fit for further experiments as the original culture, lie among the variants.

Experiences afforded by other bacteria seem to prove that the frequent repetition of the thus possible process of selection, produces a form which varies less than the original material. But it is not here the place to enter upon this important fact.

All colony cultures of *B. prodigiosus* are best made on bouillon-agar-plates, which after solidifying have been cautiously dried on a thermostat at circa 40° C. The water which then condenses on the glass cover can easily be removed; if this is neglected, *B. prodigiosus*, which is strongly motile, spreads over the surface of the agar and the colonies coalesce.

I shall now enter into a short discussion of the most important variants.

The obtained variants.

The variants derived from *B. prodigiosus* may be considered as plus- or gain-variants, minus- or loss-variants, and qualitative variants. This is exposed below in the table of descent, which shows the origin of the obtained forms; the qualitative variants (*auratus* and *hyalinus*) are placed on the same line with the normal form, the plus-variants above it, the minus-variants beneath. Hence, the arrows not only denote the descent but also whether the variability reposes on gain or loss of characters, or if it is qualitative. Dotted arrows indicate that atavism has with certainty been observed. The names indicate the chief qualities characterising the variants.

A survey of the variants without regard to their descent precedes; then follows their pedigree, which does not repose on hypothesis, but simply gives the result of the experiments.

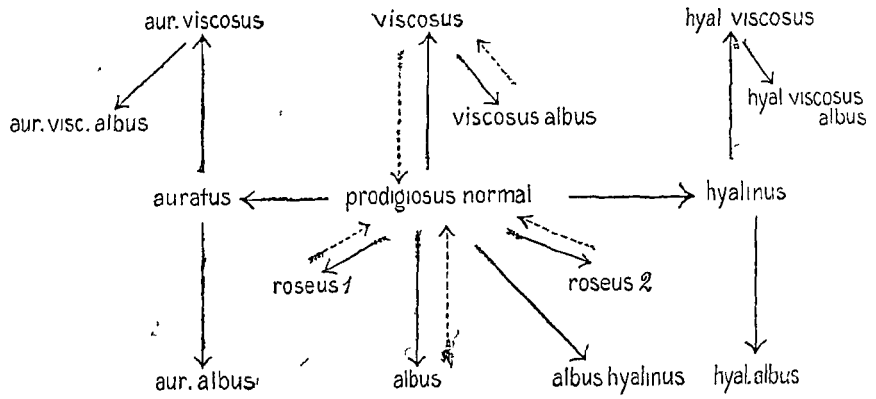
The obtained variants are:

1. *Bacillus prodigiosus*. Normal form, isolated from nature [1]).
2. „ „ *roseus* 1.
3. „ „ „ 2.
4. „ „ *albus*.
5. „ „ „ *hyalinus*.
6. „ „ *viscosus*.
7. „ „ „ *albus*.
8. „ „ *auratus*.

[1]) About 1890 from mouldering bones of a gelatinfactory near Delft.

9. *Bacillus prodigiosus. auratus viscosus.*
10. „ „ „ „ *albus* (= 7 ?)
11. „ „ „ *albus* (= 4?).
12. „ „ *hyalinus.*
13. „ „ „ *viscosus.*
14. „ „ „ „ *albus.*
15. „ „ „ *albus* (= 5?)

The relation and origin of these variants is given in the following table.



The upward arrows denote "gain-variation", the horizontal "qualitative variation", the downward arrows "loss-variation". Dotted arrows signify that atavism has been observed.

The two qualitative colour-variants, *auratus* which is orange-coloured and *hyalinus* of a deep vine-red, vary in a way quite corresponding to the normal form and like this throw off, under the same circumstances, slime-variants and white variants. Besides, the normal form may return by atavism as well from *auratus* and *hyalinus* themselves as from the variants derived from them. In the pedigree table atavism is indicated by dotted arrows for a few of the cases where it has been stated with certainty. But there is no doubt that also the other variants are disposed to atavism.

It should moreover be noted that the *auratus*-variant approaches, at least in colour, the natural variety *Bacillus Kieliensis*, but that the latter possesses a stronger power of fermentation, and produces much gas ($CO_2 + H_2$) from maltwort with dextrose or cane-sugar, the former fermenting only dextrose.

For the rest, *B. Kieliensis* itself, which varies in a way quite analogous to that of the normal form of *prodigiosus* here considered, has not yet been obtained as a variant from the latter,

A new character which may rise in addition to the already existing ones, is the production of a large quantity of slime substance by excessive growth of the cell-wall, which slime may spread through the liquids, and makes the individuals of the colonies on agarplates cohere into one tough mass. From *B. Kieliensis* was even a variant obtained whose colonies appear on the agar plates as a very consistent, almost dry zoogloea, but the analogous variant did not till now arise from the common *prodigiosus*. The *viscosus* (6), derived from the latter, is an ordinary red slime bacterium.

This red-coloured, tough-slimy form, which may be called *B. prodigiosus viscosus*, is no doubt a plus-variant. Its production has been observed under the most different nutritive conditions, between the temperatures 10° (in a cellar) and 30° C., but always and exclusively in liquid media, never on a solid one. The latter circumstance is apparently the reason why the numerous experimenters, who have studied *B. prodigiosus*, have not seen this variant. It is true that SCHEUERLEN [1]) observed that old *prodigiosus*-cultures sometimes turn slimy, but he ascribed it to their becoming alkaline and overlooked that a new constant form was produced.

The only distinct condition which seems different in the liquid cultures compared with the solid, is the access of oxygen. In the depth of the liquid this access must, of course, be very deficient for a long time, or even be entirely lacking, as the upper layers of the culture, which are rich in bacteria, take up all the oxygen. Consequently anaërobiose becomes possible in the depth, which is not the case in cultures lying free on a solid medium, and this partial anaërobiose is apparently the stimulus which induces the formation of the slime variant. That here a rather complex influence and not a direct action must be ascribed to the partial withdrawing of the oxygen, follows from the fact that the culture of *B. prodigiosus* at complete exclusion of air, as in a closed bottle, does not, even with repeated transports, give rise to the slimy variant. At temperatures of about 35° C. this variant is no more formed, although the growth of *prodigiosus* is then still very strong; at 37° the growth slackens or ceases entirely, according to the food.

In the following liquid media the production of the slime variant has with certainty been observed, as well after repeated re-inoculations as after prolonged keeping of one and the same culture at 25° to 30° C.: in broth, in broth with 1 pCt of glucose, in malt-wort, in tap-water with 5 pCt of pure gelatin and 0,02 pCt K_2HPO_4, and in

[1]) Archiv. für Hygiene. Bd. 26 p. 1.

tap-water with 2 pCt of glucose, 0.5 pCt of asparagine, 0,02 pCt K_2HPO_4, always cultivated at 30° C. and with repeated transports after two days or longer. From this we also recognise that there is no question of a direct influence of the food on the production of the variant.

The *auratus*- and *hyalinus*-variants, also, have only taken rise in liquid cultures, namely in broth and in the glucose-asparagine solution. Moreover, *hyalinus*, which is of a deep vine red, is easily obtained from a solution of pure gelatin in tap-water with 0.02 pCt. K_2HPO_4, after repeated re-inoculations, at 30° C., whereby also *hyalinus viscosus* results.

The colourless or white variants, which only differ from the original form in producing no pigment, should certainly be considered as minus-variants. They are obtained with more ease than the slime variants and, at least as to N° 4, have also been detected by other authors [1]).

Except under the said conditions, apt to keep them constant, all the cultures as well in liquid as on solid media, vary sooner or later towards white. The original form does remain preserved, but a colourless variant is thrown off, which is still more constant than the stock itself.

Not always does one and the same variant result in this case: two uncoloured constant forms, N° 4 and 5 can easily be distinguished if they originate at the same time, and their colonies are on the same agarplate so that they may be compared somewhat magnified. One, *albus hyalinus*, then looks more blueish transparent, the other, *albus*, is more of a cloudy and opake white; under the microscope the former proves to consist of smaller cells than the latter.

The cause of the production of white variants cannot be a more or less abundant access of oxygen, but must probably be sought in a stimulus, exerted by secretion products which remain enclosed in the interior of the cells.

Although the presence of ammoniumcarbonate in the medium (broth-agar), as also cultivation at temperatures higher than 30° C. e. g. at 33° C., prevent pigment production, no hereditary variation at all is caused by these influences. If the thus treated colourless cultures are transported at 20° to 25°, no white variants are obtained from them, but the normal form is found back unchanged, if at least the above mentioned precautions to preserve the constancy of the stock are not neglected.

[1]) In LEHMANN and NEUMANN's Atlas, 4th Ed. 1907, Table 30, Fig. 3, shows a coloured image of a "pure culture" of *prodigiosus*, consisting of red and white colonies.

When the white variants of the normal form are cultivated at 30° C. in bouillon or in malt-wort, the cultures will, after a few re-inoculations, turn slimy like those of the red normal form itself. Colony culture on bouillonagar proves that white slime variants are thrown off, in the same way as the normal form throws off the red ones. The white slime variants (N°. 7 ? and 14) correspond by the nature of their colonies to the two white forms, *albus* (4) and *albus hyalinus* (5), considered above.

There is still another method to obtain the colourless slime variant from the red one. If this latter is cultivated at 30° in malt-wort or in bouillon, we find after one or two transferrings, each time after two days, and when sown on bouillon-agar, many white slime colonies together with the unchanged red, moreover a considerable number of quite normal, not slimy red colonies, N°. 1, which is to be considered as atavism, but an atavism reposing on the loss of a character. The white slime variant, thus obtained by minus-variation, and found in the table as N°. 7, seems identic with the one produced by plus-variation from the not slimy white variant, which latter for that reason has not been specially mentioned.

Already in my earlier paper I spoke of rose variants, which so to say, keep the middle between the normal form and the white variant. They may be produced in various ways, for instance, by cultivating the normal form on plates of pure gelatin dissolved in distilled water (H_2O, 10% of gelatin) at room temperature, at which rapid growth and vigorous melting occur. By daily streaking off on a bouillon agarplate the same colony obtained on such pure gelatin, and provided the temperature be kept between 14° and 17° C., we find, on the fifth or sixth day, the first rose variants, either or not with the white, which under these conditions appear later. Two rose variants (table N°. 2 and 3) are easily distinguished, but it is possible that there are many more whose perception is beyond the reach of our observation. In any case, it is a fact that the character: "the faculty of producing pigment", is divisible in many ways. The hereditary constancy of at least one of these rose variants proved not to differ from that of the normal form.

Another method to obtain rose variants is cultivation of the normal form in bouillon, which by evaporation has been reduced to a threefold concentration. After a single transport already, a large number of rose variants (3) had appeared by the side of normal forms; by a much lighter colour they showed a disposition to lose their colour entirely. The variability of the different rose variants is not the same; the form, obtained by the concentration

experiment (3) produces, more readily than the rose variant (2), as well red normal forms (1) as white ones (4). For the rest, this more variable variant has also proved to remain constant when quickly transplanted.

Cases of atavism are frequently observed in these experiments. Thus, for example, the production of the normal form from *viscosus* (6) may easily be seen if the latter grows for a fortnight without transport on a bouillonagarplate; along the margin of the streaks some few normal colonies (1) will then become perceptible.

The *albus*-variants, also have a disposition to throw off a few red normal forms, but they do so only after growing for weeks or months on bouillon-agar; at first they are very constant.

The to a certain extent completely regular production of the same variants of *Bacillus prodigiosus*, suggests the existence of variability in a special and determined direction, of orthogenesis, as EIMER expressed it.

As under different nutritive conditions the same variant may appear, the food itself cannot be the stimulus; there must be, as said above, another cause in the interior of the cells, which, for *B. prodigiosus*, seems only active in an alkaline environment.

On the other hand, the food, in a wider sense, has certainly a decisive influence on the variability, albeit indirectly. So we considered already the influence of the alkaline reaction of the medium if this alkali is produced by the microbes themselves. Another example is the following. As well in malt-wort as in bouillon the *viscosus* variant is regularly produced; but from malt-wort the *auratus* variant, which so readily takes rise in bouillon, is not obtained at all. Indeed, every culture condition gives a peculiar but constantly returning mixture of variants, differing both quantitatively and qualitatively from that found under any other conditions. But the real factors here active could not as yet be detected.

From the foregoing the following results may be derived.

1. *Bacillus prodigiosus* produces as well qualitative, as gain- and loss-variants, all obtained with certainty by determined experiments; the stock-form is always found unchanged in the same culture with the variants.

All the variants are from their origin as constant as their stock.

The true factors which govern the variability in these experiments are still unknown.

2. By rapidly repeated re-inoculations and by other methods, nor-

mal form and variants may be kept constant, as it seems for an unlimited length of time.

3. All the variants vary in a way analogous to that of the normal form, thus, the *auratus*-variant produces an *auratus*-slimevariant, which must be considered as a gain-variant, and an *albus*-variant, which must be taken for a loss-variant.

The natural variety *B. Kieliensis*, which approaches the *auratus*-variant, also varies in an analogous way. The variation thus seems to be directed or orthogenetic.

4. Gain-atavism in loss-variants and loss-atavism in gain-variants, can be obtained with certainty by determined experiments. Qualitative variants, too, may give rise to atavism.

5. The experimental variants of *B. prodigiosus* have not yet been found in nature. From another bacterium, *Bacillus herbicola*, a variant, took rise which I had before repeatedly isolated from nature and which I had taken for quite another species.

6. The variants of *prodigiosus*, and this holds good for many other microbes also, differ from each other and from their stock forms in the same way as closely related natural species or varieties do among each other. But their disposition to atavism is much more pronounced.

7. The sub-variants, e. g. the rose variants of different colour-intensity, arise in the same way as the chief variants and possess the same degree of constancy.

Physics. — "*Researches on magnetization at very low temperatures.*" By PIERRE WEISS and H. KAMERLINGH ONNES. Communication N°. 114 from the Physical Laboratory at Leiden.

§ 1. *Object of the research; results.*

a. Introduction. The extension of LANGEVIN's [1]) kinetic theory of magnetism to all ferromagnetic phenomena by means of the hypothesis of the molecular field [2]) rendered the testing of deductions from this hypothesis by experimental data of great importance. The first results of this comparison were very encouraging; in some respects a remarkable correspondence was found. For instance the curves

[1]) LANGEVIN. Ann. Chim. et Phys. 8 Sér. t. 5, p. 70; 1905.

[2]) P. WEISS. Journ. de Physique 4e Sér. t. VI, p. 661; 1907.