ON FACTORS AFFECTING THE RESPIRATION OF THE AVENA COLEOPTILE

by

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I. INTRODUCTION.

§ 1. *Problem.*

The respiration of *Avena* coleoptiles has been investigated chiefly to get information on the influence of auxin on it. Already before growth substance had been isolated, Boysen Jensen and Nielsen (1926) studied the influence the coleoptile tip exerts upon the respiration of the basal parts, but they did not find any effect. Bonner (1934) first found an increase in respiration when a preparation of rhizopin was added to the coleoptiles, but he soon demonstrated that this increase was due to impurities in his preparation, pure growth substance (auxin-b and hetero-auxin) having no effect (1937). Van Hulssen (1936) found no influence of auxin-a or auxin-b upon the respiration of coleoptile cylinders. In a recent paper Pratt (1938) found that in 3—4 days old wheat embryos respiration was increased by soaking the grains in hetero-auxin solutions of concentrations as normally occur in the plants; direct addition of hetero-auxin, however, did not increase the respiration. Went (1938) points out that in these experiments growth is inhibited by the treatment with growth substance, the respiration per embryo being about the same in all cases. By computing the respiration per mg dry weight a stimulation is suggested.

These contradictory results seem to indicate, that our knowledge of normal respiration of the coleoptile is still insufficient, so it would be of interest to investigate the factors affecting its respiration.

Another reason for such investigations arose from researches on protoplasmic movement in the epidermal cells of the *Avena* coleoptile (Bottelier 1935, Eymers and Bottelier 1937). When studying the influence of temperature upon the rate of protoplasmic

movement in old coleoptiles (about 300 hours old) a Van 't Hoff curve was found with $Q_{\frac{28°}{23°}} = 1.33$, while in young plants (72 hours old) the Q_5 was only 1.05. In intermediate stages the Q_5 has the higher value at lower temperatures, which at higher temperatures turns into the lower value. The diffusion of oxygen from the medium surrounding the coleoptile towards the protoplasm proved to be the factor limiting the rate of protoplasmic streaming in the young coleoptiles, as high values (1.33) could be obtained in those coleoptiles by forcing water saturated with oxygen past them.

Analogous relations have been found by Wassink (1934) for the respiration of *Phycomyces*, where in young parts of the mycelium the $Q_5 = 1.10$ and in older parts 1.60. From the supply of glucose to the medium could be concluded that the food supply is the limiting process in young mycelia, while in old ones respiration s. str. is limiting.

So it seemed worth while to investigate whether the respiration of *Avena* coleoptiles behaves in a similar way.

§ 2. *Method.*

The plants were grown in saw dust in a dark room, kept at a constant temperature (23°) and moisture (85% rel. hum.). The coleoptiles of the age wanted were prepared for the experiments by cutting pieces of 15 mm length (including the tip) from them, dividing those pieces by cutting them lengthwise through the vascular bundles and removing the first leaves. The halves of 10 or 20 coleoptiles were put in the vessel of a Warburg manometer, in 2.0 cm^3 in glass distilled water, or in the same amount of one of the solutions used. In the small central well in the vessel 0.2 cm^3 10% KOH were added for the absorbtion of CO_2 (when KCN was added to the coleoptiles, 2% KOH was used). 7 open manometers were used, one of them acting as a blank control for temperature and barometer alterations. During the experiments the vessels were placed in a waterbath of constant temperature. The manometers were shaken at a high rate (300 times per minute) the amplitude of the oscillation, however, being small.

To determine the most suitable number of coleoptiles in one vessel, 10, 20, 30, 40, and 50 coleoptiles per vessel were compared. The respiration rate of 10, 20 and 30 coleoptiles proved to be proportional to the number of coleoptiles used, but with 40 and 50 coleoptiles this was no longer so. In the following experiments on the influence of temperature 10 or 20 coleoptiles per vessel were used, in all other experiments every vessel contained 10 coleoptiles.

II. EXPERIMENTS.

§ 1. *The Temperature Relation.*

The respiration of 72 and 96 hours old coleoptiles was compared at temperatures between 10° and 30°. 3 vessels received the halves of 10 coleoptiles, the other 3 vessels the halves of 20 coleoptiles. In this way a possible lack of oxygen, which might occur when too many coleoptiles were put in a vessel, or when the vessels were shaken insufficiently, immediately would have been detected.

TABLE 1.

Q_5 values for the respiration of *Avena* coleoptiles [1].

Temperature interval:	15°/10°		20°/15°		25°/20°		30°/25°	
Number of coleoptiles per vessel:	10	20	10	20	10	20	10	20
A.	1.21	1.42	1.34	1.34	1.38	1.37	1.39	1.31
	1.39	1.45	1.39	1.33	1.33	1.29	1.32	1.31
	1.34	1.37	1.11	1.20	1.53	1.39	1.31	1.34
	1.41	1.46	1.36	1.40	1.22	1.21	1.26	1.28
	1.38	1.42	1.43	1.46	1.32	1.33	1.24	1.25
	—	—	1.40	1.38	—	—	1.24	1.25
Mean value:	1.35	1.42	1.34	1.35	1.36	1.32	1.29	1.29
	1.39		1.35		1.34		1.29	
B.	1.78	1.58	1.48	1.61	1.42	1.33	1.42	1.41
	1.79	1.80	1.38	1.46	1.47	1.47	1.26	1.28
	1.26	1.41	1.24	1.36	1.57	1.43	1.42	1.22
	1.79	1.79	1.55	1.67	1.51	1.43	1.33	1.35
	1.52	1.59	1.58	1.61	1.40	1.39	1.29	1.31
	1.52	1.44	—	—	—	—	—	—
	1.70	1.60	—	—	—	—	—	—
Mean value:	1.62	1.60	1.45	1.54	1.47	1.41	1.34	1.31
	1.61		1.49		1.44		1.33	

In table 1 the results of these experiments are summarized. Q_5 values for the respiration measured in vessels with 10 and with 20 coleoptiles are given separately.

A difference between the Q_5 values of young and old coleoptiles is found, in the same sense as was shown for the protoplasmic streaming. The Q_5 values for the respiration, however, are much higher than those for protoplasmic streaming.

[1] In all the tables A means: coleoptiles 72 hours old, B: coleoptiles 96 hours old. The respiration values are given in mm^3 O_2 taken up in 15 minutes.

Although the energy needed for the protoplasmic movement of course ultimately must originate from the respiratory processes, this difference in Q_5 value indicates that still other factors determine the rate of protoplasmic movement. Besides, possibly only a part of the respiratory system produces the energy needed for protoplasmic movement.

The Q_5 values in old coleoptiles are decreasing rather rapidly at higher temperatures, in young coleoptiles, however, the decrease fairly matches Van 't Hoff's formula, but it is questionable, whether this fact has any significance.

A comparison of the results with 10 and 20 coleoptiles per vessel demonstrates that the diffusion in the liquid surrounding the coleoptiles is not hampered.

If the explanation for the difference in Q_5 values between young and old coleoptiles given in the case of protoplasmic streaming would also hold good here, $Q_{\frac{25°}{20°}}$ must be raised up to about 1.6 both in 72 and in 96 hours old coleoptiles, by bringing them in an atmosphere of pure oxygen. As the differences in Q_5 values between young and old coleoptiles are more pronounced at higher temperatures, for the experiments described in the following §§ the respiration at 20° and at 25° was compared.

§ 2. *The Influence of Oxygen.*

The distilled water used as a medium for the coleoptile halves was saturated with purest commercial oxygen. The air was thoroughly removed by forcing oxygen through the vessels and their connections with the manometers for about 10 minutes.

TABLE 2.

Respiration in air and in oxygen.

	Temperature	Respiration in oxygen	Respiration in air
A.	20°	13.2	12.8
	25°	17.5	17.5
	20°	12.4	12.8
	Q_5	1.38	1.37
B.	20°	6.7	6.4
	25°	9.4	9.3
	20°	6.6	6.4
	Q_5	1.40	1.45

From table 2 must be deduced that respiration is exactly the same in oxygen and in air, both in young and in old coleoptiles, in contrast to what had been found for the protoplasmic movement.

Since the oxygen supply proves not to be the limiting process for the respiration, the amount of substrate available could be the limiting factor. Although Bonner (1937) states that fructose has no significant influence upon the respiration in his experiments, and Van Hulssen found large quantities of reducing sugars present in the *Avena* coleoptile, in analogy with the experiments by Wassink, the influence of addition of glucose was studied.

§ 3. *The Influence of Glucose.*

2 vessels received no glucose, to the other 4 two different concentrations of glucose were added.

TABLE 3.

The influence of glucose upon the respiration. t = 20°.

Time in hours after addition of glucose		Respiration in water	Respiration in 2.5% glucose	Respiration in 5% glucose
A.	1	12.5	13.2	13.5
	3	10.2	15.1	18.0
	5	8.5	13.4	13.3
B.	1	7.8	11.3	10.6
	2	7.5	11.2	11.1
	3	7.3	12.5	11.8
	4	6.8	12.2	10.9
	5½	4.3	9.3	8.5

Table 3 shows that addition of glucose is followed by a considerable increase in respiration in young coleoptiles as well as in older ones. If we assume that the diffusion of glucose towards the enzyme system is the limiting process in our case, as these experiments seem to indicate, the relatively low Q_5 values found in § 1 can be easily understood. Higher Q_5 values then could be expected, if the glucose concentration is increased. From table 4, however, must be concluded that the Q_5 values are decreased by increasing the glucose concentration. This phenomenon seems to indicate that under the experimental conditions the diffusion of glucose from the medium towards the protoplasm still is the limiting process; an increase in Q_5 value then would only be possible if the enzyme system would be entirely saturated with substrate. This cannot be obtained by a

further increase in glucose concentration, since so concentrated a glucose solution will exert a dehydrating influence upon the protoplasm. Another way to attain our purpose is to decrease the capacity of the enzyme system itself by means of narcotics.

TABLE 4.

Q_5 values for the respiration in water and in glucose. t = 20° and 25°.

Coleoptiles 72 hours old.

water	2.5% glucose	5% glucose
1.47	1.33	1.25
1.52	1.29	1.13

The fact that glucose limits the respiration rate in *Avena* coleoptiles offers a new track to investigate the problem of the variations of the auxin-"standard" test reaction. As is well known, the curvature of test plants in the W e n t-test for auxin upon application of a given auxin concentration can vary greatly in the course of a month, and even of a day (K ö g l, H a a g e n S m i t and V a n H u l s s e n, 1936). V a n H u l s s e n found a correlation between respiration rate and fluctuations of the standard, a high standard corresponding with a high respiration rate. So if glucose is limiting the rate of respiration, a relation can be expected between the sensibility of the coleoptiles for auxin and their glucose content.

K o n i n g s b e r g e r and V e r k a a i k (1938) found that the variability of the *Avena*-test disappears when the test plants are deseeded one day before they are used. They point out the possibility that the variability of normal plants is due to "variations either 1) in the transport or in the action of a food factor or 2) in the transport or in the amount of the precursor or in its conversion into auxin". Our reasoning seems to point to the first mentioned possibility.

§ 4. *Narcosis with Ethylurethane and the Combination of Urethane and Glucose.*

The effect of narcosis with ethylurethane on the respiration is shown in table 5. Contrasting with the expectation narcosis with urethane causes a marked decrease of the Q_5. The concentration of urethane is sufficient to reduce the enzyme system to such an extent, that the diffusion of glucose is no longer the limiting process for the respiration, since after narcosis with 4% urethane an addition

TABLE 5.
Narcosis with ethylurethane.

temperature		water		2% urethane		4% urethane	
		respiration	Q_5	respiration	Q_5	respiration	Q_5
A.	20°	12.6		8.2		—	
	25°	18.0	1.48	8.7	1.12	—	—
	20°	11.8		7.4		—	
	25°	15.1	1.32	10.4	1.37	—	—
	20°	10.9		7.8		—	
B.	20°	9.9		4.8		4.0	
	25°	13.5	1.47	7.6	1.49	4.5	1.13
	20°	7.3		6.1		3.8	
	25°	12.3	1.58	9.4	1.34	5.7	1.10
	20°	8.3		7.8		6.5	

TABLE 6.
Addition of glucose to coleoptiles narcotized with ethylurethane. t = 20°.

	water	4% urethane	4% urethane + 4% glucose
A.	11.8	5.5	5.3
	10.3	5.1	5.2
	8.4	2.4	2.9
B.	9.0	4.1	4.1
	8.1	5.3	4.8
	9.1	5.0	4.4
	7.5	3.8	3.6
	6.6	4.0	3.8

TABLE 7.
Q_5 values for the respiration in water, ethylurethane and ethylurethane + glucose. t = 20° and 25°.

	water	4% urethane	4% urethane + 4% glucose
A.	1.37	1.30	1.11
	1.41	1.29	1.41
B.	1.48	1.06	1.22
	1.48 }	1.36 }	1.60 }
	1.51 }	1.23 }	1.62 }
	1.52 }	1.53 }	0.93 }
	1.53 }	1.39 }	1.06 }

of glucose has no effect upon the respiration at 20° (table 6). A possible explanation for this phenomenon is, that urethane also affects the enzymes activating the substrate for respiration. It therefore seemed worth while to compare the above results obtained with a general cell narcotic such as urethane with the effect of a specific inhibitor of the haemin system, e.g. KCN.

The addition of glucose after narcosis showed a very irregular influence upon the Q_5 (table 7). As a rule the Q_5 in urethane + glucose is lower or insignificantly higher than in urethane only, but in one experiment high values are obtained after addition of glucose ($Q_5 = 1.60$ and 1.62). These facts indicate that the assumption given above (§ 3) may possibly be partly correct, but, as the high values could not be reproduced, certainly things are still more complicated. All coleoptiles grown on the day of that experiment were much thicker than normal.

§ 5. *Inhibition with KCN and the Combination of KCN and Glucose.*

Table 8 shows the results of inhibition with KCN. As with ethylurethane, the Q_5 is also decreased here. Rather concentrated KCN solutions had to be used since the effect of KCN is decreasing rapidly in lower concentrations. This is possibly due to a distillation of KCN from the solution in which the coleoptiles are suspended into the KOH in the central well of the vessel, although the concentration of the KOH had already been lowered from 10% to 2%.

TABLE 8.
Inhibition of the respiration with KCN.

temperature		water		$25.\ 10^{-5}$ mol KCN		$100.\ 10^{-5}$ mol KCN	
		respiration	Q_5	respiration	Q_5	respiration	Q_5
A.	20°	13.4		4.7		4.0	
	25°	17.1	1.44	6.9	1.13	3.5	1.09
	20°	10.5		7.6		2.5	
	25°	13.8	1.42	10.8	1.29	3.3	1.27
	20°	8.8		9.1		2.7	
B.	20°	9.2		3.7		4.4	
	25°	11.6	1.41	7.4	1.28	4.1	1.11
	20°	7.2		8.0		3.0	
	25°	9.6	1.41	12.0	1.58	3.5	1.16
	20°	6.4		7.3		3.5	

$25.\ 10^{-5}$ molar KCN already inhibits the haemin system totally, since, in the beginning of the experiments the decrease of the total

TABLE 9.

Effect of glucose upon the respiration inhibited by KCN.

temperature		water		50. 10^{-5} mol KCN		50. 10^{-5} mol KCN + 2% glucose	
		respiration	Q_5	respiration	Q_5	respiration	Q_5
A.	20°	17.1		5.5		6.1	
	25°	19.5	1.32	8.7	1.18	11.0	1.36
	20°	13.2		8.7		9.4	
	20°	14.7		6.6		6.5	
	25°	18.1	1.33	7.6	1.06	9.6	1.23
	20°	12.5		7.7		9.1	
	25°	16.2	1.40	10.0	1.20	12.2	1.17
	20°	10.7		8.8		11.7	
B.	20°	7.6		3.9		6.4	
	25°	8.4	1.31	4.5	1.07	6.6	0.94
	20°	5.1		4.4		7.5	
	20°	9.5		4.6		4.4	
	25°	12.4	1.43	3.0	0.88	5.6	1.22
	20°	8.0		2.3		4.7	
	25°	10.9	1.43	3.1	1.29	7.3	1.43
	20°	7.1		2.4		5.6	

TABLE 10.

The inhibition with KCN in air and in oxygen. In C. to the vessels containing KCN, 4% glucose was added; coleoptiles 96 hours old.

temperature		water and air		10^{-3} mol KCN, air		10^{-3} mol KCN, O_2	
		respiration	Q_5	respiration	Q_5	respiration	Q_5
A.	20°	10.3		2.2		3.2	
	25°	13.8	1.42	2.7	1.23	4.6	1.31
	20°	9.0		2.2		3.7	
	25°	11.5	1.35	2.8	1.27	5.0	1.35
	20°	7.9		2.1		3.7	
B.	20°	5.6		2.5		3.1	
	25°	7.3	1.28	2.2	0.96	3.0	1.11
	20°	5.7		2.0		2.3	
	25°	7.5	1.57	2.4	1.50	2.7	1.69
	20°	4.1		1.1		1.0	
C.	20°	7.9		4.3		5.7	
	25°	10.9	1.51	6.0	1.22	9.1	1.40
	20°	6.5		5.6		7.3	

oxygen consumption is about the same as that in a 100. 10^{-5} molar solution.

Addition of glucose to the coleoptiles, when inhibited with KCN, increases both the respiration rate and the Q_5 (table 9). The Q_5, however, when only 2% glucose is added, still remains lower than in normal respiration.

§ 6. *The Effect of Oxygen upon the Respiration during Inhibition with KCN.*

As was shown in § 2, the increase of the oxygen tension in the gas phase of the vessels has no influence upon the respiration rate. After inhibition with KCN, however, respiration in pure oxygen is markedly higher than in air (table 10, A, B). The same result is obtained when this experiment is repeated with coleoptiles, suspended in a glucose solution (table 10, C). The effect is due to the inhibition by KCN only, since in 4% glucose both the respiration rate and the Q_5 are the same in oxygen and in air (table 11).

TABLE 11.

Influence of glucose upon the respiration in oxygen and in air.

temperature		water, air		4% glucose, air		4% glucose, O_2	
		respiration	Q_5	respiration	Q_5	respiration	Q_5
A.	20°	16.9		17.3		17.0	
	25°	20.6	1.36	23.3	1.36	25.2	1.40
	20°	13.3		16.8		19.0	
	25°	17.7	1.39	21.4	1.31	25.3	1.31
	20°	12.2		15.7		19.5	
B.	20°	9.9		12.4		11.8	
	25°	12.8	1.44	16.2	1.34	16.7	1.38
	20°	7.8		11.8		12.3	
	25°	10.7	1.45	16.6	1.41	17.2	1.43
	20°	7.0		11.8		11.8	

III. DISCUSSION.

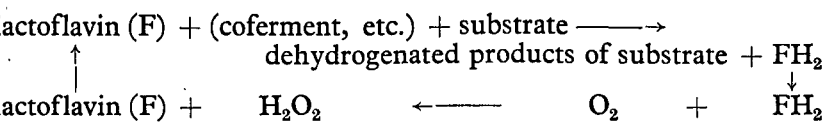
The facts mentioned in the preceding §, which at first sight seem rather contradictory, possibly find their explanation in the ideas of Theorell (1936) concerning the interaction between the haemin and the lactoflavin system in normal respiration and in respiration inhibited by KCN.

Warburg, who first supposed the lactoflavin to be oxidized

by the haemin system, in 1934 came to the opinion that in the normal cell no oxidation of the lactoflavin by the haemin system could occur, since in that case the oxidation of the lactoflavin in cells, where there spiration is inhibited by KCN, should be inhibited too. As the respiration via the lactoflavin is not affected by KCN, he concluded that the oxidation of the lactoflavin is not connected with the haemin system. Theorell points to the fact that the oxygen tension in a normally respiring cell is extremely low, but that it is increasing considerably after partial inhibition of the respiration. So he points out the possibility that at low oxygen tensions the lactoflavin system should for the greater part be oxidized by the cytochrome-c, while at higher tensions autoxidation becomes appreciable. In vitro he could obtain indeed a dehydrogenation of the lactoflavin via the cytochrome-c at low oxygen tensions, while at higher tensions an increasing part was oxidized directly.

In applying this view to the respiration of the *Avena* coleoptile one can imagine that in air the oxygen tension is sufficient to saturate the haemin system amply, but will be insufficient to saturate the lactoflavin system, which has, as appears from the experiments of Theorell, a much lower affinity to oxygen. So, when the haemin system is inhibited by KCN, the remaining flavin respiration will become sensitive to changes in the oxygen tension.

The respiratory system after inhibition with KCN in high concentration can be represented as follows:



From these formulae it is clear that with suitable concentrations of lactoflavin, glucose (substrate) and oxygen, addition of glucose as well as raising the oxygen pressure will increase the respiration. This reasoning is not in contradiction with the theory of limiting factors, as the reactions studied here are no parts of a reaction chain.

From the experiments described in the preceding §§ some facts can be derived supporting this view:

Normal respiration in 15 mm long pieces of 96 hours old coleoptiles amounts to 0.6 of the respiration of equal parts of 72 hours old ones (calculated from tables 8 and 10). After inhibition with 10^{-3} molar KCN, however, respiration is the same in 15 mm pieces of both kinds of coleoptiles ($72^h : 96^h = 1.02$, tables 8 and 10). Under our experimental conditions 72 hours old coleoptiles had a length of 14—18 mm, 96 hours old ones of about 40 mm. So

normal respiration per coleoptile in 96 hours old coleoptiles will be about 1.3 × that in 72 hours old ones, when respiration in the basal parts of the coleoptile is supposed to be equal to that in the regions near the tip. If the respiration in the latter regions, which are richer in protoplasm, is supposed to be higher, respiration per coleoptile in both cases will be about the same. After inhibition with KCN the respiration per coleoptile in 96 hours old ones will be 2.1 × the respiration in 72 hours old ones, when our first assumption is accepted, 1.6 ×, when normal respiration is supposed to be equal in both cases. So the respiration via the lactoflavin system increases strongly when the coleoptiles grow older. Now Eymers and Bottelier found that in the region of 5—10 mm from the tip the surface of the epidermal cells is increasing from 17 relative units in 72 hours old coleoptiles to 46 in 96 hours old ones; about the same values must be found for the subepidermal layers, as no cell divisions occur. As by this increase in cell surface the diffusion of oxygen towards the protoplasm is facilitated (as was demonstrated in the case of protoplasmic movement), the increase in respiration mentioned above must be a reaction of the oxygen-sensitive flavin system on an increase in oxygen tension.

To prove this assumption the influence of oxygen upon the respiration after inhibition with KCN in coleoptiles of the two ages must be compared. We then may expect that oxygen will exert the greater influence in the case of its lower initial tension in the cell, i.e. in 72 hours old coleoptiles. In agreement with this assumption we can calculate from table 10 (A, B) as the ratio between the respiration in oxygen and in air for 72 hours old coleoptiles 1.68, for 96 hours old ones 1.16. When in the latter case glucose is added to the coleoptiles in oxygen as well as in air, thus increasing the respiration and consequently the want of oxygen, the ratio is increased too and becomes 1.38 (from table 10 C).

Addition of glucose, when the respiration is inhibited with KCN, will have less influence upon young coleoptiles, where the oxygen tension is low, than upon old ones. Accordingly we find from table 9 as the ratio between the respiration in glucose and water (both inhibited with KCN) for 72 hours old coleoptiles 1.16, for 96 hours old ones 1.80.

If these ideas are correct, one must expect that a higher oxygen tension does not increase the respiration after narcosis with urethane. Our experiments on this subject are as yet not conclusive; further experimental data are wanted.

In § 4 was stated that narcosis with 4% ethylurethane is sufficient to inhibit the enzyme system to such an extent, that addition of

glucose has no more influence upon the respiration. In that case the respiration is decreased to about 50% of its normal value. When respiration is inhibited with 10^{-3} molar KCN, only 26% (72 hours old coleoptiles) or 43% (96 hours old coleoptiles) of normal respiration remains, but then addition of glucose still increases the respiration (§ 5). This phenomenon too fits in our theory: with urethane indeed the whole enzyme system is inhibited to a certain extent, but with KCN the haemin system is blocked only, and the flavin system, which is the glucose sensitive system, remains unaffected.

As in 96 hours old coleoptiles 43% of the respiration remains after inhibition with KCN, the lactoflavin must be present in relatively large quantities in the *Avena* coleoptile.

No satisfactory explanation can as yet be given for the difference in the Q_5 values of normal respiration of 72 and 96 hours old coleoptiles. Most probably this difference is caused by an internal diffusion resistance for the substrate in young cells. After cell elongation the cell volume increases while the amount of protoplasm remains almost the same; then the protoplasmic layer becomes thinner and consequently the diffusion resistance is decreased. One of the experiments described in § 4 may give an indication in this direction, viz. the one in which high Q_5 values were obtained when glucose was added to coleoptiles narcotized with urethane. The coleoptiles grown on that day were of the usual length, but much thicker than normal. As the same sample of seed was used throughout the experiments, this can only mean, that the diameter of the cells was larger than normal. This caused an increase in cell surface, while the protoplasmic layer became thinner, and consequently diffusion was easier in that case. Of course further experimental data are wanted to prove this assumption.

The fact that the Q_5 becomes higher when glucose is added after inhibition with KCN (§ 5) points in the same direction.

IV. SUMMARY.

The Q_5 for the respiration of *Avena* coleoptiles is higher than the Q_5 of their protoplasmic movement. The rate of the protoplasmic streaming therefore is not determined directly by the rate of the respiration.

The Q_5 is higher in 96 hours old coleoptiles than in 72 hours old ones.

Under the experimental conditions the diffusion of oxygen

towards the cells is not limiting the respiration, in contrast with what has been found for the protoplasmic movement.

Addition of glucose increases the respiration and decreases the Q_5.

Narcosis with ethylurethane to such an extent that glucose is no longer limiting the respiration also causes a decrease of the Q_5.

After inhibition of the respiration with KCN to the same extent as with urethane or more, addition of glucose still increases the respiration. Besides, the respiration has become sensitive to changes in the oxygen tension.

The phenomena appearing after inhibition with KCN can be explained by assuming, according to the views of Theorell, that in normal respiration the lactoflavin system is reoxidized by the cytochrome-c only, but after inhibition with KCN the oxygen tension in the cell is increased to such an extent, that the lactoflavin system can partly be oxidized directly by free oxygen.

In the *Avena* coleoptile relatively large quantities of lactoflavin must be present, since in 96 hours old coleoptiles after total inhibition with KCN 43% of the initial respiration remains.

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