Physiology. — "*The temperature-optimum of physiological processes.*" By Miss. J. VAN AMSTEL and Prof. G. VAN ITERSON JR. (Communicated by Prof. M. W. BEIJERINCK).

(Communicated in the meeting of October 29, 1910).

Since the appearance of our first paper on the temperature optimum of physiological processes, [1]) a communication was published by Mr. A. A. L. RUTGERS [2]) "Influence of temperature on the geotropic praesentation time of Avena sativa".

Beside the discussion of the highly interesting and accurate observations concerning the said subject, this paper gives a comparison of the results obtained by Mr. RUTGERS with those of other experimenters, and our paper, referred to above, is more specially submitted to criticism in it. Considering that the detailed record of the values on which our preliminary publication is based may still stay out some time, we thought it desirable already now to vindicate the conclusions which we have drawn from our results against the objections advanced by Mr. RUTGERS. At the same time we use this opportunity to indicate some new considerations in support of our views.

It may be called to mind that we had studied the relation between temperature and fermentation velocity, such as it appears after mixing a yeast suspension with an excess of glucose solution and after the so-called "Antrieb" is past [3]). Thereby it was found that in contradiction with what after the theory of DUCLAUX-BLACKMAN might have been expected, already before a lasting noxious influence of the temperature on the fermentation function becomes perceptible a distinct deviation from the rule of VAN 'T HOFF sets in, which is even so great that the velocity-temperature-curve changes in respect to the temperature axe from convex to concave.

For different fore-heating periods (5, 10, 15 and 20 minutes) one and the same curve for the relation between velocity and temperature was found, as might be expected à priori. At noxious temperatures this relation for the 4 mentioned times of fore-heating is also represented by 4 different curves, and by now stating at a determined time of foreheating and a determined temperature what part of the yeast

[1]) These Proceedings June 25, 1910 p. 227.

[2]) These proceedings Oct. 29, 1910 p. 476. In Mr. RUTGERS' Dissertation the same subject is treated elaborately.

[3]) For the sake of simplicity we leave out for the moment the inversion of cane sugar, which was studied by us in a corresponding way.

has lost the function, it was possible from each of these 4 curves to calculate the curve which would have occurred if the temperature had not exerted a (lasting) noxious influence. Indeed, by these 4 ways was actually obtained the same curve, showing a pronounced optimum and forming the continuation of the simple curve found for harmless temperatures for the 4 fore-heating periods. It should besides be noticed that also the velocities above the noxious temperature were first measured after the "Antrieb" was finished, which proved to be the case when about 20 cM^3 CO_2 had evolved.

We think that from these results the conclusion must be drawn that the theory of DUCLAUX-BLACKMAN should be rejected, for after this theory, when no injury of the active agent by the high temperature was occasioned, the relation between velocity and temperature would be represented by a continuously rising curve, and its growing concave beneath, as well as its falling above the optimum would be caused by the injury of the active agent during the fore-heating.

Against this conclusion now Mr. RUTGERS advances in the first place the following:

In his researches the praesentation time wanted for the perception of the gravity by coleoptiles of oat-germplants for temperatures of 0°, 5°, 10°, 20°, and 25° C. proved to be independent of the time of fore-heating (the successive fore-heating periods differed at least 1 hour from each other). At 30° C., however, the praesentation time proved to *decrease* with the time of fore-heating; after about 12 hours, the shortest praesentation time was attained which, after 24 hour's fore-heating was still maintained. On the other hand, above 30° the praesentation time *increased* with continuation of the fore-heating: at 35° C. it attained a maximum after 18 hours, which it had still preserved after 24 hours; at 37° and 38° C. however, it increased continuously, as long as the fore-heating was continued to 24 hours).

We should bear in mind that the praesentation time cannot be compared with the velocity of a process, but to a certain extent to the reciprocal value of a velocity. So the curve "praesentation time-temperature" shows a "temperature minimum" (with a slight difference only for the different periods) of fore-heating instead of a "temperature optimum".

From the fact that at 30° C. at first a greater value for the praesentation time is found, which decreases with continuation of foreheating, RUTGERS concludes that the higher temperature only reaches its full favorable influence after a prolonged remaining

at that temperature, and on the ground of the literature thereabout he thinks this must be considered as a common phenomenon.

That at temperatures above 30° with continued fore-heating an *increase* of the praesentation time is obtained, should now, according to RUTGERS, be ascribed to the fact that the favourable action of the continued fore-heating is quite annulled by the noxious influence arising at the same time. Such a noxious action might, according to RUTGERS, in his experiments already be active at 30° C., but there the favouring by continued fore-heating would be greater than the injury. RUTGERS thinks this must be concluded from the fact that the curve, found by him by extrapolation for a time 0 of fore-heating, at 30°, indicates a lower value of the praesentation time than that practically found after 1 hour's fore-heating.

If now it is right that also at higher temperature than 30° a favouring influence by continuation of the fore-heating may be admitted, the values found for the praesentation times after 1 hour, 2 hours, etc. of fore-heating, would, without that favourable action, be higher than they are now.

After RUTGERS it is on this account not allowed to draw conclusions after different times of fore-heating about the praesentation time or the velocity of a physiological reaction (indifferently whether this is done by calculation as in our method or by extrapolation as in that followed by BLACKMAN and others), if no account is kept with the favourable influence of continued fore-heating. He thinks that if this is done, BLACKMAN's theory for the explanation of the temperature-optimum proves to be right and that for the relation between temperature and praesentation time, resp. the velocity of the reaction at a fore-heating time of 0, a curve without minimum resp. optimum will be found.

It may be remarked here at once that RUTGERS has not confirmed by observations this modified theory of BLACKMAN. For this a special study of the noxious influence by continued fore-heating would have been required and sufficient; such a study, however, has not been made and hence, the testing of his theory with his own results is impossible.

Mr. RUTGERS has thus only pointed to the "possibility" that this modified theory of BLACKMAN might give an explanation of the phenomena.

We now wish to prove that this modified theory is not confirmed by *our* observations, without the support of new accessory hypotheses.

To this end we state that, according to RUTGERS, the considerable deviations from the rule of VAN 'T HOFF, too, may be explained by

the fact that hereby likewise favourable and injurious influences of continued fore-heating would appear side by side.

Could the favourable influence be eliminated we should, in accordance with this view, by our observations, already below 45° have found four different curves for the four different periods of fore-heating, and the zero-curve calculated herefrom or found by extrapolation, would have answered VAN 'T HOFF's rule. That we now only found one single curve might be explained by the circumstance that here the favouring influence annuls the noxious. Now it is in itself already an adventurous supposition, that at the 4 different periods of fore-heating those two influences always annul each other, but its possibility is not excluded. However, the fact should be emphatically pointed out that a *lasting* noxious action on the yeast at those temperatures at which the curve is simple was not observed during the time of our experiments: when the yeast, after being heated for 20 minutes at 45° C., was cooled to 20° C., it showed the same fermentation velocity as if no heating had taken place. Yet, in order to accept BLACKMAN's theory, it should be admitted that at the fore-heating ± 35 % of the yeast had lost its activity.

Now, here again an outlet might be found by introducing a second new hypothesis. It might be admitted that the inactivation of the fermentation function had taken place "invertibly", but then it must also be admitted that for the inactivation at temperatures at which the single optimum curve must be replaced by many, an uninvertible annihilation exists together with the invertible inactivation. Moreover, then still the fact remained to explain that the 4 optimum curves for the time 0, which we calculated from the different curves observed at noxious temperatures, only taking the "lasting" noxious action, into account, fall together into one. Neither this is to be conceived without the help of a third hypothesis, for, if this holds good for the 4 zero-curves found after RUTGERS' calculation this will not in general be so with the curves calculated after our method.

These three new hypotheses should moreover not only be accepted as valid for the alcohol fermentation, but also for the inversion action of cane sugar.

Let us now consider the fundamental assumption whereon this part of RUTGERS' opposition is based. After his view a favourable influence increasing with the time of fore-heating might also in our experiments have been of some weight.

We think, however, the motives for this conception insufficient. For it reposes in the first place on a conclusion derived by this experimenter from modifications stated in the praesentation time for

30°, and fore-heating times of from 1 to 24 hours, and, further on the consideration that we were always obliged to let an "Antrieb" pass before we obtained constant values of the fermentation velocity. In the first place, now, we do not think the comparison of his experiments with ours justified: our longest fore-heating times lasted 20 minutes, of adaptation or growth phenomena there is hardly question in so short a time whereas such processes will no doubt occur in the long periods of RUTGERS' experiments[1]). We even see in this circumstance the probable explanation of the differences in the course of the curves obtained by him for the relation between praesentation time and time of fore-heating at a temperature of 30° and higher.

Moreover, even if we accepted a favourable influence, by continued fore-heating, as vigorous as was observed in this experiment of RUTGERS, that influence, during 20 minutes, i.e. our longest time of fore-heating, would not have been of much importance. For in the 20 minutes which succeed the hour of fore-heating in his experiments, the praesentation time changes only from 210" to 217", a modification falling within the limits of observation errors.

Furthermore, the rising of the fermentation velocity during the "Antrieb" must by no means be exclusively ascribed to the action of the continued fore-heating: an "Antrieb" also originates without heating. Hereby a number of factors will play a part: so, with our method of working, the glucose solution did not immediately penetrate into the cells. In fact, all kinds of experimental mistakes accumulate in this factor.

We do not, however, intend to neglect the here meant time factor; it is quite certain that first the "Antrieb" must be finished before the temperature is able to exert its full influence.

Let us therefore consider the significance of this factor with reference to the table below, which relates to special experiments performed for the study of that factor.

It is clear that this table gives but a very imperfect image of the rate of velocity during the "Antrieb". What will be the initial velocity at the beginning of that "Antrieb"; to this we shall certainly get no satisfactory answer based on these experiments[2]). It

[1]) Compare here in particular the Dissertation of Mr. RUTGERS.

[2]) Supposed that this "initial" velocity proved to have the value 0 (and to this our results do point), or that it was perfectly constant for all temperatures (for instance that of 20° C., as may be expected at another arrangement of the experiments), what, then, remains of the determination of a temperature curve for *that* initial velocity?

Fermentation velocity in cM³. CO_2, evolved per second.

cM³. CO_2.	40 grs. yeast 78% cM³. H_2O 25 cM³. gluc. sol. temp. 40°.	12 grs. yeast 32.8 cM³. H_2O 10 cM³. gluc. sol. temp. 45°.	6 grs. yeast 34.8 cM³. H_2O 10 cM³. gluc sol. temp. 45°.	16 grs. yeast 31.4 cM³. H_2O 10 cM³ gluc. solut temp. 45°.	
0—5	0.152	0.135	0.106	0.157	0.17
5—10	0.250	0.209	0 144	0.250	0.26
10—15	0.357	0.253	0.190	0.312	0.33
15—20	0.417	0.333	0.190	0.357	0.38
20—25	0.500	0.357	0 200	0.333	0 41
25—30	0.625	0.333	0.208	0 385	0.41
30—35	0 625	0 357	0.208	0.357	0.35
35—40	0.625	0.333	0.200	0 385	0.35
40—45	0.625	0 312	0.208	0.417	0 38
45—50	0.625	0 333	0.200	0.417	0 41
50—55		0 333	0.200	0 417	0.41
55—60		0 333	0.208	0 454	0.47
60—65				0 417	0.41

seems however, that we can get out of this difficulty by an other v

For all non-lasting-noxious temperatures the fermentation velc — and after our experience the same holds good for very diffe physiological processes [1]) — attains after a relatively short perio which an irregular rate is observed, a constant value whic maintained much longer than the first irregular course. That cons value, now, proves at a fixed regulation of the circumstances w govern the process, exclusively dependent on the temperature. H it is evident that the relation should be sought between *that* velo and the temperature and to fix for that velocity the tempera

[1]) It is a fact that also for physico chemical reactions a certain time mu allowed to pass before the process proceeds with the theoretically expected vel This is particularly obvious in photo chemical processes; not before the e the so-called "photo-chemical induction time" the quantity of converted n becomes proportional to the product of time and light intensity (compai instance NERNST, Theor. Chemie, 2 Aufl., S 603). Now, certainly nobody studying the temperature-coefficient of the photo-chemical process, will be des to trace the initial velocity existing at the beginning of that induction time.

curve; then the question rises whether with exclusion of noxious influence by high temperature, the temperature-curve would either or not show an optimum.

Such was the problem proposed in our paper and the result was that without a noxious influence still an optimum curve was found. Were it possible to heat the yeast without injury it would be evident that the constant fermentation velocity, setting in after the "Antrieb" is past, reaches an optimum for a certain temperature.

A second objection is advanced by Mr. RUTGERS against our conclusions. In our paper we called to mind that beneath (lasting) noxious temperatures considerable deviations from VAN 'T HOFF's rule appear and in our opinion the theory of BLACKMAN should already be rejected on that account.

As an explanation for our results RUTGERS now suggests the possibility that the diffusion velocity through the cell-wall may have acted in our experiments as a limiting factor in the sense of BLACKMAN's theory. To this we observe in the first place that there is much more reason to suppose this factor playing a part in the experiments of KUYPER and BLACKMAN on the respiration and carbonic acid assimilation of higher plants, wherewith these investigations kept no account.

We think, however, that for the fermentation it must be admitted that the diffusion through the wall of the yeast cells does not restrict the fermentation velocity. Supposing that sugar diffuses through the wall with a velocity only 10^{-3} times as great as the diffusion velocity in water, then a calculation, which we hope to give later, proves that more than 10^3 times as much glucose may enter through the wall as was maximally fermented in our experiments.

Moreover, if RUTGERS' supposition was right, our curve beneath the optimum would represent the relation between the quantity of the matter diffusing through the wall and the temperature, while it is known that this relation is represented by quite another curve. [1])

Certainly the possibility exists that to explain an optimum — also in absence of injurious influence by the heating — some limiting factor must be taken in consideration. But this can certainly not save the theory of BLACKMAN to explain the occurrence of that optimum. For the bent of the velocity-temperature curve is, according to that hypothesis, solely to be attributed to dying of the function at the fore-heating, consequently to the time factor.

[1]) Compare for instance WÜLLNER, Experimental Physik, Bd. I, S. 456.

Here we wish still emphatically to point out that it has never been our intention to underrate the great signification of the introduction of the conception "limiting factors" by BLACKMAN; what by this investigator has in general been observed about this point, preserves its full vigour, also when accepting our conclusions.

A third objection which Mr. RUTGERS brings forwards against our views as to the course of the temperature curve beneath the optimum, is that also at various common chemical reactions quite analogous deviations from the rule of VAN 'T HOFF have been stated. We think, however, that this well-known fact must not be advanced against our conclusion. For, admitting that for physiological processes, also at harmless temperatures, the rule of VAN 'T HOFF is of no consequence what then remains of BLACKMAN's theory?

For in this case there is not the least objection to suppose that also in absence of noxious influences the value of the temperature coefficient lowers to 0 or even becomes negative. Even some physico-chemical processes are known which proceed with a decided temperature optimum. So, the number of crystal germs produced at different temperatures in undercooled solutions, as TAMMANN's [1]) researches prove. The most remarkable results in this respect are those of C. ERNST, whereby the relation between the velocity of the catalysis of hydro-oxygen by colloidal platinum is described. For this catalytic process, which exhibits in many respects interesting analogies with physiological processes, ERNST comes to the result that beneath a certain temperature, for different times of fore-heating one single curve is found for the relation: reaction-velocity-temperature, whilst besides, with different fore-heating times, also different curves are found.

He further shows that fore-heating at higher temperatures causes a (lasting) noxious influence on the reaction velocity, which injury he also determines after the method followed by us. When now he calculates the curve: velocity-temperature for a fore-heating time 0, he also obtains a curve showing an optimum. The results obtained by this anorganic process are thus quite in accordance with those found by our experiments.

It must still be remarked that during the time taken by his experiments performed to observe the temperature optimum (with fore-heating periods of 4 and 5 hours), ERNST did not detect a favourable influence by continued fore-heating. A slight decrease of velocity, hardly perceptible after 4 days, but after 14 days somewhat visible, found

[1]) Zeitschr. f. Phys. Chem. Bd. 25, 1898, S. 441.

its explanation wholly in the change of the concentration during the reaction.

Here also Mr. RUTGERS is obliged, if he wishes to maintain the favourable influence, to admit an "invertible" noxious action, which levels that very influence.

To be sure we should by no means wish to omit mentioning that under the greatest possible reserve ERNST gives an explanation for the occurrence of the optimum curve in absence of injury, whereby this curve is again represented as the resultant of a rising and a descending curve. But the factor rising with the temperature as well as that falling with it, would according to ERNST, be of quite another nature than the factors which play a part in the theory of DUCLAUX-BLACKMAN. For the increasing of the reaction-velocity with rise of temperature must in his opinion be a consequence of the greater value of "absorption velocity" at higher temperature; the decrease should be ascribed to the reduction of the quantity of the gas absorbed by the platinum surface at such a temperature. Of the before mentioned *time factor* there is no question here whereas in DUCLAUX-BLACKMAN's theory the decrease should exclusively be ascribed to it.

Referring to the preceding we still wish expressly to state that Mr. RUTGERS is quite wrong in presuming that it would be our intention to contest BLACKMAN's view, "that for explaining the reaction-velocities on botanical territory the physico-chemical laws must be the real base". (See page 154 of his Dissertation). If one wishes for example, to compare our optimum curve, occurring when no noxious action is present, with the reaction curve for the process studied by ERNST, there is of course not the least objection. In our opinion it may be expected that specially the investigation of coagulation phenomena of proteids under the influence of temperature on one hand, and that of adsorption phenomena on the other, will give an explanation for the origin of such a curve.

Summarising we come to the following conclusions:

1. The theory of DUCLAUX-BLACKMAN to explain the occurrence of an optimum temperature is not accepted unmodified by RUTGERS.

2. That it is confirmed by the facts, when modified as proposed, has not been proved by RUTGERS.

3. Results found by us for physiological processes can only be brought into accordance with the modified theory by introducing three new hypotheses. For none of these efficacious arguments can be given.

4. For the study of the relation between reaction velocity and

temperature one has to take the (practically) constant velocity, v when the experiment is rightly performed, sets in after a short p

5. The thus studied velocity shows also in absence of (l noxious influences, an optimum for a certain temperature, wl contrary to the theory of DUCLAUX-BLACKMAN for the expla of the occurrence of a temperature optimum at physiological e ments, this theory should hence be rejected.

6. The diffusion through the wall of the yeast cells is no li factor in the processes studied by us.

7. The fact that also physico-chemical reactions deviate fro rule of VAN 'T HOFF cannot be opposed to our conclusion tl the case of the alcohol fermentation, the considerable deviatio that rule beneath noxious temperatures is in contradiction wi theory of DUCLAUX-BLACKMAN.

8. On the contrary, it speaks greatly in favour of our obser and conclusions that the catalysis of hydro-oxygen-mixtures by co platinum (which process presents also in other respects much a to physiological katabolisms) shows a temperature optimum in a of (lasting) noxious action, so on exclusion of the time facto behaves with respect to the temperature perfectly in the sam as was found by us for the alcohol fermentation and the inv of cane sugar by yeast invertase.

Delft, October 1910.

Physics. — "*Isotherms of monatomic gases and of their mixtures.* VI. *Coexisting liquid and vapour densities of c calculation of the critical density of argon.*" By C. A. CROM Comm. N°. 118[a] from the Physical Laboratory at L (Communicated by Prof. H. KAMERLINGH ONNES.)

(Communicated in the meeting of September 24, 1910).

§ 1. The experiments published a short time ago[1]) by whi vapour pressures of argon above —140° C. were determined a means of deriving the difference between the coexisting liqu vapour densities at the same temperatures as those at whi vapour pressures were measured. As was mentioned in § 2 of the paper just referred to, both the position of the argon me in the low temperature reservoir and that of the mercury me

[1]) These Proceedings May 1910. Comm. Phys. Lab. Leiden, N°. 115. Fo this paper which is frequently quoted in the text will be called Comm. 11