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in a system of bright and dark lines, as soon as the brightness in the neighbourhood of the lines is not perfectly symmetrical with respect to their centres.

At any rate we may conclude from what preceeds that we cannot be too critical while observing maxima and minima of brightness, and that in many cases we shall even have to convince ourselves of the existence or non-existence of real maxima and minima of brightness corresponding to the observed maxima and minima.

Bacteriology. — *On the relation of the obligatous anaërobics to free oxygen.* By Prof. M. W. Beijerinck.

The relation of the living cell to free oxygen is best to be judged from the influence of this gas on the *growth* and on the *mobility*. Of course, only the first method is of universal application.

As to the mobile microbes, some time ago I gave the name of „figures of respiration" [1]) to the peculiar groupings, which originate in preparations destined for the microscope, in consequence of the access of oxygen only along the edge of the examined drop under the cover-glass, the microbes being thereby enabled to seek that quantity of oxygen which is best adapted to their respiration. Three types may here be distinguished according as the microbes seek the highest tension of the oxygen along the edge, a middle tension at some distance of it, or the smallest tension in the centre of the preparation. These types I called the aërobic, the spirillous and the anaërobic type.

Further experience has shown that the anaërobic type, characterised by the accumulation of the moving microbes at that spot of the preparation where the oxygen tension is minimum, — commonly near the centre, — does not exist as a special type, but becomes visible only under particular circumstances, and further, that when the aëration of the preparation is sufficiently small, all anaërobics, examined till now, appear to belong to the spirillous type, that is to say, they not only don't fly those places in the preparation, where a small oxygen tension still exists, but they even seek them.

This tension, beneficient for the anaërobics, is however very slight, whence follows, that by using only a moderate number of microbes, consuming but very little oxygen, there may enter at the edge more oxygen than is wanted. In such a case the tension, most approaching

[1]) Centralblatt für Bacteriologie Bd. 14 pag. 837, 1893.

the required optimum, will be found in the centre. The accumulation of the microbes will then also be localised to the centre, causing the semblance of an anaërobic type as a special case. It is clear, that if this is the right explication, the true representatives of the second type, viz. the spirilli, must under certain conditions also accumulate at the centre, namely then, when all the spirilli together cannot absorb the total quantity of oxygen entering along the edge of the preparation, and this is indeed easily to be observed, by using a small number of spirilli and a large coverglass.

So, there is no sufficient reason to divide the mobile bacteria into three types according to their relation to free oxygen, as I formerly did, but only into two. It also seems to me that the names for the types, already mentioned, are not quite applicable, and that it is preferable to call *aërophilous* all organisms which seek the highest oxygen tension [1]), and *microaërophilous* those which require a lower tension. To this latter group then, belong the obligatous anaërobics as far as now observed, and the aërobic spirilli with regard to their mobility.

I am obliged here to speak of „aërobic spirilli", as I have formerly shown that there also exists an obligatous anaërobic spiril, namely the organism of the reduction of sulphates, *Spirillum desulfuricans.* Though this kind is very mobile, yet the growth is so slow, that I have not succeeded in collecting a sufficient number of individuals to get distinct figures of respiration, — a difficulty which exists also more or less with other obligatous anaërobics.

The conviction that free oxygen is beneficial to all that lives, and in the long run probably even necessary, is based on the relation of the *growth* of the obligatous anaërobics to this gas, and here the mobile forms as well as those which are not mobile may enter into consideration. Before however describing the experiments which seem decisive, I must fix the attention on the following circumstance.

For alcohol yeast and the other facultative anaërobics, it must be admitted, that the possibility of their temporary anaërobiosis, is determined by the presence of a *provision of oxygen* in loose combination with the living matter of the cell itself, by which combination some cell divisions are rendered possible without the supply of new oxygen. Consequently there must be a difference between aërated and not aërated cells.

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[1]) Here is only question of experiments in common air, not in pure oxygen.

If it is accepted that this relation holds also good for the obligatous anaërobics, then it is to be expected that their provision of oxygen will be much smaller than that in the yeastcell, so that it is necessary to take much more efficacious measures to render the influence of the oxygen visible in the former than in the latter case. It is therefore desirable, in some cases even necessary, to use for the experiments materials, taken from such cultures as have long been continued in absence of air, by which the provision of oxygen has been lessened. So far as I am now able to judge, strongly aërated anaërobics are, as to their growth, aërophobic, i. e. they grow best there where the oxygen tension is minimum or zero. As contact with air is in itself not sufficient to cause aëration, — spores for instance seem less fit to be aërated than vegetative cells, — there now and then occur strange incidents which make the experiments troublesome.

The way in which I arranged my growth experiments is as follows.

Material of the species to be examined, is introduced, in a not aërated condition, and if possible, in the state of spores, into the culture mass still in fusion, in such a quantity, that the germs, developped into colonies, may render that culture mass, after solidification, rather opaque.

If such a culture mass, from which the free oxygen is completely withdrawn, is contained in a deep experiment-tube, where the air can only find access from above, then, if the growth is favoured by a certain oxygen tension, there must result at the very place where this tension becomes optimum, an opaque and distinctly visible *niveau* of colonies, which are greater than the colonies beneath and above this niveau.

The easiest way for completely removing the oxygen, is to sow simultaneously an aërophilous species, not acting injuriously on the development nor disturbing the observation of the anaërobic. Such an aërobic must have the following qualities: The oxygen must be completely absorbed, without exciting so much growth in the surface of the culture mass, that the colonies of the anaërobic become indistinct. Besides, an easy recognition in the microscopic preparation, and a simple separation of the aërobic and the anaërobic must be possible.

In trying various species of microbes, I found some kinds of yeast to be most efficient for the research of the anaërobics of putrefaction and of sulphate reduction, as for these processes no carbohydrates are essential, in which case yeast does not grow strongly, whilst it is distinctly recognisable under the microscope. Besides, yeast can easily be separated from the anaërobics of proteine putrefaction, because it dies at 50° à 60° C., whilst the spores of the latter can be heated to 90° à 100° C. without dying. For the examination of those anaërobics which require sugar in their food, as for instance the butyric ferments, it is preferable, for oxygen absorption, to make use of certain blastomycetes (which grow and reproduce like yeast, whilst alkohol fermentation is absent) or aërobic bacteria, which don't produce acid, nor liquefy gelatine. Good results were obtained with a red blastomycete, isolated from garden-soil, and with *Bacillus fluorescens non liquefaciens.*

It is good (but not always necessary), to place the prepared experiment-tubes, in an exsiccator which is vacuated. For this vacuation a KÖRTING-waterjetpump with manometer will suffice, by which at the same time the pression of the gas used may be measured.

Another very suitable method to state the influence of oxygen on the growth, is to cultivate in the „humid room" on the object-bearer under the cover-glass, in a not too small quantity of the nutritious liquid, but in such a way as to keep the preparation thin enough for the microscope. In this way it is possible to observe, in the same preparation, first the figure of respiration and afterwards the growth.

The species of obligatous anaërobics which I have examined are the following.

Butyricferment (Granulobacter saccharobutyricum). This anaërobic is extremely common in garden-soil. Fit material for figures of respiration is to be obtained as follows. Water with some kalium phosphate and magnesium sulphate and 5 or 10 pCt. glucose is boiled in a little flask with so much fibrine that a thick paste is formed. During the boiling an infection with garden-soil is practised, in which only spores of bacteria remain alive. In the thermostate a vegetation of aërobics develops first, which, by the absorption of the oxygen, introduces butyric fermentation. Sometimes this fermentation will follow, even in absence of aërobics, i. e. notwithstanding the entrance of air into the mass of fibrine, thus showing that some aëration is certainly no bar to this process. If perhaps an aërobic grows too strongly, reinfection in another flask with the same mixture, will suffice to make it disappear and at length still to obtain an almost pure butyric fermentation. If in the infection material there are too few spores, as well of the butyric ferment as of aërobics, some aërobic bactery, or a blastomycete must be purposely added for the absorption of the oxygen.

In this way a culture is obtained containing only the „oxygenform" of butyric ferment, i. e. only mobile staves and no clostridia. With them a figure of respiration may be produced consisting of a single fine line of quickly moving little staves, situated at some distance from the edge of the cover-glass and the meniscus of the preparation, which places the microaërophily of this ferment beyond all doubt.

If to the fermenting mass pure calcium carbonate is added, by which the acid is neutralised, the growth of the bacteria becomes much stronger, and the staves give place to clostridia rich in granulose, and which at length produce spores. Although the opaqueness caused by the chalk, spoils in some degree the purity of the figures of respiration, yet the experiment succeeds well enough, and leads to the same result, i. e. proves that the clostridia of the butyric ferment are microaërophilous in the same way as the oxygenform. With boiled milk, [1]) in which a spontaneous butyric fermentation had originated, the above observations could equally be made. That the same may be said with regard to the *butylic ferment* (*Granulobacter butylicum*) formerly described by me [2]), I need hardly add here, as it was the continued study of this very ferment, which rendered the here described relations clearer to me.

Anaërobics of putrefaction of proteids. The most striking instances of obligatous anaerobics are met with in the putrefaction of pepton, or, generally speaking, of proteine substances. If one wishes to isolate the microbes concerned, efficacious measures must be taken for the exclusion of oxygen, as the quantity of air which these ferments admit, without their growth being impaired, is certainly much smaller than with the butyric and butylic ferments. Hence here in particular it was of importance to study their relation to oxygen.

Before entering upon my immediate subject, I think it necessary to say something about the different species concerned in the process of putrefaction, the literature on this subject being quite unsatisfactory.

Bacillus putrificus coli BIENSTOCK [3]) is an anaerobic, found back by nobody, so that it cannot be typical for the putrefaction of proteids. Besides, an exact microscopic examination shows that more

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1) Milk, boiled in a not too undeep flask, will sometimes get into butyric fermentation, even with free entrance of air, without the presence of aerobics.

2) Archives Néerlandaises T. 29, pg. 1. As this ferment produces much more propylicalkohol than butylicalkohol it would have been better to call it *Granulobacter propylicum.*

3) Zeitschrift fur klinische Medicin, Bd. 8. pag. 1. 1884.

than one species must here be active. That however, the number of typical bacteria should be very great, I think doubtful, for the following reasons: The course of the process of putrefaction is quite the same when the material, after infection with soil, is for some moments heated to 90 à 100° C. as when this is not done. Hence it follows that only spore-forming microbes are typical for the process. The experiment shows further that exclusion of air acts favorably on its course, so that all aerobic microbes appear to be indifferent, except in so far as by absorption of oxygen, they favor the development of the properly so-called putrefaction bacteria.

By these two data the process was so much simplified from a bacteriological point of view, that there appeared some chance of further unravelling it. Though hitherto I have by no means entirely succeeded, I think, nevertheless, that what follows holds good.

Three species of obligatous anaerobics are in particular concerned with the putrefaction of proteids. In the first place *Bacillus septicus*, secondly a group of extremely variable forms, related to the tetanus-bacillus, and to which I will give the name of „skatol-bacteria", and thirdly, an immobile, well-characterised species, called by me *B. pseudopulcher*. For separating these different species, I used a culture gelatine of the following composition: Destilled water, 10 pCt. gelatine, 3 pCt. pepton siccum, 0,05 pCt. dinatrium phosphate, 0,05 pCt. magnesium sulphate, using at the same time yeast or a blastomycete for withdrawing the oxygen. When put into a deep experiment-tube, the anaerobics develop even with free entrance of air.

B. septicus PASTEUR, is, according to my experience, one of the most spread species of bacteria, to be found wherever animal substances are tainting, and very common in dust and in the soil. It is an easily recognisable and well defined species. A virulent form of it goes in the German literature by the name of *B. oedematis maligni* [1]). Material of the latter, occurring in the laboratoria, I compared, also with a view to their relation to oxygen, with cultures of *B. septicus*, repeatedly isolated by me from putrefying albumen solutions, or fibrine, infected with garden-soil, but I could discover no difference.

The skatol-bacteria are to be known by the globular spores which are found in proteine putrefactions, in the swollen ends of thin, commonly long staves. One of the forms isolated retained

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[1]) A bactery, accepted by the medical men as a particular species, *B Chauveaui* (of the French) or *B. emphysematos* (of the Germans) is, in my opinion, only a variety of it.

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at first, even in the pure cultures, globular spores, whilst in other isolations the spore-form proved not to be constant. The dimensions of spores and staves are most variable. Motion, if present, is slow, in pure cultures sometimes absent. Glucose, added to the above mixture, gives rise to the production of gas. The colonies cause the culture gelatine more to weaken than to liquefy; they are sometimes colorless, commonly, however, surrounded with a brownish aureole. The study of this species is difficult on account of the great variability in form and functions, which renders the experiments doubtful and often suggests infection with allied forms, to which their common occurrence gives particular cause.

While skatolbacteria never fail in putrefying substances, *B. septicus* may be absent and its place be taken by *B. pseudopulcher*. This name was chosen on account of its resemblance to a common earth-bactery, related to *B. megatherium*, and which I baptised *B. pulcher* [1]). Motion is never observed in *pseudopulcher*; the spores are oblong, larger than in *B. septicus*, frequently to be found in long rows within the threads, generally, however, they occur in short staves. The colonies, which liquefy strongly, have a smooth surface, by which they may be easily distinguished from *B. septicus*. They are characterised by a heavy sediment, consisting of staves and spores. This sediment is different, or wanting in *B. septicus*. The pure cultures develop gases but not many stinking products. There is often a distinct smell of cheese to be observed. The study of this bactery is still imperfect and I mention it only because it might be taken for *B. septicus*.

For the object I have in view, I studied in particular *B. septicus*, while I think, that there is not one indication in bacteriological literature which suggests any benificient effect of free oxygen on the functions of this bacillus. For the skatolbacteria on the other hand, such indications exist. It seems at least according to some authors, that certain varieties of the nearly allied and commonly obligatous anaërobic tetanus-bacillus, are suscept to change into aërobics, a transformation which I witnessed myself by other varieties isolated from putrefying albumen. Moreover *B. septicus* is a „bona species", i. e. recognisable for everybody.

B. septicus is exceedingly mobile and generally consists of staves, covered all over with ciliae. Spores grow easily, especially when there is contact with air. They are more oblong than globular,

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[1]) At present in trade by the name of „alinit."

mostly enclosed in the somewhat swollen ends of the staves, and surrounded by a hollow space. Though this bacillus is evidently polarically constructed, it moves spore-end or tail-end forward, and may suddenly change the direction of the movement. When a little air accedes, the staves may grow out into long threads and the motion ceases.

With a total withdrawal of oxygen there is a disposition for the formation of clostridia, but without a marked difference between an „oxygenform" and a „clostridiumform" as found in *Granulobacter butylicum.*

If the nutrient matter is merely albumen or pepton, gases are produced, whose quantity increases more or less by the addition of glucose.

Fibrine and proteids produce volatile sulphides, sometimes in great profusion; production of merkaptan, too, is observed under unknown circumstances. The colonies liquefy the gelatine of the above composition; their surface is quite characteristically pointed, evidently because many small shoots pierce slightly into the gelatine, before the melting sets in. This may be compared with the behaviour of anthrax, where, however, there is no melting at all. Commonly whether spores or vegetative cells are sown out, only few germs develop, which proves, that the nutrient matter itself, — even the best I could procure, — acts in a high degree as a „bactericid" in relation to *B. septicus.* The growth is slow, even at brooding temperature, when compared to allied aërobics.

Concerning the necessity of oxygen for *B. septicus* and the skatol-bactery, I could state what follows.

B. septicus I observed as well with regard to the figures of respiration, as to the growth. In both ways the microaërophily could with certainty be stated. As this bacillus is extremely mobile, and as the spores render the swarms of bacteria very opaque, the study of the figures of respiration offers no difficulty.

A small number of bacteria accumulating in the centre of the preparation, produce the impression of aërophoby. If on the contrary, the bacteria are very numerous, a circular accumulation is formed at some distance from the edge of the cover-glass and the meniskus, pointing out the place where the tension of oxygen is optimum. If we examine the inner field, i. e. the part surrounded by the accumulation and totally deprived of oxygen, there, too, all is in motion. This motion is however much slower, more staggering and uncertain, than in the accumulation itself. I think that this inner part is continually supplied with individuals from the accumulation, which individuals, after some time return to the latter, to take in a new provision of oxygen. Outside the accumulation, near the edge of the cover-glass, where the pression of oxygen increases, the number of bacteria diminishes quickly, together with the mobility of the remaining ones. At the edge itself all is in complete rest, and no motion sets in when the surrounding is freed from oxygen. Still I have no reason to consider the resting individuals as dead; I even think they function as an „oxygen filtrum", thus protecting the more inwardly swarming.

If some grains of fibrine are introduced into preparations of which the figures of respiration are being studied, and if placed at c. a. 25° C. in a „humid room", a considerable increase of bacteria may readily occur. Watching the process micros- and macroscopically, we find the growth almost exclusively limited to the accumulation parallel to the edge, which accumulation grows more and more dense by the increase of the spores, whilst the central part continues as clear as at first. Consequently, it may be taken for granted that *B. septicus* requires oxygen for its growth, as well as for its mobility.

On this occasion I wish to correct a mistake in my description of *Spirillum desulfuricans.* I there stated erroneously [1]) that *Spirillum tenue,* which is typical for microaërophily as to mobility, is also microaërophilous with regard to growth, so that, when sown in a fit culture mass, it shows its maximum growth, not at the surface, but at a certain distance below it.

This has proved to repose on „trophotropy", signifying that growth is more favored by the influence of the food than by the oxygen. It occurs only when a bad culture ground is taken for the experiment, and it is by the aërophily of the growing spirilli that it must be explained. For the intense growth will cause at the surface a rapid exhaustion, so that, if no abundance of food is present, and if the food can only come up slowly from the depth by the process of diffusion to the place of consumption, then, not the surface itself, but a deeper layer will, under the joined action of oxygen and food, be most favorably situated for growth and increase. Thus in fact, *Spirillum tenue* is aërophilous as to growth and microaërophilous as to mobility.

Beside to this peculiar form of „trophotropy" in the growth, one has to pay attention, when studying the figures of respiration, to a phenomenon of almost the same nature with respect to the mobility, and which may be called „trophotaxis". It consists in the accumu-

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[1]) Archives Néerlandaises, T. 29, pag. 272.

lation of the mobile microbes, which are more attracted by the food than by the oxygen, not in the meniscus and at the edge of the preparation, but at some distance. I observed this in an aërobic species, which I have called *Bacillus perlibratus*, where trophotaxis may become so strong, that microaërophily is mimicked, and was erroneously described by me as such [1]).

With abundant food, however, nothing is to be seen of these phenomena, so that by attentive observation microaërophily may always distinctly be recognised.

I now return to the anaërobes of the putrefaction of proteids, and in particular to the second important form, the skatolbactery. Of this polymorphous form I examined, as already said, material closely allied to the tetanus-bacillus, which material is strictly anaërobic, and perhaps ought to be considered as the most characteristic for the process of putrefaction in general. I isolated various varieties and by means of growth experiments I was enabled to state microaërophily. The mobility of my varieties was too insignificant to be of use for the production of figures of respiration. When using the above mentioned pepton gelatine as nutrient matter and *Saccharomyces apiculatus* for the absorption of oxygen, most convincing „niveaus", of a light brown color and with much growth, originate in deep experiment-tubes at a certain distance from the surface, in the surface itself the transparent clear colonies of the apiculatus-yeast develop vigorously, the skatolbacteria not being able to develop there.

The spore-formation seems here also to be favored by a small quantity of oxygen. Certain it is that spores are the most profusely formed in the niveau, and as their production goes parallel, first to the weakening and then to the complete liquefying of the gelatine, it is clear that also the latter process must begin in the niveau, to become only much later perceptible in the depth, and without reaching the surface at all.

I wish to terminate this survey of the obligatous anaërobics, studied by me, with the statement that the existence of microaërophily could also be proved for *Spirillum desulfuricans* by means of growth experiments.

This species is, in opposition to *S. tenue*, strictly anaërobic and belongs morphologically to quite another group than the butyricferments and the bacteria of putrefaction, which is clearly demon-

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[1]) Centralblatt für Bacteriologie, Bd. 14, pag. 839, 1893.

strated as well by the vibrio- or spiril-form, as by the absence of spores [1]).

If sown in pepton gelatine, with MOHR's salt and an aërobic bactery (*B. termo*) for the absorption of oxygen, in deep experiment-tubes, the microaërophily becomes visible by a black „niveau" of ferrosulfid, first formed at some distance beneath the surface and thence, only slowly, growing towards the depth and upward. When microscopically examined this niveau proves to be richest in reducing spirilli, so that evidently not the function of the sulphate reduction as such, but the growth of the *Spirillum*, active in this process, is furthered by a low oxygen tension.

It is fit here to make a few remarks concerning the relation of facultative anaërobics to free oxygen. By far the greater part of facultatives is aërophilous. I mention for instance *Mucor racemosus*, all alcohol yeasts, *Bacterium coli commune*, *B. lactis aërogenes*, *Granulobacter polymyxa*, *Bacillus tuberculosis*, *B. prodigiosus*. If the production of figures of respiration is possible, then the width of the moving bacteria zone is very great, even in dense swarms, which indicates a slow consumption of oxygen. This is especially the case with the fermenting species, as *coli* and *aërogenes*, and sometimes, too, with not fermenting species, such as *Bacillus tuberculosis* [2]).

Microaërophilous are among the facultatives, so far as I think I can assert now, only the true lactic ferments, which may be brought to two groups of which the most important representatives are: *Bacterium lactis* (of buttermilk), and *Bacillus longus* (of cheese and of the yeast industry).

As these forms have no motion and produce little living matter in growing, the total quantity of absorbed oxygen is very small, whence the experiments are difficult and subject to doubt. If sown, however, in a proper solid culture mass, to which calcium carbonate is added, in a deep experiment-tube, it may be observed that, under favorable circumstances, at a certain depth below the surface, the formation of acid is the most vigorous. This is caused by the existence of a niveau, very rich in bacteria if compared with deeper layers where less, as well as with those nearer to the surface, where more oxygen is present. After some time, however, so many colonies originate, as well at the surface as in the depth,

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1) As I think the only well-described instance of a spore-free obligatous anaërobic.

2) The mobility of *Bacillus tuberculosis* has first been seen by Mr. MAC GILLAVRY. The figures of respiration are troublesome to obtain and only with quite young cultures, as for instance of flesh bouillon agar, not older than 24 hours.

that the microaërophily grows indistinct, without changing into aërophily.

Recapitulating, and adding some instances not yet mentioned, I come to this conclusion :

Aërophilous are: All aërobic bacteria (except some spirilli), most facultative anaërobics, probably all cells of higher animals and plants, most infusoria.

Microaërophilous are: The few hitherto examined obligatous anaërobics, to which belong also the chromatia and other sulphur-bacteria, and *Spirillum desulfuricans.* Of the facultatives probably all lactic ferments, besides some (perhaps many) species of monads, and some infusoria.

Aërophilous with regard to growth, microaërophilous with regard to motion are: Some true spirilli, perhaps also some monads.

Though nobody will be surprised that, in reason of the above observations, I believe that all living organisms known at present, require free oxygen for their existence, I am far from pretending to have furnished the entire proof for that belief. It may even be asked whether there is cause to speak of „want" of free oxygen, and if „use of it if accessible" were not more adequate.

With regard to the examined obligatous anaërobics I have only shown that an extremely small quantity of free oxygen is propitious to their growth and mobility, but not yet that in the long run they would perish without it [1]).

I must however insist on this being positively the case with the aërophilous facultative anaërobics, such as alcohol ferments, *B. coli commune*, etc. If these are prevented from laying in a „provision" of oxygen, on which to live when this gas fails, the growth soon ceases and, even with the best food, life too [2]). This fact is very singular, for the extremely small quantities here concerned, are nothing as to the development of energy.

Consequently it is not clear why the combined oxygen, which abounds in the food, cannot here fill the place of free oxygen. With

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[1]) Experiments in this latter direction have not yet given any sure results and have only proved that, with apparently efficacious precautions, anaërobiosis without access of air can long go on. So I could, without supply of air, make seven butylic fermentations go on successively, but at the seventh there arose some doubt whether the bacteria had varied or that an infection from without with butyric ferment had occurred.

[2]) For this reason I formerly proposed to call these organisms "temporary anaërobics", but now that I am more and more convinced that also the "obligates" can exist only temporarily without free oxygen, I no more attach much value to that term.

the unknown signification of the latter it is, to be sure, quite uncertain whether there must exist a minimum limit beneath which the possibility of life is totally excluded; but as this limit does certainly exist for the facultatives, one is by analogy inclined to accept its existence everywhere, consequently for the obligatous anaërobics, too. That is, for them also, to recognise free oxygen as a necessity for existence.

This opinion has the more weight now that it has been proved how easily may be shown that they not only *use* free oxygen but if possible, *seek* it and that it may promote even such important functions as growth and mobility.

Without doubt, this points to something more than „use", albeit the term „want" goes perhaps too far. As it is however a fact that the obligatous anaerobics can produce thousands of new generations without a renewed contact with free oxygen, the hypothesis demands the acceptance of a peculiar exciting action of the free oxygen, stored up as a provision in the body of the bacteria.

This action cannot be compared to that of kalium, or of magnesium, or of the other elements necessary for life in small quantities. In the first place, because the latter must be present in quantities of another order, quantities gigantic compared to that of the oxygen provision; secondly and especially, because these elements may be withdrawn from the most different chemical compounds. The very necessity of the oxygen being free, causes the difficulty of giving a definite representation of its function. Some light would go up if it could be proved, that in the food a loosely bound form of oxygen might occur, accessible to the anaerobics, and PASTEUR has indeed supposed that the oxygen, which is found in beer wort, and cannot be separated from it by pomping or boiling, makes the anaerobiosis of beer yeast possible.

Facts are however not in accordance with this hypothesis. Now, as in the case of beer yeast and the other facultative anaerobics, we are obliged to admit the existence of a store of free oxygen in the cell itself, which, in a way hitherto unexplained, makes a temporary anaërobiosis possible, analogy, supported by the observations here described, leads to the same conclusion for the obligatous anaerobics.

Physiology. — *On the influence of solutions of salts on the volume of animalcells, being at the same time a contribution to our knowledge of their structure.* By Dr. H. J. HAMBURGER.

(Will be published in the Proceedings of the next meeting).