*Acta Botanica Neerlandica* **11** (1962) 331–410

# RESPIRATION OF THE IRIS BULB IN RELATION TO THE TEMPERATURE AND THE GROWTH OF THE PRIMORDIA

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(*received August 21st, 1962*)

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## CHAPTER I

# INTRODUCTION

### I.1. OUTLINE OF THE INVESTIGATION

Much is already known about the development of the growing-point, which is situated in the centre of the iris bulb, into either a plant and a flower or into a new bulb. BLAAUW (1935) studied the morphological development of the so-called Dutch irises extensively. Also, Blaauw and his co-workers published a large number of articles on the influence of the temperature on development (see HARTSEMA, 1961). In fact, temperature is undoubtedly the most important environmental factor in the development of the growing-point. Its influence should not only be regarded as an accelerating or inhibiting factor in a completely fixed developmental scheme, but — and this is even more important — as a directive factor in the morphogenetic differentiation of the growing-point, i.e. in the formation of bulb scales, leaves and flowers. In newly lifted bulbs we find only the beginning of this development. Temperatures of 25° or 30° C slow up the development rate of the growing-point and then the bulbs are said to be "retarded".

Blaauw's work, which has opened up fruitful possibilities for practical use, should be seen as a very important step towards the understanding of the physiology of the biological development.

For further investigation it is important to study the metabolic activity of the iris bulb and its dependence on temperature. This field is, however, still relatively unexplored. For this reason the respiratory activity of the bulb as part of the metabolism has been chosen as a subject for study in its relation to temperature. Respiration is defined here as the carbon dioxide output and the oxygen uptake, i.e. the gas exchange of the bulb.

Besides investigating the respiratory activities it is also important to measure the growth rate of the primordia and the influence of temperature on it. This may enable us to answer the question of whether the respiratory activity is correlated with the growth and if so, to what degree.

For the time being the study of the complete bulb has been given prominence. The study of the respiration and also of the growth of the separate parts of the bulb entails new problems, which, though they are considerable, are as yet of secondary importance in our investigation. Closer examination becomes desirable when the next step on the path of biochemical research is taken. The present investigation is no more than a reconnaissance.

In the introduction (I.2.) the life history of the growing-point, i.e. the biological background, has been discussed in greater detail. In Chapter III the influence of temperature on the initial growth of the primordia and on the retardation of the growth of the primordia has been touched upon. In Chapter IV attention has been paid to the respiratory activity of the bulb after lifting, when stored at temperatures that retard the development. In Chapter V the effect of different temperatures on the respiratory activity of retarded bulbs has been investigated. This Chapter also describes some investigations of the rise in the respiratory activity as a result of wounds or the peeling of the tunicated bulbs. In addition the influence of the oxygen concentration on the respiratory activity has been investigated and the respiratory quotient has been measured under various conditions. In Chapter VI some data outlining the influence of the temperature treatment on the sugar content of the bulb are to be found. Chapter VII consists of a discussion of the results.

## I.2. THE BIOLOGICAL BACKGROUND OF THE INVESTIGATION

An iris bulb roughly consists of a small disc, around which the scales and a few sheath leaves are arranged. In the centre of the sheath leaves lies the growing-point, which at the moment the iris bulb is lifted, shows hardly any differentiation. The future development of this growing-point with three or four primordia depends on the size of the bulb and on the temperature. In small bulbs it develops into a very short stem with three leaves at the most and furthermore a new bulb is formed in the centre of the old. In bigger bulbs a tall stem with a larger number of leaves and a terminal flower develops.

The present investigation was restricted to the intermediate sizes of bulbs, the so-called arbitrary sizes, in which the nature of the development of the growing-point depends on temperature. The influence of temperature on the development of the growing-point has been studied in this laboratory. The scheme in Fig. 1 shows the possible trends of development. If a bulb after lifting is treated at a temperature higher than about 20° C, the result will be the formation of leaves, thus excluding the formation of a new bulb. A temperature less than about 17° C leads to the development of bulb scales.

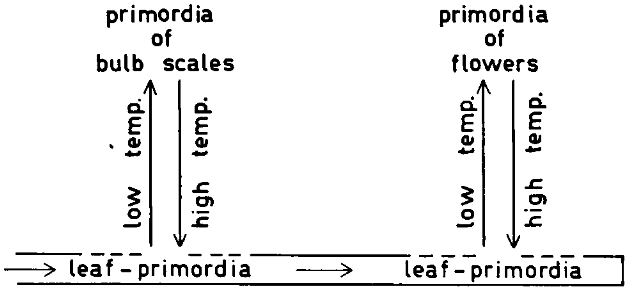

Fig. 1. Scheme of the morphological differentiation phases in the development of the central growing-point in the iris bulb.

When the first phase of the differentiation of the leaves has been completed, two possibilities are open. Either the growing-point can continue to initiate leaves, or a flower can be initiated. This, too, depends on a previous temperature treatment. Although a higher temperature will retard the rate of development it will also cause the initiation of leaves to continue. A lower temperature will induce the initiation of flowers (see Hartsema & Luyten, 1940; Blaauw, 1941).

At the present time the scheme in Fig. 1 summarizes the observations that have been made in this laboratory. However, the fact should be stressed that the figure shows the influence of the temperature on the differentiation of organs and has no reference to the rate of development. For a correct interpretation of the experiments described in this dissertation the following details ought to be mentioned.

Though a higher temperature such as 25° or 30° results in the initiation of leaves in the newly lifted bulb, the development rate of the growing-point is seriously retarded. In 'Wedgwood' bulbs, for example, the shoots found after one year at 25° and 30° were 44 and 14 mm respectively. These bulbs are said to be retarded, because in this condition the iris bulb can be stored for a year without losing its viability, and still develop into a normal flowering plant when brought to other temperatures. These retarded bulbs are pre-eminently suitable objects for research. In Chapter III. 1. the rest phase and the problem of retardation of the bulb are further discussed. The most important aspect of the present study was the investigation of the temperature treatment necessary to end the resting phase of growth and respiration.

## I.3. OLDER INVESTIGATIONS

Although there is an extensive literature on the influence of temperature on the growth and the development of the growing-point in the bulb (see: HARTSEMA, 1961) little has been published so far on the metabolic activities, especially on the respiratory activity of the bulb. We shall mention the most important publications in which reference is made to the respiration of the bulb.

Probably the first time a measurement of the respiration of bulbs was mentioned in the literature was by STICH (1891). In his article some data were published about the influence of the oxygen concentration on the respiratory quotient of narcissus bulbs. He found that the oxygen consumption rather than the carbon dioxide output dropped markedly at oxygen concentrations of less than 10 per cent. Consequently the respiratory quotient increased to more than 1. In addition Stich found that wounding of the tulip bulb and the potato tuber caused the respiratory quotient to drop below unity. He observed that when the potato was wounded it showed no increase of the output of carbon dioxide when the tuber was brought into a hydrogen atmosphere. Thus the wound respiration of the potato tuber proved to be dependent on the presence of oxygen.

SMIRNOFF (1903) published a short treatise on the respiration of *Allium* bulbs after wounding and on the "respiration" without oxygen. Wounding evidently causes the respiration to rise, to reach an optimum and then to fall. Probably what the author observed was an increase in the carbon dioxide output as a result of the removal of the bulb tunics.

A publication on the respiration and the deterioration symptoms in hyacinth bulbs at higher temperatures (DOLK & VAN SLOGTEREN, 1930) appeared for the first time at the Laboratory of Flowerbulb Research, Lisse. In order to control the yellow disease [*Xanthomonas hyacinthi* (Wakk.) Dowson] in hyacinths van Slogteren at that time devised a hot-air treatment at 38° C. This made it necessary to study the effect of a 38° treatment on the sound bulb. As far as the respiration was concerned Dolk came to the conclusion that the curve showing the relationship between the carbon dioxide output and the temperature showed an almost linear course up till 40°, with a sharp optimum at 40°, which is the approximate lethal limit. Obviously the hot air treatment at 38° came close to the lethal limit. The influence of the time-factor on this optimum was not investigated. Furthermore the experiments of Dolk & van Slogteren indicate that an oxygen concentration lower than 14 to 15 per cent limits the aerobic respiration at a temperature of 38° C. The authors came to the conclusion that bacterial development on the surface of the bulb resulting from a high air-temperature and air-humidity, may have promoted an anaerobic state in the bulb. In a single experiment the respiratory quotient at 38° was found to be about unity.

The respiration investigations were later continued and extended at the laboratory by Algera. The background of his investigations was the development of the plant and the flower, a development in which the temperature plays a determining part. Continuing the experiments begun by PINKHOF (1929), ALGERA (1936, 1947) studied respiration and carbohydrate metabolism in the tulip bulb. Algera's investigations therefore concerned themselves with the relationships between the carbohydrate content, the respiratory activity, the development of the plant and the temperature treatment of the tulip bulb. Algera paid special attention to the metabolic activities during the elongation period of the initiated organs. The lower the temperature was at the preliminary treatment of the bulb the higher were the temperatures at which an optimum development of the plant could be reached, i.e. low temperatures could speed up the development afterwards. WENT (1948) gave the period of low temperature treatment the very appropriate name of phase of preparation for elongation. HARTSEMA, LUYTEN & BLAAUW (1930) speak of an inhibited optimum. ALGERA (1936, 1947, 1950) considers the sugars in these circumstances to be the factor which limits the optimum growth and the respiration. The sugars that were formed and accumulated at a lower temperature therefore constituted a potential source of energy for a faster development later.

MANN and LEWIS (1956) published data about the respiratory activities and the temperature treatment of garlic bulbs (*Allium sativum* L.). This investigation is particularly relevant, because the problem as it occurs there seems to be very similar to that of the iris bulb. Just after lifting, when the bulbs were in a resting state, they were transferred to a range of temperatures from 0° to 25° C, with intervals of 5°. The carbon dioxide output was measured occasionally for half a year. At about 10° the greatest increase in respiration occurred. This development in activity seemed to be related to the gradual disappearance of the state of rest in the bulb. Mann & Lewis did not determine sugar contents. However, from earlier investigations by WÄCHTER (1908) we know that in onions (*Allium cepa* L.), closely related to garlic, no differences in the sugar content are reported to occur, in spite of temperature differences ranging from –7° to 19° C. At temperatures ranging from 35° to 44° C, however, the proportion of reducing sugars to invertable sugars does reportedly change in favour of the latter.

In view of EYSTER's information (1949) about the starch synthesis from sugars in leaves of onion plants it is not impossible that the starch synthesis of the onion bulb will also be inhibited.

VAN LAAN (1955) has tried to find a correlation between the enzyme activity in the bulb tissue and the preceding temperature treatment of the iris bulb. Although the heat treatment of the bulb after lifting (32° C for 10 days) seems to stimulate the dehydrogenase activity (colouring with 2.3.5. triphenyltetrazoliumchloride), it slows up the catalase activity and seems to stabilize the amylase activity, whereas a cold treatment (10° C) stimulates the dehydrogenase, the catalase and the amylase activity. These are quite isolated facts, as further knowledge of the reaction mechanisms concerning the development of the bulb is lacking.

Isenberg, Jensen & Odland (1954) reported that spraying of onion plants with an aquous solution of a suitable maleic hydrazide concentration could check the sprouting of lifted bulbs. The respiratory activity ($0_2$ absorption) and the dehydrogenase activity (reduction of 3.5. diphenyltetrazoliumchloride), even after a storage period of 23 weeks proved to be stimulated at a low concentration and to be retarded at a higher concentration of the spray. The succinic dehydrogenase particularly seemed to be inhibited at higher maleic hydrazide concentrations.

## CHAPTER II

# MATERIAL AND METHODS

### II.1. Material

Of the genus *Iris* there exist only two sections that produce genuine bulbs and no rhizomes or tubers (Dykes, 1924). They are the *Reticulata* and *Xiphium* sections. For the experiments bulbs were used that belonged to the *Xiphium* section. They were the hybrids Wedgwood and H. C. van Vliet. Today these hybrids may be termed cultivars. The origin of these cross-breeds belonging to the so-called Dutch irises cannot be entirely determined. According to Blaauw (1935) the Dutch irises are the result of crosses between *Iris Xiphium praecox, Iris tingitana,* and a cross between *Iris Xiphium* (original form) and *Iris Xiphium lusitanica,* called var. *Cajanus.* The cultivar 'Wedgwood' particularly seems to have taken the favourable early flowering quality from the *Iris tingitana.* The cultivar 'H. C. van Vliet' is a form issuing from later crossings of the crosses mentioned. It flowers at a later date than 'Wedgwood'. However, on account of their not exactly determined origin it is best to speak of *Iris* 'Wedgwood' and *Iris* H. C. van Vliet'.

After being lifted, which occurs from August 15—31, the bulbs were stored at 25° or 30° in controlled temperature rooms, which were ventilated. The air humidity in the storage rooms was very low (25 to 30 % relative humidity at 25°). From the rooms material was taken when needed. Unless otherwise stated experimental material was taken from the 25° storage rooms. Thus material of an equal quality was available throughout the year.

At the storage temperatures used, the bulb material remained in practically the same stage of development during the storage period. Except in the very beginning of the period little difference was observed in the respiratory activity between the beginning and the end of the storage period.

### II.2. Experimental arrangement and gas train

In measuring the carbon dioxide output 500 ml oblong glass containers were used. Two baskets of wire gauze containing the bulbs were made to fit in the glass container so that the bulbs could be transferred easily from the storage rooms into the respiration containers and back. For carbon dioxide output measurements 8 to 10 bulbs were used, whereas for measurement of oxygen uptake, whether combined with the carbon dioxide measurement or not, about 100 bulbs were needed, because of the lack of sensitivity of the method. In the latter case a metal respiration container with a capacity of 5350 ml was used. The turbulence of the circulating gas in the respiration container was promoted by fixing cross plates immediately after the inlet and just before the outlet. The temperature of the bulbs could be regulated by submerging the respiration container in thermostatically regulated water baths. Consequently the heat transfer from the water in the constant temperature bath to the bulb went via the glass or metal wall and an isolated layer of air between them. In view of temperature effects, which will be discussed later, it was important to find out how soon the bulb had taken on a new temperature. This is shown in Fig. 2. The temperature was measured with the aid of thermocouples. One thermocouple was placed in the water bath near the respiration container and the other in the centre of the bulb. From the observations it may be concluded that the temperature in the bulb in a respiration container of 500 ml differed only little from the temperature of the water bath in 2 hours, and that in 3 hours there was no longer any difference at all. When a 5350 ml respiration container was used this was 5 and 9 hours at a temperature change from 25° to 40° C. In this temperature change small deviations may occur because of the heat produced by the respiration of the bulbs. No greater deviation, however, was found than about 0.3° C.

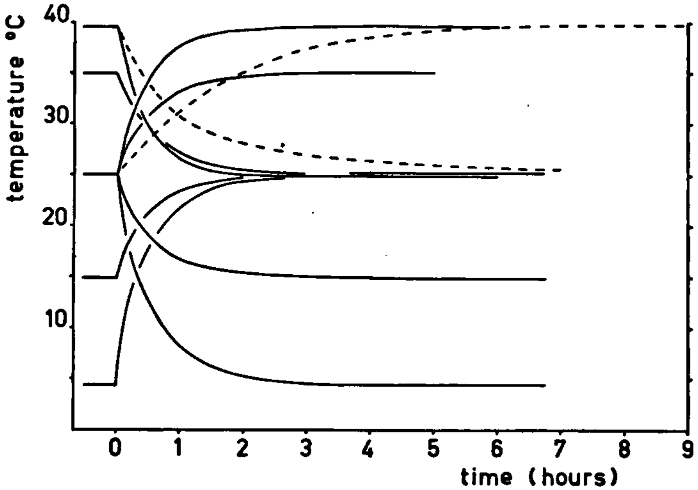

Fig. 2. The time-course of the newly adopted temperature inside the bulbs after temperature changes of the thermostat bath. Capacity of the respiration vessels: 500 ml (solid line) and 5350 ml (broken line).

Before passing through the respiration container the gas was freed of carbon dioxide by passing it through soda asbestos, which has a great capacity for absorbing carbon dioxide, and then dried by passing it through silica gel. The rate of air flow was 3, 6, 9 and 12 l/h, as required, seldom more. The air was delivered from a compressed air supply (open air from Lisse) or from metal bombs. A very constant air flow can be maintained by gradually reducing the pressure. Small differences were adjusted by means of a needle valve. The overpressure to which the bulb and the rotameter were exposed was not more than some centimetres of waterpressure. The air-rate was measured with the help of rotameters from the firm of Rota, Oeflingen (Germany). The use of rotameters has the advantage that slight faults like resistance through condensed water or small leakages in the gas train could be recognized in time by an irregular behaviour of the floater in the rotameter. The capillary flow meters from which the air-rate is read by means of a manometer are too inert to register these slight irregularities. By means of a spiral glass tube immersed in the water bath and placed before the inlet of the respiration container the right temperature of the air led over the bulbs was assured. After passing through the respiration container the air was dried with silica gel before being analysed. A small absorption of carbon dioxide gas by the silica gel did not cause any practical difficulty, because the new equilibrium was reached very soon. The silica gel can be rapidly regenerated. The drying tubes contain only about 23 grams of silica gel and their volume was about 35 ml. In order to check the gas flow a water seal was added after the gas analyser.

The bath temperature generally kept constant within ± 0.2° C, mostly within ± 0.1°. The carbon dioxide concentration in the respiration container normally was kept below 0.05 per cent by regulating the rate of the gas flow (mostly 3—9 l/h). In the oxygen measurements a scale range of the apparatus of 0—0.05 % is not possible, because the oxygen measuring method is not sensitive enough. Here the scale range was 20—21 %, i.e. 1 % difference. For this reason the carbon dioxide measurement was adapted to the measuring range of 0—1 % in combined measurements. It should be emphasized that dry air was continuously led over the bulbs. Thus the chance of infection was slight and the practical storage situation was best imitated.

The $CO_2$ production and the $O_2$ consumption were nearly always based on the intensity of the gas exchange of the bulb at 25° C, the temperature at which the bulbs were also stored. This activity was put at 100 and later changes were expressed in percentages of this activity at the beginning. In most figures this relative respiration rate is given. We regarded this method as justified because the most important consideration was to find out what happened when the bulbs were transferred from the retarded storage condition to other temperatures. In Chapter V.1. it will become clear that the respiration could be considered constant over a relatively short period of time when stored at 25°. Practically in all experiments an equal number of bulbs was used, the fresh weight, too, was approximately equal. Only in a few cases an absolute quantity of carbon dioxide is taken as a measure for the respiratory activity. When this is done the recorded percentage of carbon dioxide is given in the graphs under condition of an air flow of 3 l/h. If in those cases it proved to be necessary to compare some experiments, the carbon dioxide output was corrected in view of the differences in fresh weight of the equal number of bulbs at the beginning of the experiment. This was for instance done in the experiments shown in Figs. 5 and 6A.

In the cases in which a respiration quotient was measured the air was first analysed for carbon dioxide by means of the URAS apparatus (to be discussed below) and then led through the oxygen analyser (Magnos 1, see below).

## II.3. GASOMETRIC ANALYSIS OF CARBON DIOXIDE AND OXYGEN

In order to obtain continuous measurements of the gas composition which was altered as a result of the oxygen consumption and the carbon dioxide production of the bulbs it proved to be necessary to make use of an automatically recording gas analyser. For the carbon dioxide measurements an infra-red gas analyser was used (called URAS), constructed by the Badische Anilin- & Soda-Fabrik (Ludwigshafen am Rhein, Germany), type AWM and a gas analyser from the firm of H. Wösthoff (Bochum, Germany), type 501–1. The oxygen analysis was done by means of a Magnos 1 apparatus from the firm of Hartmann & Braun (Frankfurt am Main, Germany). We shall briefly summarize our experience with these analysers.

### II.3.1. *Infra-red gas analyser* (URAS)

Publications have regularly appeared about the biological application of this measuring apparatus, so we can omit a detailed description. For this we may refer to the following publications: EGLE & ERNST (1949); EGLE & SCHENK (1951); TRANQUILLINI (1952); BAUMEISTER (1952); KOEPF (1953) and GAASTRA (1959).

The method is based on the absorption by $CO_2$ in the infra-red part of the spectrum. Through an ingenious find by Luft & Lehrer (D.R.P. 730478 of 9/3/1938) this principle was made ready for stable measuring. The apparatus proved to be quite satisfactory in the experiments. Three analysis tubes were inserted into the apparatus, which made the following scale ranges possible: 0—100 %; 0—10 %; 0—1 %; 0—0.05 % and 0—0.005 % $CO_2$. The accuracy was about 2 per cent of the full scale. Besides absolute measurements, difference measurements can be made with this apparatus, because the control tube, normally filled with air that is free from $CO_2$, or with $N_2$, can also be filled with a definite percentage of carbon dioxide. This apparatus can be widely applied because slight alterations in a certain gas concentration can be measured, provided the air is previously dried. Silica gel is entirely satisfactory as a drying agent. The measurements are independent of the rate at which the air is led through the apparatus, though naturally there are practical limits. It did not seem desirable to lead the gas through at an air-rate of less than 3 l/h, because then there would be a danger of the absorption tubes not being flushed sufficiently, causing dead corners to be formed, as a result of which deviations in the readings could occur. It is important to note that there were little changes of the zero indication as well as of the indication of the galvanometer after being adjusted at a certain level. Thus there was no need for frequently repeated zero checking, which always requires time, nor for the frequent passage of a test-gas. To our mind it is an advantage to have the apparatus working continuously.

### II.3.2. *Micro-gas analyser* (Wösthoff)

The method used in this analyser is based on the fact that an alkaline solution, before and after the reaction with a certain amount of carbon dioxide gas, shows a difference of electric conductivity. This is measured by means of electrodes and the difference depends on the amount of $CO_2$ that was made to react with a certain amount of alkali per time unit. The method succeeds or fails depending upon the solution of the technical problem of how, at a very uniform rate, to bring the constant flow of alkaline solution into contact with a constant flow of gas. The Wösthoff apparatus meets the need excellently. The difference value of the two electrodes is made visible and is registered by an electronic compensation recorder. It seems that as yet little has been published about the biological application of this method (See Kilbinger, 1953). The scale ranges of the apparatus used were 0—0.05 % and 0—0.20 % $CO_2$. The accuracy amounted to about 2 per cent of the full scale. This method is restricted to a measuring of absolute quantities of carbon dioxide only. The air need not be dried carefully before the analysis. The pump system of the apparatus for sucking the air to be analysed makes it possible to use a minimum supply of air. As the demand of the sucking pump was very low (50.7 ml of gas per minute), the minimum air stream across the bulbs had to be about 3 l/h. In the experiments it was made to flow independently of the analyser by the insertion of an "overflow". The Wösthoff apparatus also provided us with very stable readings.

It is hard to say which of the two carbon dioxide analysers was more convenient. Slight differences may, however, be essential in certain applications, and therefore we shall summarize their slight advantages and disadvantages below. For the sake of simplicity the URAS analyser will be denoted by U and the Wösthoff analyser by W.

1. W is easier to move; U should preferably be permanently fixed.
2. U makes it possible to measure slight differences, whereas W is limited to absolute measurements.
3. Short-time alterations in the gas composition are generally shown better by W than by U. This at any rate becomes apparent if the experiment is performed with the greatest sensitivity, in which case the analysis tube in U will have a content of about 150 ml and the gas rates will be low.

4. As in U the gas to be analysed passes unchanged through the analyser the gas can be used again for $O_2$ analysis and it is possible to determine a respiratory quotient in the same test-sample.
5. The relationship between the recorder readings and the percentage of carbon dioxide gas, seen graphically, is almost linear in W, whereas in U it has a clearly exponential course. In the U method the exact quantity can only be found by using a calibration curve.
6. In W every four days the container must be refilled with a new sodium hydroxide solution, whereas in U no further direct measures for maintenance are required.
7. In W the zero adjustment and the span adjustment can be regulated independently, whereas in U the two indications are not quite independent of each other. The adjustment of the scale range therefore takes more time in U.
8. With W it is possible to measure small gas samples by means of pipettes (discontinuous method), whereas it is practically impossible to do so with U.

Because the URAS and Wösthoff method gave equal results, the method used is not indicated in the experiments.

### II.3.3. *Oxygen analyser* (MAGNOS I)

The measuring method is based on a property of the oxygen gas that distinguished it from other commonly occurring gasses, i.e. the paramagnetic susceptibility of the gas. When analysed the gas is led into a ring-shaped chamber, in which a horizontal cross-tube is placed. In normal circumstances no gas would flow through the cross-tube, because there is equal pressure on both sides. If, however, a magnetic field is applied perpendicular to the cross-tube and if the magnetic property of the oxygen is weakened on one side by heating it by means of a heating spiral, then the gas will flow through the cross-tube, which is called a magnetic wind. This magnetic wind is caused by non-homogeneity in the magnetic field and its rate is proportional to the oxygen content of the air. The heating wire wrapped round the cross-tube cools off because of the gas flow and this causes a change in the electric resistance in the wire, which can be measured, since the metal wire is part of a Wheatstone bridge circuit (LEHRER & EBBINGHAUS, 1950). Another difficulty that arose was how to change the measuring range of 0—1 % to a range of 20—21 %, which naturally occurs in air. The problem was a technical one, viz. how could the right zero suppression be found without the basic effect being influenced? Ebbinghaus succeeded in doing this by turning the ring chamber until the cross-tube was in a vertical position. Because the density of the heated gas will be less, a compensatory current against the magnetic wind (a chimney effect) will occur in the cross-tube when it is in a vertical position. Consequently the gas current is stopped and the zero level considerably suppressed. The sensitivity of the measuring method will not be affected (EBBINGHAUS, 1953;

EBBINGHAUS, STRUGGER & PERNER, 1953). Thus a scale range of 20 to 21 % has been realised with an accuracy of 1 % from the full scale, according to the authors. As the measurement is susceptible to temperature changes, the apparatus was placed in a container with self-regulating heating, thus keeping the environmental temperature constant (40° C). Before entering the analyser the gas should be thoroughly dried. In our experiments the gas was pre-dried with silica gel, the last traces of moisture being removed by means of a tube with magnesium perchlorate. The apparatus was calibrated at a flow-rate of 3, 6, 9, 12 l/h of air. Though the differences in the readings are slight when the gas is led through at these different rates, corrections will be necessary for highly precise measurement. In most of our experiments they were made. Any changes that may occur in the pressure of the barometer will also have to be taken into account. The relation between the barometer pressure and the readings of the apparatus showed that this factor should not be neglected. Changes of the air-pressure of 1 % result in a deviation in the readings of 2 % of the scale range of 20 to 21 %. A higher air pressure gives a lower reading. Besides, the sensitivity of the apparatus changes in accordance with the air pressure. With an increase in the air pressure of 1 % the sensitivity over a scale range of 20 to 21 % is increased by about 3 %.

In order to prevent deviations it is necessary to remove the carbon dioxide before analysing the gas. In our experiments this was done by means of absorption tubes with soda asbestos. We would like to emphasize the consequences for the calculation of the true quantity of oxygen that is absorbed by the bulbs, for by removing the carbon dioxide gas the percentage of oxygen as well as that of nitrogen in the gas mixture increases proportionally. In the theoretical calculations — not given here — it becomes clear that the oxygen intake registered by Magnos should be multiplied by a factor 1.25 to 1.26, in order to get the real oxygen intake. With an oxygen intake of 0.1, 0.5 and 1.00 % the calculated corrective factor will be 1.263, 1.258 and 1.252. For absolute oxygen measurements in determining the respiratory quotient this should be taken into account. The correction is independent of the respiratory quotient of the material.

In general it may be said that measuring with Magnos requires more care than the carbon dioxide measurements with URAS and Wösthoff analysers.

Further details about similar analysers are to be found in MÜLLER (1958) who worked with a "Differenz-Magnos" and in BIALE (1960) who mentions experiments with a Beckman oxygen analyser, which has the advantage of being insensitive to the content of carbon dioxide in the gas to be analysed.

### II.3.4. *Calibration of the scale ranges*

In order to render the absolute accuracy of the measurements as perfect as possible, much attention should be paid to the calibration of the apparatus. Initially, in the carbon dioxide analysis, we used calibrating gas of a definite composition, furnished by a factory (Badische Anilin- & Soda-Fabrik; and later: Hartmann & Braun). Later the need was felt to make our own gas mixtures for calibrating. This was done in two different ways. First of all gas mixtures were composed with the aid of rotameters. The right mixture was achieved in one or two steps. It should be remembered that the rotameters must be adjusted according to the nature of the gas that is conducted through them, for its specific gravity and its specific viscosity play a part in the rotameter indication. To calibrate the rotameters a soap-film calibrator was used, such as has been described by GOODERHAM (1944). This has proved a simple, accurate and reliable method with results that can be reproduced. The way in which the rotameters were arranged for the mixing of the gas cannot be discussed here because our space is limited. In addition to the calibrating method mentioned above two Wösthoff pumps for the mixing of gas were used (type 511-1 and type A31/2). One of them was able to prepare mixtures of two gases in the proportions of 1 : 9 to 9 : 1, while with the other a scale range of mixing proportion between 1 : 34.7 and 1 : 2812.0 was made possible. By combining the pumps all kinds of concentration ranges were made possible. The use of mixing-pumps was far simpler and required less time than rotameter mixing but the result was similar. For the mixing of oxygen, the pump served as well. As the natural percentage of oxygen in the air a percentage of 20.94 % was taken (SCHOLANDER, 1942, 1947). When air taken from cylinders was conducted through the apparatus, the reading proved to be a little lower than with air from our own compressed air supply (open air, Lisse) in most cases, from which it was concluded that the oxygen percentage was a little lower. This should be taken into account when experimenting.

In the Magnos the calibration line showed a slight curve, but this deviation was so small (at the most 2 % of the full scale) that it could be neglected.

## II.4. METHOD OF DETERMINATION OF SUGAR CONTENT

In order to find out whether the respiratory intensity depends on the sugar content in the bulb an introductory investigation of the nature and the quantity of the soluble sugars was carried out. For 6 days the bulbs were exposed to various temperatures that were known to influence the respiration. Before and after treatment the sugar content was determined. The reserve carbohydrates present, like amylum and possibly also inulin, were not considered.

With the aid of the copper reduction method according to Somogyi and Nelson the reducing power of crude extracts from iris bulbs was determined before and after hydrolysis (SOMOGYI, 1951; NELSON, 1944). Analysis, too, was made of sugars from the same extract after a chromatographic separation of the sugars and after eluting the separated sugars from the paper. The method that was used will be briefly described.

After removing the tunics, the bulbs were cut into small pieces, and 100—150 grams of the cut tissue was placed in 200 ml boiling 96 % ethanol in order to stop enzyme action. Next the tissue was ground in a homogenizer and another 50 ml of alcohol was added. After this the thick suspension was heated in an incubator at 60° for 20 minutes and then filtered on a Büchner funnel by suction. The residue was rewashed and re-extracted, this time with 70 % alcohol. A third extraction of the pulp with a soxhlet apparatus proved to be no longer profitable in view of the fact that only an extra of 2 to 2.5 % sugar was extracted, and this took a lot of time. The total filtrate of about 700 ml was concentrated by evaporation to 100 ml. After standing for some hours the fluid was decanted and bottled for storage.

First of all the sugar content of the crude extract was determined. The reducing power was determined before and after hydrolysis. The amount obtained before hydrolysis will mainly refer to glucose and fructose, the amount obtained afterwards to glucose, fructose and sucrose together. The increase in reducing power will partly be also the outcome of the hydrolysis of other fractions which might possibly be oligosaccharides (see VI.1.). Hydrolysis was done with HCl (final concentration 3 %) for ten minutes at 70° C, after which a neutralisation was effected to a pH of 7 before the sugar content was determined. Hydrolysis was also effected in a simple way, i.e. with the aid of invertase, but this method did not always give reproducable results.

The sugars were chromatographically separated by means of the descending technique, and as recommended by JERMYN & ISHERWOOD (1949) the non-aqueous phase of the mixed solution of ethyl acetate: acetic acid: water (3 : 1 : 3) was used as a solvent. This solvent proved to be more satisfactory for a good separation than butanol:acetic acid:water. For paper we used Whatman 1 filter paper. After the separation the sugars were eluted from the pieces of paper with hot water. The volume was reduced by evaporation after filtration and was brought back to the same volume of 20 ml in all cases. The glucose and fructose extracts could be directly used for the determination of Somogyi and Nelson, whereas the sucrose extract had to be hydrolysed first. Reagent grade sugars were used as standards.

Unfortunately in the first part of the experiments it was omitted to determine, as a control, the reducing power of an equal number of blank pieces of paper. Later these controls proved sometimes to possess a small reducing power. The error, however, is slight. But it should be taken into account that the sucrose figures may be a bit too high. That is why the glucose and fructose figures, which happened to be very low and with which a slight mistake could have a serious effect, were not mentioned in the results that will be given later. A stronger evaporation of the original liquid in order to increase the glucose and the fructose concentration entailed difficulties because the extract became too treacly and also because tailing of the spots occurred in the chromatogram.

## CHAPTER III

# GROWTH OF THE GROWING-POINT AND OF THE ROOT PRIMORDIA IN RELATION TO TEMPERATURE AND OXYGEN CONCENTRATION

### III.1. REST PHASE OF LIFTED BULBS AND THE STORING OF THE IRIS BULB

As has been mentioned, it is possible to retard the development of the inner growing-point of the iris bulbs in a dry condition after they have been lifted. They are usually called "retarded" bulbs. This term (derived from popular language) only means that the rate of the normal development can be slowed up for a long period of time. We can also employ the frequently used term "rest phase". We know, however, from general research on flower bulbs as well as from research on irises that during this phase very fundamental reactions take place, which determine the life-history of the growing-point. For this reason objections can be raised against the term rest phase; it is based only on external observations, so it is a false appreciation. However, if we keep this in mind, we may use the term, for want of a better one.

From BEYER's investigations (1952, 1955) it became evident that the retardation of the development was quite simple to bring about. After lifting, the bulbs only need to be stored at a temperature of 25° or 30°. We shall not discuss the possible practical applications, e.g. export of bulbs to the southern hemisphere and bringing about florescence of the plants at other than the normal times. Certain cultivars, among which are 'Wedgwood' and 'H. C. van Vliet' can be retarded very well and can be stored for about one year.

TABLE 1

Influence of storage temperature on the retardation of the development of the shoot in 'Wedgwood' bulbs. Length of the outermost foliage leaf in mm. Mean values of 5 bulbs. After lifting (about 20/8) the bulbs were stored at 30° C, and at 6/9 the bulbs were exposed to different temperatures

| | 1956 | | | | 1957 | | |
|---|---|---|---|---|---|---|---|
| | 20/8 | 6/9 | 6/10 | 5/11 | 11/1 | 7/2 | 6/4 |
| 20° | 1.9 | 2.4 | 13.1 | 28.6 | 42.2 | 51.1 | 85.4 |
| 25° | | | 1.7 | 5.8 | 18.5 | 24.1 | 31.8 |
| 30° | | | 1.8 | 2.2 | 9.0 | 10.8 | 17.5 |
| 35° | | | 2.0 | 2.0 | 2.1 | 2.2 | — |
| 40° | | | † | — | — | — | — |

† dead

In table 1 the highly retarded growth has been shown at higher temperatures. As a standard measure for the development of the central growing-point the increase in length of the first leaf has been chosen, which will be shown to be representative for the growth of the shoot in the bulb, as described in Chapter III.2. The figures show that the retardation of the development is more complete according to the height of the temperature at which the bulbs are stored. There is a limit at 40° C at which the temperature becomes lethal. Because the bulb tends to dry up at a higher temperature, in practise a storage temperature of 25° or 30° is generally taken. At 30° the development is more retarded than at 25°. This can be seen in table 1, in the diagram of the growth of the shoot in Fig. 5 and in table 4. Fig. 5 shows that in a year the first leaf has grown no more than about 37 mm at 25° and 13 mm at 30°. Thus the growth has been thoroughly retarded. As the bulbs are stored at lower temperatures the growth activity increases accordingly. This will be shown in the next Chapter.

## III.2. TEMPERATURE AND GROWTH OF THE PRIMORDIA OF THE GROWING-POINT

In this Chapter some experiments to determine the influence of the temperature on the development of the growing-point in the bulb are described. For this retarded bulbs have been used, in which the development has practically come to a stop and in which by a change of temperature a new growth activity is caused.

In Chapter V analogous experiments to determine the influence of the temperature on a new increase of the respiratory activity are described. In the discussion (Chapter VII) these data are used to give a more detailed description of the correlation between the growth and the respiration.

After the bulbs were stored for a month (on 27/9/57) the length of the first leaf of a number of them was measured, while small batches of bulbs were simultaneously transferred from 25° and 30° storage to temperatures of 1, 5, 9, 13, 17, 20, 25, 30 and 35° C. After 6 days, 3 weeks and 6 weeks the bulbs of these batches were used to measure the length of the first leaf again. Each batch consisted of 5 bulbs. The experiment was repeated when the bulbs were stored for 62 and 210 days at temperatures of 25° and 30°. The bulbs belonged to the cultivars 'Wedgwood' and 'van Vliet'. Fig. 3 shows a representative result of 'van Vliet' bulbs. The length of the first leaf at the start of the experiment, i.e. the moment when the bulbs were transferred to the various temperatures, is shown by the level denoted by arrows.

The most important conclusion that can be drawn from the experiments was that the optimum of the shoot growth lies at 13—17°. Furthermore it can be said that the development of the shoot is more retarded at 30° than at 25°. These conclusions are valid for 'van Vliet' bulbs as well as for 'Wedgwood' bulbs, and could be

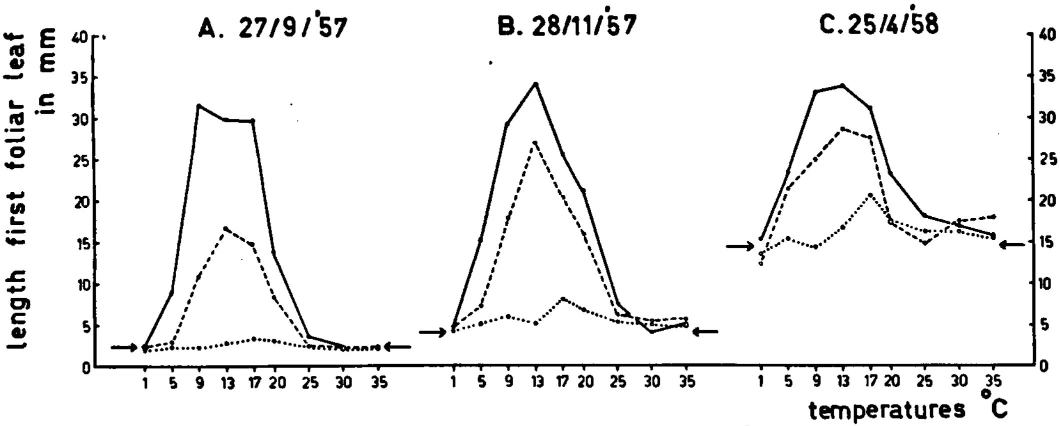

Fig. 3. The effect of the temperature on the growth of the first leaf-primordium in the central growing-point of the iris bulb. A, B and C indicate the dates at which the 'van Vliet' bulbs were transferred from the 25° storage to different temperatures. The arrows in the figure show the length of the leaf at these dates. Length of the first leaf in mm after growing-periods of 6 days (dotted line), 3 weeks (broken line) and 6 weeks (solid line).

confirmed in other seasons. At the same temperatures 'van Vliet' bulbs can be retarded better than 'Wedgwood' bulbs. (A temperature of 35° retards very well, but is avoided in practice, because of the higher desiccation).

The results are based on the measurements of the first foliar leaf and the question may arise in how far the growth of the first foliar

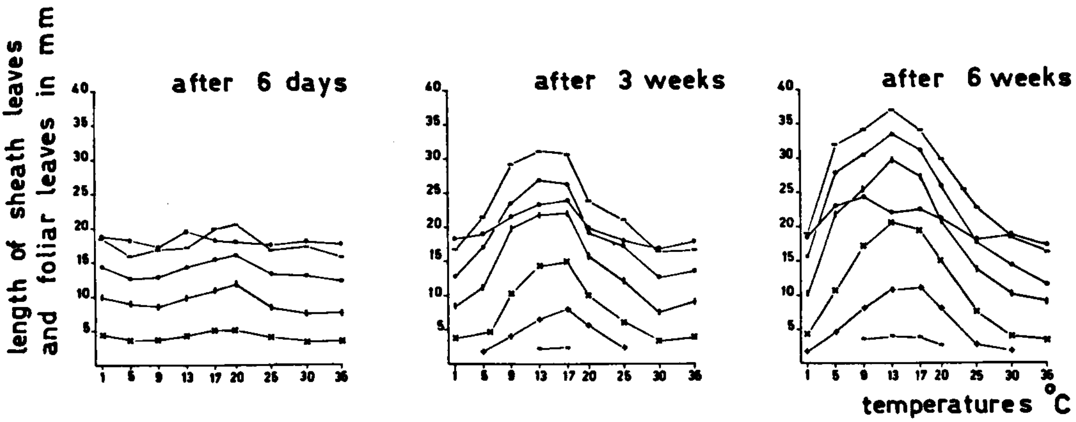

Fig. 4. The effect of the temperature on the growth of the different leaf primordia in the central vegetation-point of the iris bulb. Length of the parts of the shoot in mm after growing periods of 6 days, 3 weeks and 6 weeks. The 'van Vliet' bulbs were transferred to different temperatures after a 25° storage period of 6½ months. (closed circle: first sheath leaf; hor. mark: second sheath leaf; open circle: third sheath leaf; vertical mark: first foliar leaf; multipl. mark: second foliar leaf; plus mark: third foliar leaf; uniformity mark: fourth foliar leaf).

leaf is representative for the whole shoot. In Fig. 4 analogous measurements of the growth of the three sheath leaves and the existing foliar leaves are shown graphically. In this case each batch consisted of 10 bulbs. The optimum temperature for the growth of all the shoot parts proved to be similar. It is therefore justifiable to take the observations concerning the first foliar leaf as representative for the growth of the whole shoot. It must be emphasized that these results only concern dry bulbs without roots. Similar material was used in the respiration experiments.

### III.3. OXYGEN CONCENTRATION AND THE DEVELOPMENT OF THE PRIMORDIA OF THE GROWING-POINT

In this Chapter the influence of the oxygen on the growth of the shoot in the bulb is illustrated by means of a few experiments. In Chapter IV.3. we shall later discuss the influence of the oxygen concentration on the respiration. The oxygen content was varied from 100 % (pure oxygen), 21 % (air), 10 % (mixture of $O_2$ and $N_2$) and 0 % (pure nitrogen). Again the increase in length of the first foliar leaf served as a standard measure for the growth of the primordia and again bulbs from the 25° storage were used. For the gas treatment the bulbs were put in pots through which the gasses or gas mixtures concerned were led at the rate of 6 to 12 l/h. An

TABLE 2

Growth of the first leaf primordium at 25° and 15° in an atmosphere of air or nitrogen.

| | Treatment | | | Length first leaf in mm (mean values of ten bulbs) |
|---|---|---|---|---|
| 'Wedgwood' | control beginning (25° storage) 28/10/61 | | | 2.9 |
| | control end (25° storage) after 16 days | | | 4.9 |
| | after 16 days | 25° | air, free | 5.0 |
| | | | air, ventilated pots | 4.4 |
| | | | nitrogen, ventilated pots | 3.2 |
| | | 15° | air, free | 16.7 |
| | | | air, ventilated pots | 16.7 |
| | | | nitrogen, ventilated pots | 2.9 |
| 'van Vliet' | control beginning (25° storage) 28/10/61 | | | 2.5 |
| | control end (25° storage) after 16 days | | | 3.0 |
| | after 16 days | 25° | air, free | 3.2 |
| | | | air, ventilated pots | 3.2 |
| | | | nitrogen, ventilated pots | 2.7 |
| | | 15° | air, free | 17.8 |
| | | | air, ventilated pots | 15.0 |
| | | | nitrogen, ventilated pots | 2.3 |

accumulation of carbon dioxide was therefore prevented. The growth was measured at temperatures of 25° and 15°.

In the experiment of table 2 the difference in growth of the first leaf of bulbs which had been in air and in nitrogen for a period of 16 days has been determined. As a control the increase in length of the first leaf of bulbs, which were not used for the experiment, but which remained in the storage room, was also registered (indicated in the table by "storage"). For the experiment the bulbs were taken, sometimes in pots, into another experimental room at 25° or 15°. "Free air" means that the bulbs lay freely in air, not in pots, in this experimental room.

The most important conclusion is that in a nitrogen atmosphere there is no growth which normally occurs in air at 15°. At 25° not even the slight growth occurs that is observed in bulbs in air during the experimental period. The slight difference between the measurements in nitrogen and in air at 25° proves to be statistically significant (the probability of more extreme values is lower than the 1 % level for 'Wedgwood' bulbs and lower than the 2 % level for 'van Vliet' bulbs).

Another question is to what degree the growth and the oxygen concentration are related. In table 3 experiments are given with 'Wedgwood' and 'van Vliet' bulbs from the 25° and 30° storage. The greatest mean increase in length was observed with an oxygen concentration of 10 %. In air this increase is less and it is still more

TABLE 3

Growth of the first leaf primordium at 15° in atmospheres of pure oxygen, air, 10 % oxygen in nitrogen, and nitrogen.

| | Bulbs from 25° storage | | | Bulbs from 30° storage | | |
|---|---|---|---|---|---|---|
| | Treatment | | Length first leaf in mm (mean values of ten bulbs) | Treatment | | Length first leaf in mm (mean values of ten bulbs) |
| 'Wedgwood' | control beginning (25° storage) 24/11/61 | | 5.9 | control beginning 8/1/62 . . . . . . . | | 4.1 |
| | control end after 14 days . . . . . . | | 7.6 | control end after 17 days . . . . . . | | 2.9 |
| | after 14 days 15° | 100 % $O_2$ | 13.5 | after 17 days 15° | 100 % $O_2$ | 12.9 |
| | | 21 % | 17.9 | | 21 % | 19.0 |
| | | 10 % | 20.8 | | 10 % | 22.7 |
| | | 0 % | 5.8 | | 0 % | 3.0 |
| 'van Vliet' | control beginning 24/11/61 . . . . . . | | 3.4 | control beginning 8/1/62 . . . . . . . | | 2.2 |
| | control end after 14 days . . . . . . | | 4.1 | control end after 17 days . . . . . . | | 2.2 |
| | after 14 days 15° | 100 % $O_2$ | 12.1 | after 17 days 15° | 100 % $O_2$ | 11.9 |
| | | 21 % | 15.8 | | 21 % | 17.6 |
| | | 10 % | 17.3 | | 10 % | 21.3 |
| | | 0 % | 3.5 | | 0 % | 2.2 |

reduced in an atmosphere of pure oxygen. For the calculation of the significance, the test of Wilcoxon has been used (see: WIJVEKATE, 1961). In the case of 'Wedgwood' bulbs from 25° storage the difference between the growth at 10% and 20 % $O_2$ seems to be significant (the probability of more extreme values is between the 2 % and 1 % level). In the experiments with 'van Vliet' bulbs stored at 25° the above-mentioned difference is not significant. A highly significant difference was noticed when bulbs from the 30° storage were used (the probability of more extreme values is lower than the 1 % level). It is concluded from these experiments that the first growth is greatest with an oxygen concentration lower than normal, but possibly this is valid only under certain conditions related to the "retarded" state of the bulb. In an oxygen concentration of 100 % the growth was retarded significantly in all experiments. In judging the situation in the experiment with 10 % oxygen it should be kept in mind that the oxygen content at the place of the shoot in the bulb may possibly have been even somewhat less than 10 %.

From the experiments it may be concluded that:

1°. No growth of the primordia takes place in the absence of oxygen.
2° The increase in length of the shoot is greatest with an oxygen tension somewhat lower than in air. Probably this holds true only under certain conditions of the stored bulb.
3°. A higher oxygen concentration than in air inhibits the increase in length of the growing-point in the bulb.

### III.4. TEMPERATURE AND GROWTH OF THE ROOT PRIMORDIA

Under the conditions of the respiratory experiments, just as with the dry storage of the bulb, no root development is externally discernible. The air humidity is too low for this. Nevertheless the possibility exists that some incipient activity of the root primordia has taken place under the influence of a temperature treatment, which may also influence the respiration. For this reason the optimum temperature range for the root growth was investigated. This was done under optimum conditions by planting the bulbs in wet sand. In interpreting this it should be kept in mind that the experimental conditions for the bulb during these experiments were quite different from those during the shoot growth and the respiratory experiments.

Both 'Wedgwood' and 'van Vliet' bulbs were used in the experiments. The bulbs were planted in boxes filled with wet sand and placed in experimental cubicles with temperatures of 1, 5, 9, 13, 15, 17, 20 and 25° C (sometimes 30° as well). These experiments showed that the optimum for the growth of roots was higher than 13—17° C, as was found for the growth of the shoot. The optimum for the growth of the roots was not very clearly localized, but a temperature of about 20° was most favourable for the development. This result did not only apply to the total length of the roots that were formed, but equally to the fresh weight and the dry weight.

When comparing the results of the root growth and the shoot growth, we see that both the conditions for starting the growth and the optimum temperature for the growth seem to be different.

## CHAPTER IV

# RESPIRATORY ACTIVITIES DURING THE REST PHASE AND STORAGE

### IV.1. TEMPERATURE AND RESPIRATORY ACTIVITY DURING STORAGE

At a first approach the carbon dioxide output of the bulb has been measured at temperatures of 25° and 30° (storage temperatures). The result shown in Fig. 5 is based on measurements in the season of 1957/58. In the season of 1956/57 the result was basically identical. In the experiments of Fig. 5 the carbon dioxide output was continuously measured in the beginning (indicated by the solid lines in the figure), whereas later the bulbs were stored in chambers and were only moved periodically for the carbon dioxide to be measured (dotted lines).

After a very short peak in the very beginning of the storage period the respiratory activity decreases strongly. This sharp decline after lifting of the bulbs has also been observed by RODRIGUES PEREIRA (1962), but in his case the decline was less pronounced. After this drop the respiration slowly increases again. The increase is so slow that the carbon dioxide output can be considered to be practically constant for a number of days. We are therefore justified to call this a steady state condition. The experiments described later were always started with material in the steady state condition and were never continued for more than two weeks. In the experiment of Fig. 5, however, the respiratory activity was observed over a longer period, a period that may be called a rest phase at 25° and 30°, possibly with the exception of the beginning. In Fig. 5 we see that in the beginning the respiratory activity is higher at 30° than at 25°. At the starting point we may assume a $Q_{10}$ of 2.6. In a few weeks' time after the first sharp drop, the respiratory activity proves to have decreased more at 30° than at 25°. The difference becomes more pronounced as the storage period goes on. To assume a $Q_{10}$ is not permissible now because the time-factor is not eliminated. It should be kept in mind that the previous history of the objects that are to be compared has not remained the same. The result that the respiratory activity during storage becomes greater at 25° than at 30°, is completely reproducable.

In a later season the influence of the temperatures of 25° and 30° was once again investigated, and in addition two bordering temperatures of 20° and 35° were used. This was done in the storage period 1960/61. The result is shown in Fig. 6A. We find that apart from the sharp drop in the beginning the respiration rises again at 20°. This takes place much sooner than at 25°. The experiment

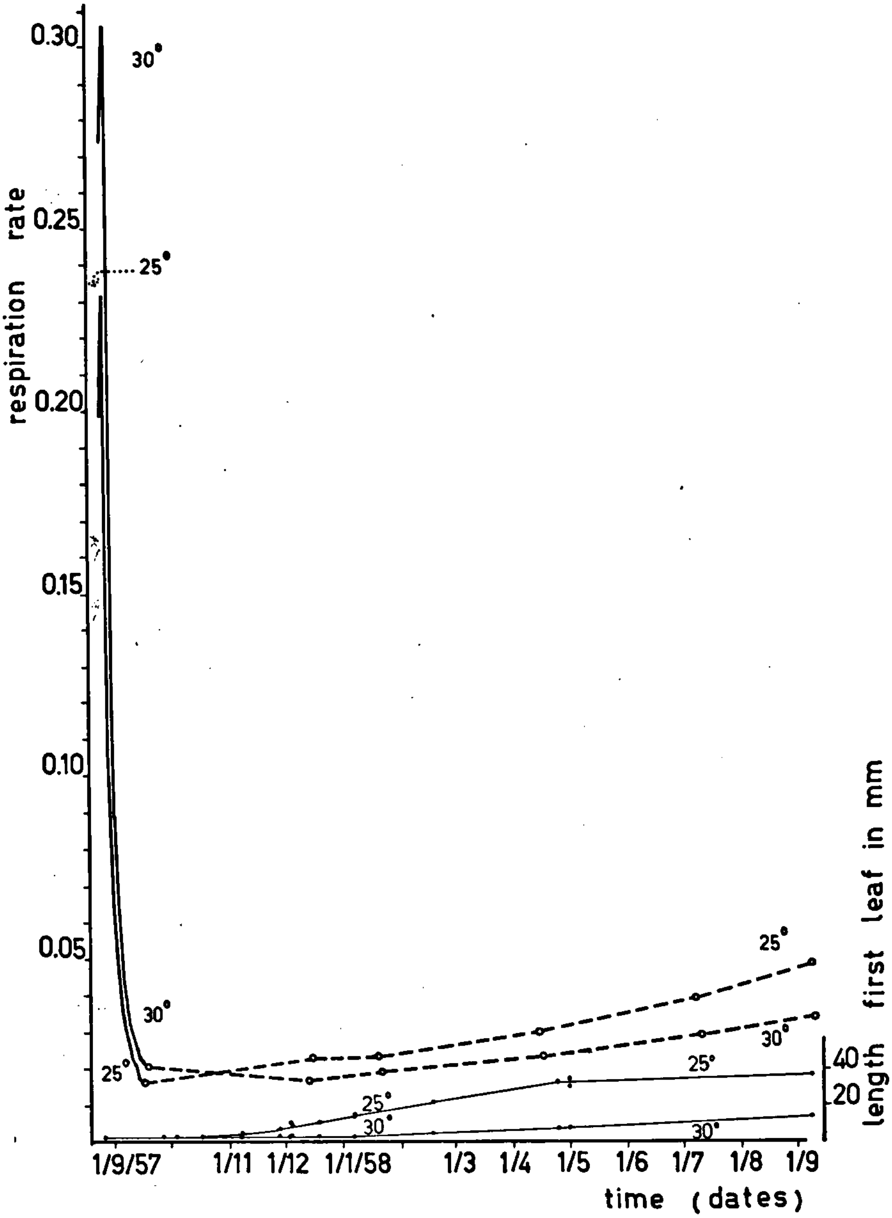

Fig. 5. The course of the respiration rate and the growth of the first leaf-primordium of 'Wedgwood' bulbs after lifting during a storage period of a year at 25° and 30° C.

Ordinates: respiration rate ($CO_2$ production in % of the gas at an air-rate of 3 l/h) and length of the leaf in mm.

Abscissa: time (dates).

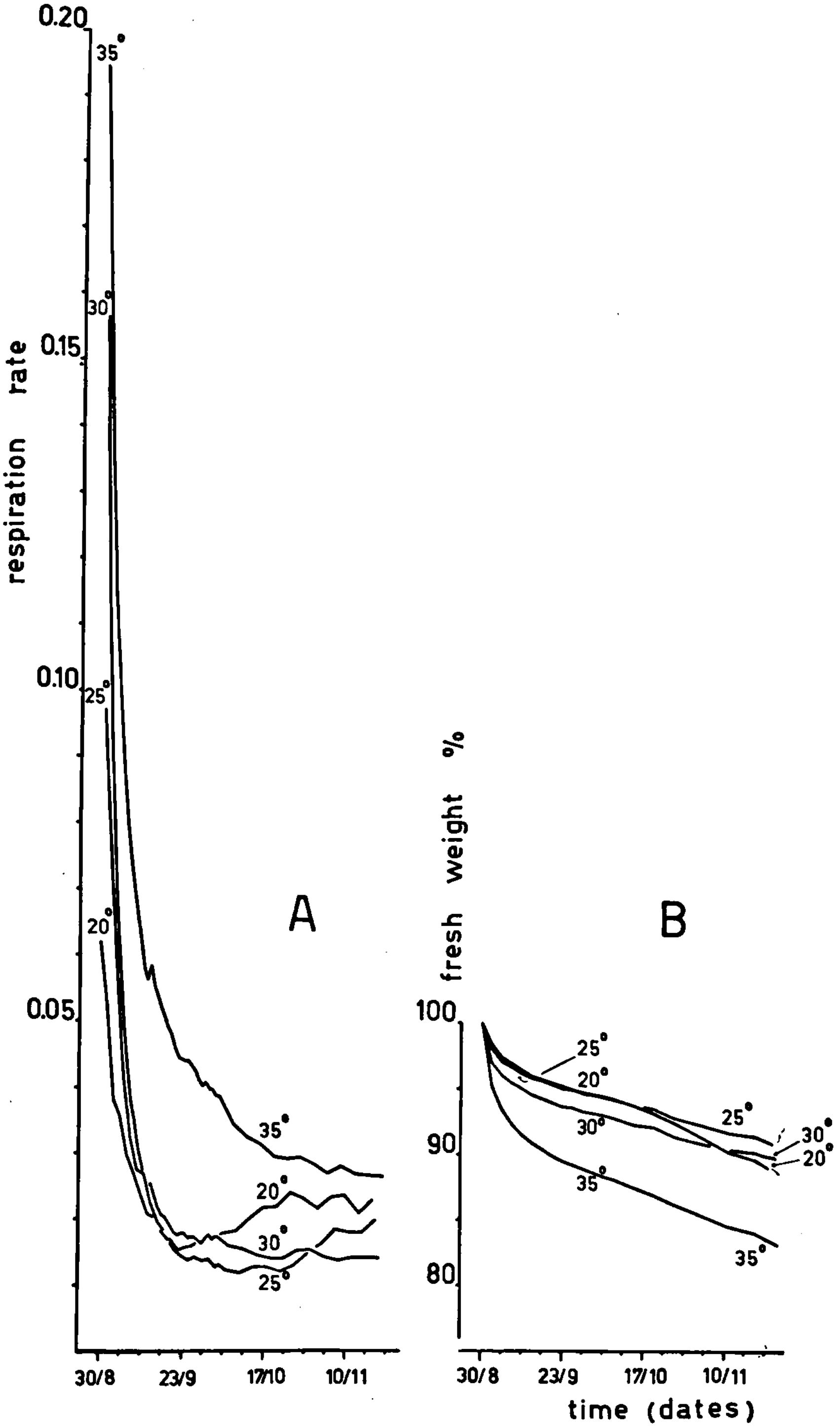

Fig. 6. A and B. The respiration rate and the decrease in fresh weight of 'Wedgwood' bulbs after lifting during storage at temperatures of 20°, 25°, 30° and 35°. Series A.: $CO_2$ production in % of the gas in an air current of 3 l/h. Series B.: Registration of the fresh weight in % of the original values.

at 30° lasted too short in this case to observe a rise, as illustrated in Fig. 5. At 35° no tendency for an increase in activity is observed, but rather a tendency to decrease. For the sake of completeness the length of the primordia is also given in table 4 at the end of the experiments given in Fig. 6.

TABLE 4

Length of the first leaf primordia in the bulbs at the end of the experiments given in fig. 6A and 6B. Length in mm. Experiments lasted 83, resp. 89 days.

| | Experiment 6A | Experiment 6B |
|---|---|---|
| 20° | 17.6 | 22.4 |
| 25° | 5.1 | 7.7 |
| 30° | 2.4 | 2.4 |
| 35° | 2.1 | 2.3 |

We have seen that it is characteristic for the respiratory activities of the iris bulb that only in the beginning a normal relation between the activities and the temperature exists. After a strong decrease in the respiratory activity in the beginning of the storage period, a rise in the respiratory activities occurs sooner or later according to the temperature. At 35° this was, however, not investigated over a long period. At 30° the respiratory activity proves to be lower than at 20°, 25° and 35°. These differences cannot have relation to the temperature coefficient, but are a result of fundamental changes in respiration mechanism.

It may be asked whether the respiratory activity of the iris bulb in the absolute sense is high or low. At 25° or 30° the rate of carbon dioxide output proves to be of the order of 6 $mm^3$ $CO_2$/h/g fresh weight. This corresponds with approximately 0.02 $mm^3$ $CO_2$/h/mg dry weight (calculated according to a dry weight measurement of August 1957 which gave a dry weight of 31 %). This activity is of the same order of magnitude as that of potatoes in storage (APPLEMAN & MILLER, 1926; CHOUDHURY, 1939). In comparison with respiratory activities of other tissues and organs this is comparatively low (BONNER & GALSTON, 1952; JAMES, 1953; GODDARD & BONNER, 1960), which is not so surprising if it is remembered that the iris bulb is an organ in a relative rest phase.

## IV.2. THE WATER-LOSS OF THE BULB AND THE RESPIRATORY ACTIVITY

The respiratory activity of a tissue is sometimes thought to be related to the water content (see HUBER & ZIEGLER, 1960). As the bulbs are exposed to temperatures of 25° and 30° and to a low air humidity during the storage period, the question arises of whether in the iris bulb the water content and hence the loss of water during the storage of the dry bulb determines the respiratory activity. As the loss of fresh weight was known, but not the change in dry weight during the storage period, the loss of water was calculated by substracting the loss of dry matter due to the respiration from the total loss of weight.

A loss of dry substance during storage occurs because carbon dioxide escapes, while there is a gain of substance on account of the oxygen taken in. If it is assumed that glucose serves as a respiratory substrate and that it is completely oxidised the bulb should become lighter by 0.273 gram per one gram of carbon dioxide gas released. The curves of the carbon dioxide gas emission during the seasons 1956/57 and 1957/58 were used to calculate the total quantity of carbon dioxide separated during the various periods. For this purpose the curves were contoured with a planimeter. A representative example of the calculations resulting from these experiments is to be found in table 5.

TABLE 5

Loss of fresh weight, dry matter and water of 'Wedgwood' bulbs during 25° storage. Loss calculated at initial fresh weight. Initial fresh weight: 139.6 g. Final fresh weight: 95.0 g. Number of bulbs: 8.

| Period | Number of days | % loss of fresh weight | % loss of dry substance by respiration | % water loss |
|---|---|---|---|---|
| 18/ 9/57– 8/ 9/58 | 355 | 31.9 | 2.69 | 29.2 |
| 18/ 9/57–16/12/57 | 89 | 6.9 | 0.45 | 6.4 |
| 16/12/57–20/ 1/58 | 35 | 3.1 | 0.21 | 2.9 |
| 20/ 1/58–15/ 4/58 | 85 | 8.2 | 0.59 | 7.6 |
| 15/ 4/58– 8/ 7/58 | 84 | 8.7 | 0.74 | 8.0 |
| 8/ 7/58– 8/ 9/58 | 62 | 5.0 | 0.70 | 4.3 |

The experiments show that the loss of substance through respiration forms only a comparatively small part of the total loss of weight and varies from 6 to 14 per cent. The loss of water calculated proves to run parallel to the loss of fresh weight and the rates do not widely diverge. Therefore, when studying the relationship between the water content and the respiratory activity, it is justifiable to replace the loss of water by the loss of fresh weight.

If the data are plotted in curves, it becomes clear that the relationship between the fresh weight of the bulb and the time of storage is shown to be practically linear except for the beginning of the storage period. Shortly after lifting the decrease of weight is faster than later. The calculations show that the iris bulb loses about 25 to 30 per cent of its fresh weight after being stored for a year. In view of the storage conditions we may say that the bulb resists desiccation very well.

In the season of 1960/61 the first phase of the desiccation of the bulb during the dry storage after lifting was more accurately measured than before (see Fig. 6B). If the results in table 5 are taken into account, we may assume that the greater part of the loss of weight can be interpreted as loss of water. Little difference in loss of weight was observed between storage at 25° and 30°. At 35° the loss of weight is greater. A clear dip in the slope of the curve at 20° arouses the suspicion that a special change has possibly occurred here. This may be connected with an increasing evaporation at this temperature, because the shoot has grown best at this temperature (see table 4). As has been found in one case, the loss of water is slightly obstructed by the presence of the tunics round the bulb as well. If the tunics are removed the decrease of weight gets more important. There was no important difference noticed between the drops in weight at 25° and at 30°.

If the course of the respiration curve of Fig. 6A is compared with the loss of weight (proportional to the loss of water) of Fig. 6B, the following conclusion is reached. During the first period after lifting the loss of water somewhat deviates from that of the next period, in which we have found a linear ratio between the loss of water and the time. In the first storage period there is a possibility that a relationship exists between the water content and the intensity of the respiration. In the second period, the main storage period, this is not the case; there the respiration is controlled by the temperature.

## IV.3. OXYGEN CONCENTRATION AND THE RESPIRATORY ACTIVITY

The influence of oxygen on the respiration has also been investigated. For these experiments bulbs from 25° storage were used, since such bulbs may be considered to be in a steady state condition, as have been argued above.

Our first approach to the problem of the influence of different storage conditions was an investigation of the influence of pure $0_2$, 20 % $0_2$, 10 % $0_2$ and pure nitrogen on the respiration. For this purpose gas mixtures of 10 % or 20 % oxygen in nitrogen were prepared by means of Wösthoff mixing-pumps. This mixture was led into a 10 l bottle, out of which the necessary quantity of gas was carried off into the respiration container by means of a membrane pump. On account of the instability of the membrane pump small fluctuations occurred in the gas transport, which resulted in some irregularities in the carbon dioxyde registration (Figs. 7A and D). The experiments will be described in brief.

In Fig. 7A the carbon dioxide emission of 'Wedgwood' bulbs is registered at a temperature of 25°. At a given moment the air was replaced by a gas mixture of 20 % $0_2$ and 80 % $N_2$, and after 3 days air was led through again. The respiration showed a downward tendency, which may be regarded as a normal phenomenon (see V.1.). No difference was observed between this experiment and others in air.

When a gas mixture containing 10 % $0_2$ in nitrogen was led across the bulbs, a different result was obtained. The intensity of the carbon dioxide output decreases gradually and only increases when the bulbs are exposed to air (see Fig. 7D). The experiment has shown that the respiration decreases in intensity with 10 % $O_2$,

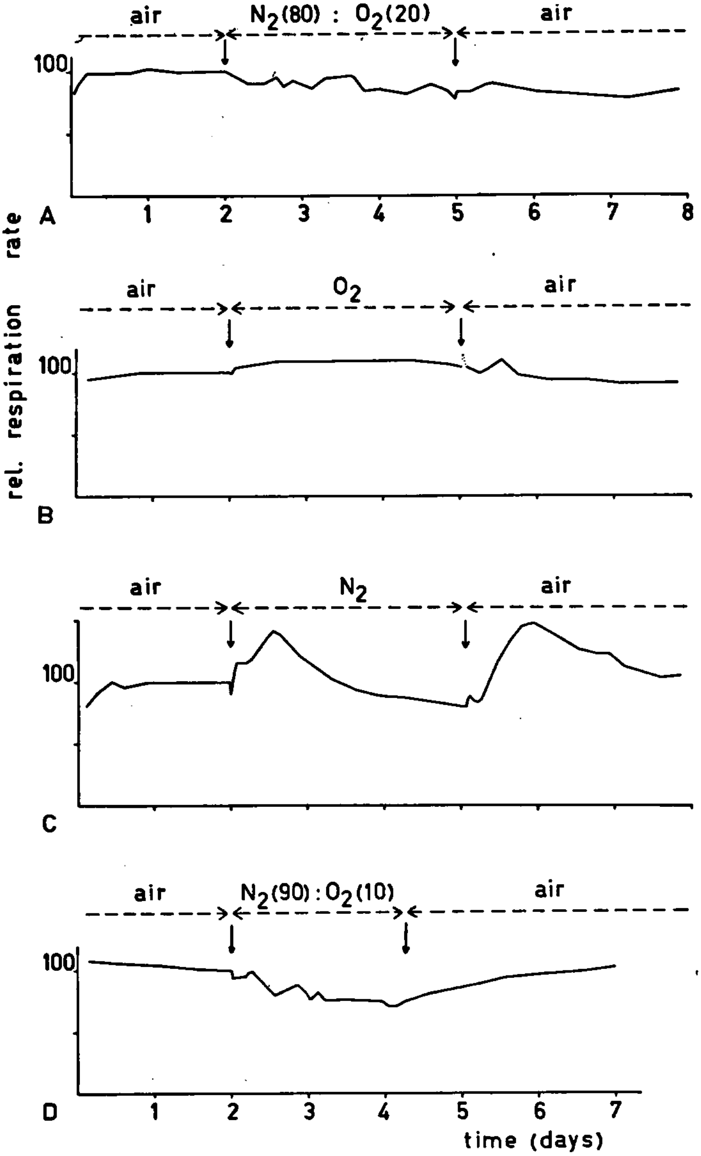

Fig. 7. A, B, C and D. The respiration rate of 'Wedgwood' bulbs in atmospheres of air (A), oxygen (B), nitrogen (C) and 10 % oxygen in nitrogen (D) at a temperature of 25°.

but this does not necessarily mean that fermentation has already started to take place.

If the bulbs are brought into a nitrogen atmosphere marked reactions are to be seen (Fig. 7C). After the transfer the carbon dioxide emission increases temporarily, then decreases. After a transfer from nitrogen to air a temporary increase is observed as well. These transient effects when the bulbs are transferred from an aerobic phase to anaerobic phase and vice versa have also been observed in several objects. The anaerobic carbon dioxide emission of the iris bulb after 3 days is 15 to 20 % lower than the carbon dioxide emission under aerobic conditions.

In Fig. 7B an experiment is shown in which at a given moment the air is replaced by pure oxygen. After 3 days air is again led over the bulbs. A pure oxygen atmosphere does not increase the carbon dioxide emission by more than 15 to 20 %. Thus, a five times higher oxygen concentration only slightly influences the respiration.

Summing up we can say that the influence of the oxygen concentration on the intensity of the $CO_2$ emission does not vary much if the oxygen content is over 10 %. At an oxygen concentration lower than 10 % the carbon dioxyde emission is very probably influenced. A fermentation can occur, because in the absence of oxygen an obvious carbon dioxide output is observed. If the bulbs are transferred from air into a nitrogen atmosphere (and vice versa) marked transition phenomena are observed.

## CHAPTER V

## RESPIRATORY ACTIVITY OF RETARDED BULBS AS INFLUENCED BY TEMPERATURE CHANGES AND OTHER FACTORS

### V.1. TEMPERATURE AND CARBON DIOXIDE PRODUCTION

In the experiments described in this chapter bulbs were used that were stored at 25° unless otherwise stated. The bulbs were placed in the respiration container and allowed to acclimatize for some days at the same temperature as that of the storage room, i.e. 25°. This was desirable because the registration of the carbon dioxide production nearly always showed a very faint hump in the beginning. After this temporary increase the carbon dioxide production showed a slight decreasing tendency, which was so slight, however, that the level could be considered to be practically constant. In the experiment we always waited until the hump was past. Fig. 8 once more confirms what can already be seen in Fig. 5, i.e. that at 25° little respiration changes occur. It can be said that the respiration is on a steady metabolic state.

From this steady metabolic state the bulbs were transferred into other temperatures, without changing any other conditions. These

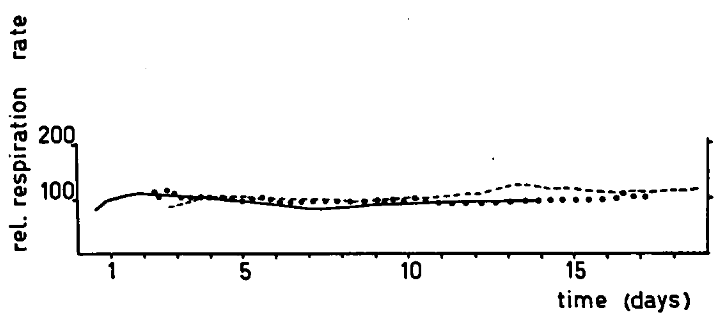

Fig. 8. The constant respiration rate of stored 'Wedgwood' bulbs at temperatures of 25°. Three different experiments. Respiration rate in terms of $CO_2$ production.

transitions took place rather suddenly because the respiration container was put from one water bath into the other. As shown in Fig. 2 the bulbs assumed the new temperature after about 2 hours in a respiratory container with a capacity of 500 ml, whereas at its most unfavourable it lasted about 5 hours with a respiratory container of 5350 ml capacity. The gas exchange of the bulb was recorded continuously whenever possible. As each experiment took at least about a fortnight, 11 experiments took at least half a year. That is the reason why there is such a large time lapse between each experiment. As the steady metabolic state always shows that the condition of the bulbs must have been about similar, we consider a comparison of the experiments justified. As far as has been investigated the experiments proved that only a quantitative difference in effect could be observed when the same experiment was repeated at various times of the season. However, for the sake of completeness we shall mention the dates of the experiments (table 6).

The results obtained in 1956/57 are shown in Figs. 9 and 10. At 25° the carbon dioxide production shows a slow decreasing tendency. However, in most experiments the curves at 25° remained much more constant than the example from the series given here (see Fig. 8).

TABLE 6

Initial date of the respiration experiments with 'van Vliet' bulbs in the season 1956/57

| Experiment | Dates |
|---|---|
| 5° | 16/ 4/57 |
| 10° | 12/ 2/57 |
| 15° | 30/ 1/57 |
| 20° | 14/ 1/57 |
| 25° | 15/12/56 |
| 30° | 2/ 1/57 |
| 35° | 31/ 3/57 |
| 40° | 26/11/56 |
| 45° | 15/ 4/57 |
| 50° | 26/ 4/57 |

When the bulbs are transferred to lower temperatures than 25°, the carbon dioxide production shows a decrease in the beginning, as was to be expected according to the van 't Hoff rule, after which, however, it increases again. For this reason we assume that certain limits to the respiration are removed at temperatures below 25°, or an extra respiration develops. After 6 days the carbon dioxide production

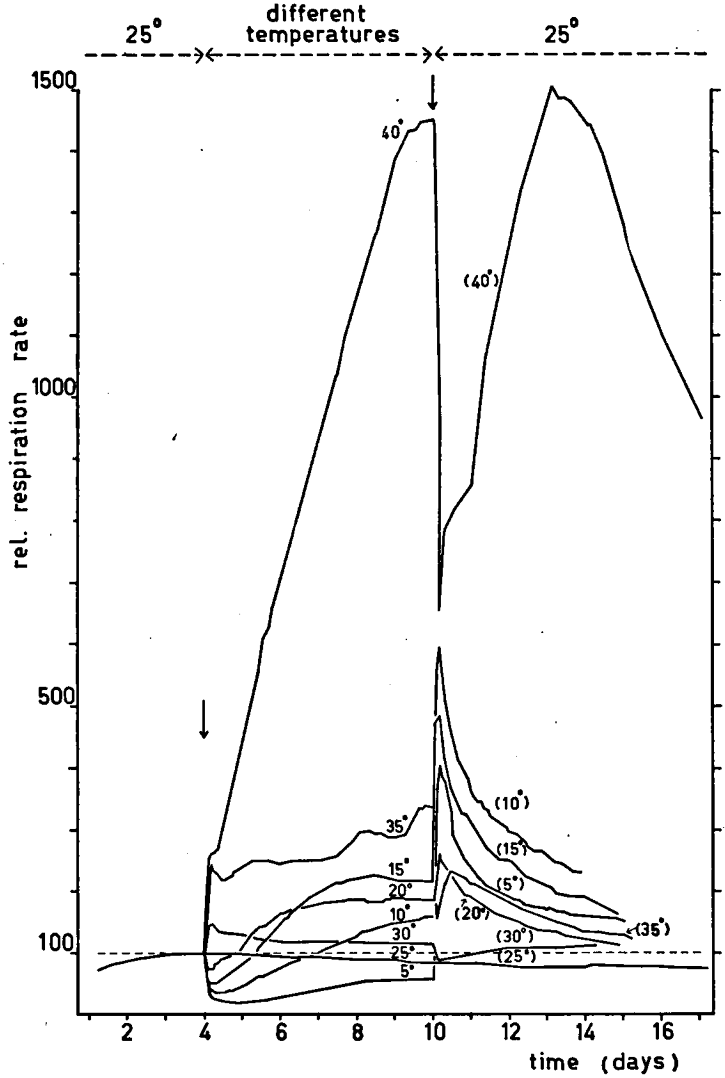

Fig. 9. The course of the respiration rate ($CO_2$ production) of 'van Vliet' bulbs stored at 25° and transferred to different temperatures. After 6 days the bulbs were again transferred to 25°.

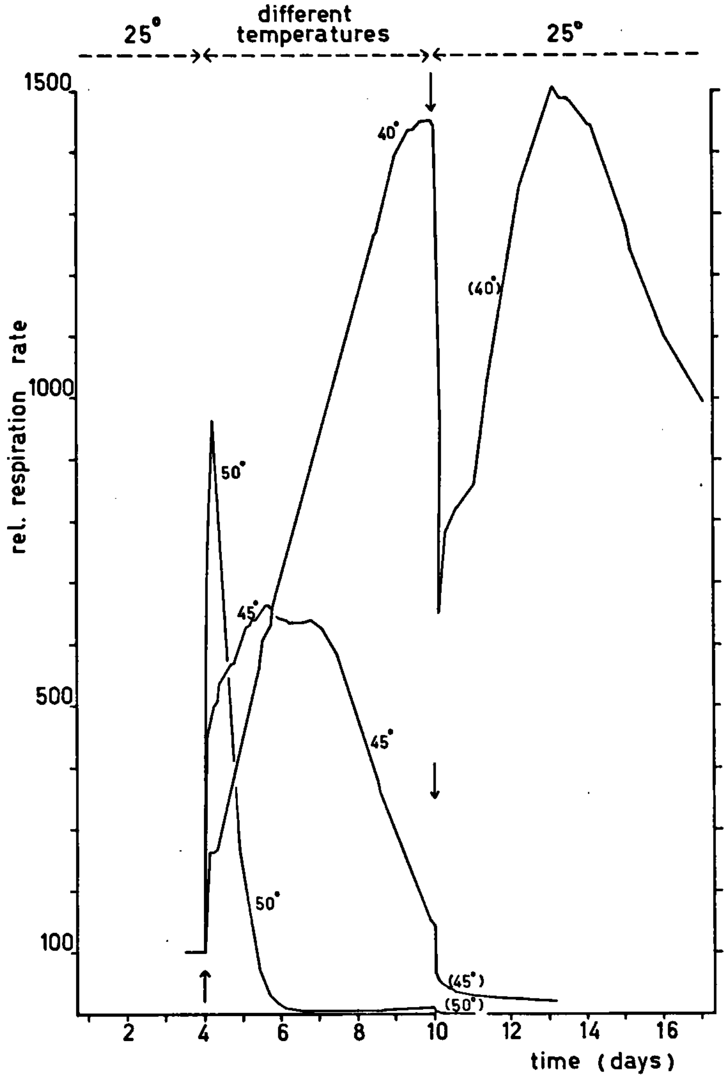

Fig. 10. As in figure 8.

proves to be greatest at 15°. The optimum of this respiration, which develops at low temperatures occurs at approximately 15°, at any rate during the period when the observations were made.

At temperatures above 25° different effects were obtained. At 30° a short rise is followed by a decline and after some days the respiratory value approaches that of the original activity at 25°. At 35° the activity curve proves to be somewhat irregular, in most cases, however, a nearly constant level can be observed.

At 40° at first a pronounced rise occurs in accordance with the temperature coefficient of the process, after which a steady rise can be observed, which may continue for several days. At 45° we may note a sharp rise as well, but it is less pronounced. After that a regression of the curve can be observed. At 50° there is an immediate rise of the respiration rate and the carbon dioxide production, after passing an optimum, changes into a sharp decline. It should be realised in these experiments that a 40° treatment will be lethal for the bulb within a few days. At 45° or 50° the bulb is killed even sooner. No doubt the sharp decline at 45° and 50° is connected with deterioration phenomena of the bulb.

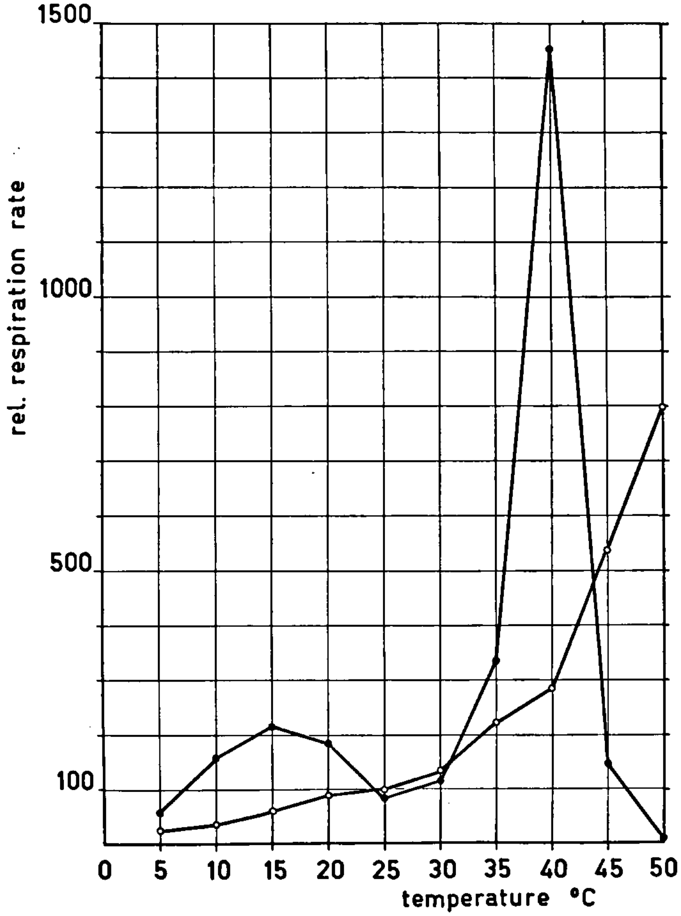

Fig. 11. Relationship between the rel. respiration rate ($CO_2$ production) and the temperature after a ten hours' (open circles) and six days' treatment (closed circles) of 'van Vliet' bulbs.

The temperature effect is probably demonstrated best if we compare an instantaneous picture of the relation between the intensity of the respiration and the temperature after a period of 10 hours with that after a period of 6 days (see Fig. 11). The difference in the two curves is caused by secondary changes in the respiratory system. The secondary rise should therefore be distinguished from a rise of the primary respiration, which is in accordance with the temperature coefficient of the respiration process. In Fig. 11 it can be seen that an optimum occurs at 15° and at 40° after six days. These respiratory activities will be denoted from now on by "15° effect" and "40° effect". In Chapter V.4. these effects will be discussed further.

If after a 6 day temperature treatment the bulbs are restored to a temperature of 25°, it will be observed that the respiratory activity decreases again in all cases except after transfer from 30°. If the causes of the decline of the respiration at temperatures over 40° are not taken into account, a tendency of the activities to return to their original value at 25° can be observed. Therefore the increases in activity caused by the temperature prove to be reversible. We may draw the following conclusions from the observations mentioned.

1. During storage at 25° the respiratory intensity of the bulb is constant and at a low level. At temperatures under 25° activities develop which are retarded at 25°. The optimum of this activity proves — at any rate during the time of observation — to be at 15°. This may be considered either as an increase of activity by means of the development of a new respiratory mechanism, or as a removal of a respiratory blockage.
2. At 30° the respiratory intensity is even lower than at 25° when the normally expected temperature coefficient ($Q_{10} = 2$) is taken into account. From this we conclude that the respiration is repressed most at 30°. At temperatures over 30° the activity increases again.
3. Near the lethal temperature limit, i.e. at approximately 40°, the respiratory activity shows a sharp rise. At 45° and at 50° the respiratory mechanism is soon damaged permanently. This is for instance shown in the sharp drop at 50°.
4. The process (or processes) which is connected with the increase in respiratory activity, at higher as well as at lower temperatures is entirely or partly reversible. Thus, if any activity has developed, it can be reversed by transferring the bulbs to temperatures of 25° or 30°.

The data of the experiments in the seasons 1954/55 and 1955/56 with bulbs of the variety 'Wedgwood' were essentially the same as those of the experiments carried out with 'van Vliet' bulbs. The reactions were generally more extreme with 'van Vliet' bulbs than with 'Wedgwood' bulbs. The respiratory activity after a 6 days' treatment of 15° or 40° was much higher in 'van Vliet' bulbs. These differences in the final respiration level, however, are not fundamental. Thus the conclusions mentioned in the four above items are just as valid here.

Where a comparison can be made, the data about the respiration rate at different temperatures published by RODRIGUES PEREIRA (1962) are in accordance with the more extensive observations given here.

Two more points must be mentioned here. Though there were quantitative differences, the following observations were made in the 'Wedgwood' cultivar, as well as in the 'van Vliet' cultivar.

In the 40° curve a short peak is observed on the first day, shortly after the bulbs are transferred to 40°, whereas a trough is observed in the curve when the bulbs are brought back to 25°. A similar phenomenon is noticed with the curve of 35°. These effects are called the "peak effect" and the "trough effect". They will be discussed in greater detail in Chapter V.5.

Another point that may be mentioned is the fact that in the 50° experiment a slight rise in the carbon dioxide output occurs again after the sharp drop. These symptoms were observed after the bulb had been damaged by the temperature treatment and they are therefore probably connected with an autolysis of the cells. At a temperature higher than 60° the process seems to stop. As far as could be judged from a few preliminary experiments the temperature coefficient of the process will probably be extremely high. The phenomenon was not studied further.

## V.2. TEMPERATURE AND CONSUMPTION OF OXYGEN

It is important to know whether the effects in the carbon dioxide production described above are analogous to those in the uptake of oxygen. Thus we may get information which will enable us to decide whether the carbon dioxide results from respiration, from fermentation processes or from isolated decarboxylation reactions. In the first experiments oxygen consumption and carbon dioxide were measured separately and the results could be compared only in parallel experiments. Later on oxygen and carbon dioxide were measured simultaneously, as a result of which the respiratory quotient could be accurately determined (see V.7.). It should be realised that though the experimental procedure was not changed fundamentally, the respiratory container was much larger because it was necessary to use a greater number of bulbs in the measurements (see II.2.). The existing carbon dioxide tension was considerably greater (see page 369). In order to save time the entire range of temperatures was not studied.

First of all it was investigated whether the 15° effect on carbon dioxide production would be paralleled by a similar effect on oxygen uptake, i.e. whether at this temperature the greatest increase in the respiratory intensity would occur after the bulbs were transferred from the 25° storage to this temperature. Fig. 12 shows the results of the experiments at 10°, 15° and 20° C and indicates that oxygen consumption and carbon dioxide production are similarly affected; a

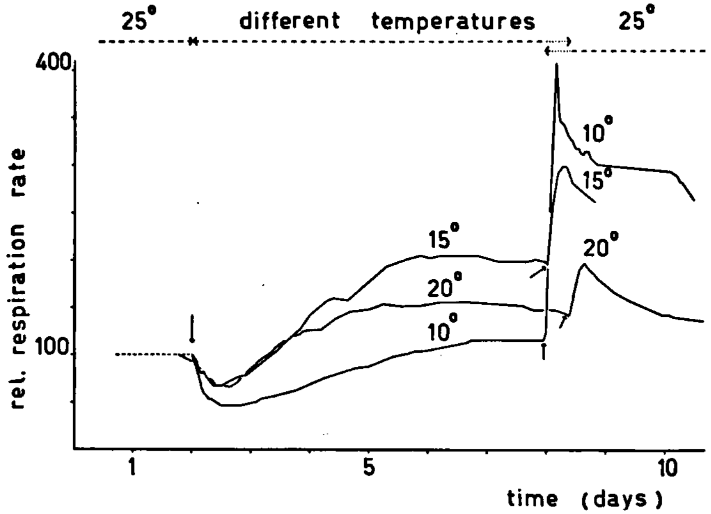

Fig. 12. The course of the respiration rate ($O_2$ uptake) of 'van Vliet' bulbs stored at 25° and transferred to temperatures of 10, 15 and 20° C. After 6 days the bulbs were again transferred to 25°.

temperature of 15° proved to be in fact an optimum both for oxygen uptake and carbon dioxide production.

Next it was investigated whether a 40° effect on $O_2$ consumption could be registered. Fig. 14 shows that this can actually be done. As regards the oxygen consumption the 40° effect again proves to be quantitatively much more pronounced than the 15° effect (compare Fig. 13 with Fig. 14).

These observations give no information about the question whether the oxygen consumption is relatively lowest at 30°, as was found in the carbon dioxide production. These results were not yet wholly

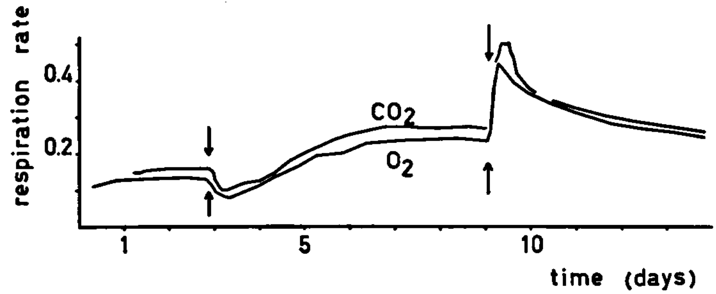

Fig. 13. The 15° effect of 'van Vliet' bulbs. $CO_2$ production and $O_2$ consumption were measured simultaneously. Respiration rate in % of the gas in an air current of 6 l/h.

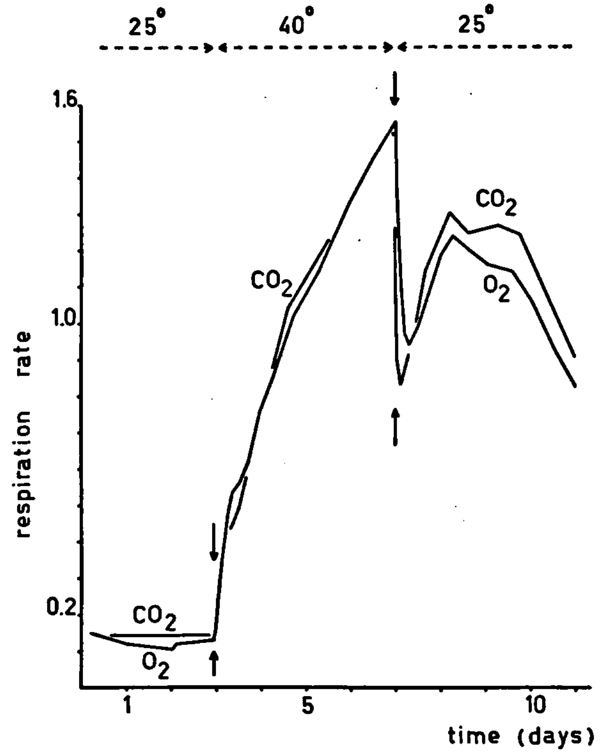

Fig. 14. The 40° effect of 'van Vliet' bulbs. $CO_2$ production and $O_2$ consumption were measured simultaneously. Respiration rate in % of the gas in an air current of 6 l/h.

convincing. Experiments in which bulbs that were stored at 30° were used gave an affirmative answer, whereas the result of an experiment with bulbs stored at 25° did not lead to such a clear-cut conclusion. Probably the time when the bulbs were used for the experiment plays a part here.

Summing up we can say that the rules for carbon dioxide production and those for the consumption of oxygen do not differ fundamentally. In how far the R.Q. changes will be stated later (V.7).

## V.3. CHANGES IN RESPIRATORY ACTIVITY CAUSED BY FACTORS OTHER THAN TEMPERATURE

It was important to find out in how far the temperature effects had a character of their own and whether a relationship between the temperature and other factors which stimulated the respiration exists. For this reason some other factors were investigated as to their influence on the respiration.

### V.3.1. *Influence of the bulb-tunics*

The iris bulb is wrapped in 2 to 4 thin membranes enclosing the bulb tightly. They are remainders of dead leaves which have never been of a fleshy nature as is often the case with other bulbous plants. Apart from the fact that these tunics lend a certain mechanical protection to the bulb, they are an additional safeguard against the loss of water of the bulb (IV.2). It is not known whether the tunics have any influence on the gas exchange. The effects on the respiration described above could even be caused by the presence of a diffusion resistance of the tunics. It was important to investigate this because in all experiments the tunics were left intact, and because in the literature too there are indications that there are reactions in the respiration when the skin of the tulip bulb is removed (ALGERA, 1947) and when the bulb membranes round the onion are removed (SMIRNOFF, 1903). Very recently RODRIGUES PEREIRA (1962) also observed this respiration effect in the iris bulb.

In our experiments it was investigated how the carbon dioxide production and the oxygen uptake were affected when the tunics were removed. In addition it was investigated what the influence is of the oxygen on the respiratory effect that occurs on the removal of the tunics and whether the regression of this effect can have any relationship with the formation of a layer of cork. Finally it was investigated whether after removal of the tunics the 15° and the 40° effects disappeared as well.

Some examples of experiments concerned with the removal of the tunics are given in the Figs. 15, 16 and 17. The carbon dioxide production shows an enormous increase. In about 24 hours a maximum is reached, after which the carbon dioxide production gradually

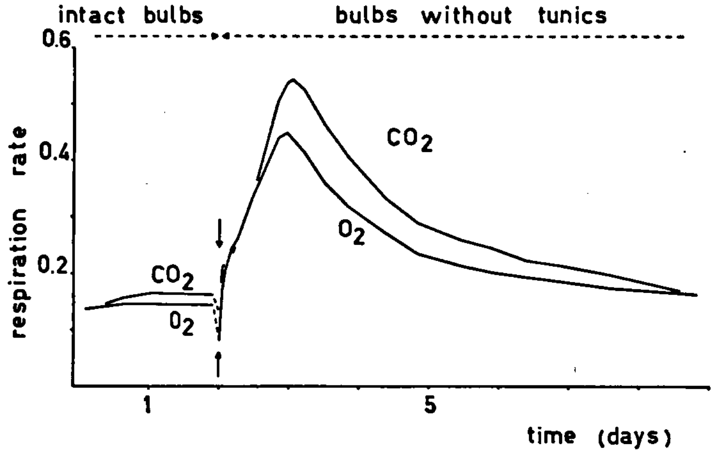

Fig. 15. The respiration rate of 'van Vliet' bulbs before and after the removal of the bulb tunics. The tunic effect. $CO_2$ production and $O_2$ consumption were measured simultaneously. Temperature 25°.

decreases. This regression continues for days (Fig. 16). Though the carbon dioxide production has not been measured for more than 22 days, the original level will certainly be reached again after a long period. So the removal of the tunics causes a real, though temporary effect. The experiment illustrated in Fig. 15 shows that the oxygen consumption temporarily increases to an enormous degree as well. This indicates that it is by no means a question of an isolated emission of carbon dioxide, but a reaction of the complete respiration mechanism. We shall call this effect the "tunic effect".

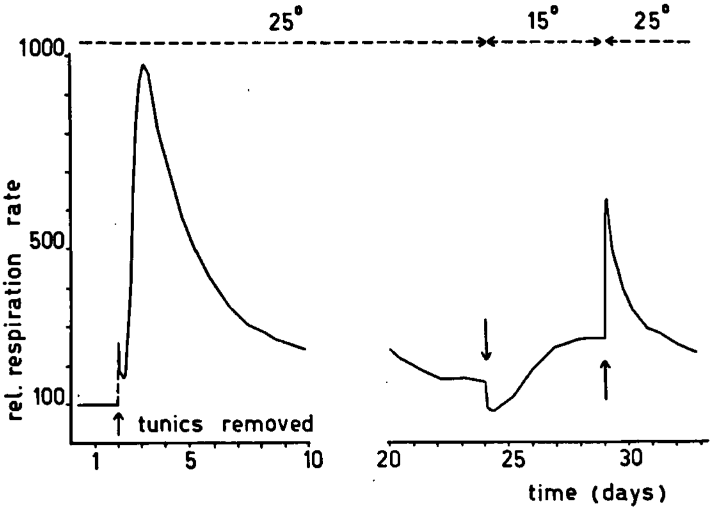

Fig. 16. The 15° effect of 'van Vliet' bulbs after the removal of the bulb tunics. Respiration rate in terms of $CO_2$ production.

There is a difference in the amount of the effect if we compare the experiments in which the $CO_2$ uptake only is measured (Figs. 16 and 17) with the experiments in which $CO_2$ and $O_2$ have been measured simultaneously (Fig. 15). It is observed that in the first case the effect is markedly greater. In the experiment described in Fig. 15 the absolute carbon dioxide concentration in the respiration vessel was about ten times larger; this was a necessary consequence of the technique used for the simultaneous measurement of oxygen. This may be the cause of the quantitative difference between the effects. If this is true, the concentration of the carbon dioxide or some other gas should have an influence on the respiration.

When the bulbs are provided with a nitrogen atmosphere at the moment when the tunics are removed, the tunic effect hardly occurs at all (see Fig. 18). When after some days air is admitted again part of the effect does occur, though the increase and the maximum are not so pronounced. So the tunic effect depends on the presence of oxygen. When the effect is allowed to develop in air to a certain

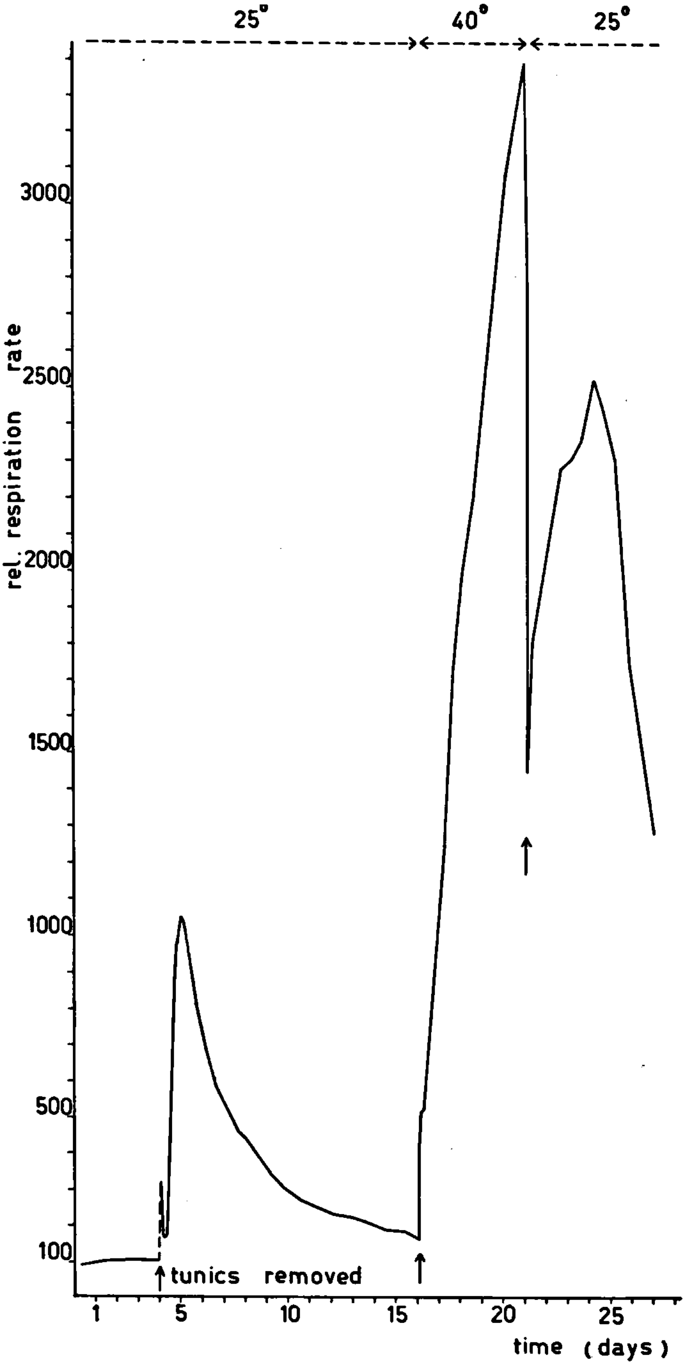

Fig. 17. The 40° effect of 'van Vliet' bulbs after the removal of the bulb tunics. Respiration rate in terms of $CO_2$ production.

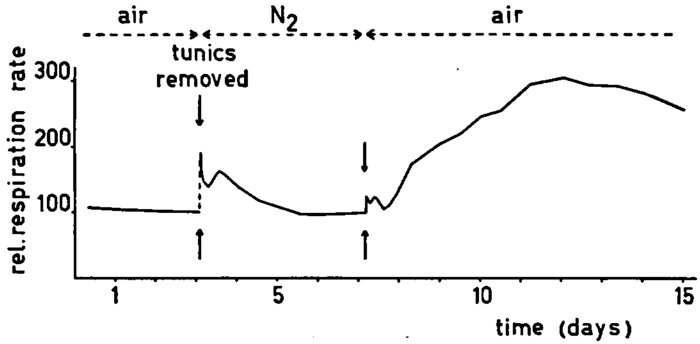

Fig. 18. Influence of an anaerobic atmosphere on the tunic effect of 'van Vliet' bulbs. Respiration rate in terms of $CO_2$ production. Temperature 25°.

point, and at a given moment nitrogen is led across the bulbs, the respiratory rise is replaced by a sharp drop (Fig. 19). The regression of the normal tunic effect is accelerated if oxygen is removed. We may safely draw the conclusion that the tunic effect can be considered as a change in the complete respiration mechanism which is dependent on the presence of oxygen.

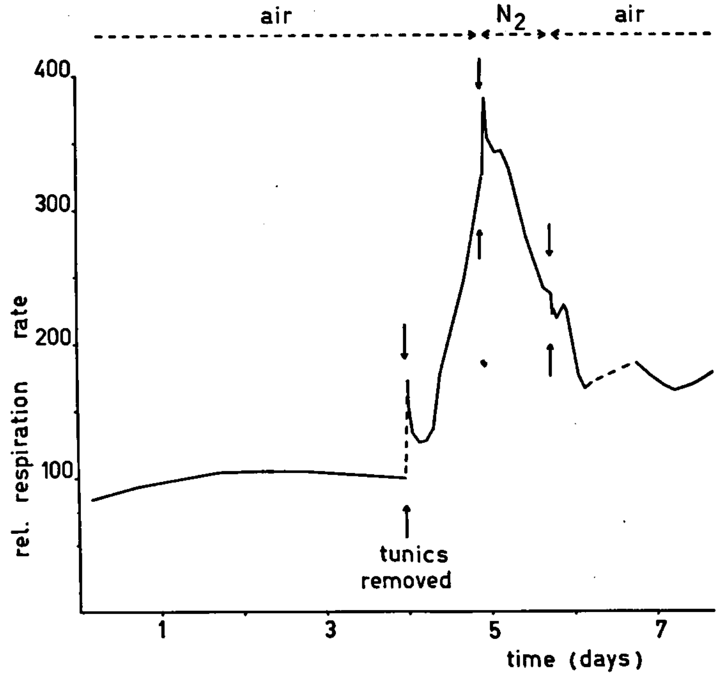

Fig. 19. Influence of an anaerobic atmosphere after a partial development of the tunic effect of 'van Vliet' bulbs in air. Respiration rate in terms of $CO_2$ production. Temperature 25°.

The regression of the tunic effect might be the result of the formation of a cork-layer or of another anatomical formation near the surface of the bulb, which would restrict the oxygen diffusion (compare this problem with observations on potatoes: ROSENSTOCK, 1955 a). In anatomical observations of tissue-slices of the outer scale, however, we did not observe any formation of a cork-layer.

As pure oxygen compared with air produces only a small effect on the respiration of the bulb with its tunics intact, it is improbable that the rise of the respiration after the removal of the tunics is the result of a better supply of oxygen.

The 15° and 40° effect are seen to occur freely after the removal of the tunics. This was shown by experiments illustrated in the Figs. 16 and 17. In these experiments the bulbs were only exposed to other temperatures after the reactions on the removal of the tunics had for the greater part disappeared. The absolute amount of the 15° and 40° effect appeared to be greater than normal. If, however, the 15° and 40° effect are expressed in percentages of the original respiratory intensity before the change of temperature, there is little quantitative difference between the normal effect and the effect after the removal of the tunics. There is no reason for regarding the secondary rise in respiration described above as a result of the normal presence of the tunics. These results raise the question whether there is a relationship between the tunic effect on the one side and the 15° and 40° effect on the other. This will be discussed in VII.8.

### V.3.2. *Influence of mechanical treatment and damage*

The question has arisen in how far the tunic effect can be ascribed to mechanical damage of the surface of the outer scale when the tunics were peeled off. In this case the effect may be ascribed to a wound respiration. The peeling off of the tunics was done with the utmost care and although it is not impossible that small wounds were caused here and there, these were so slight that it is almost impossible that such a great effect could be the result.

In order to find out more about it, an experiment was carried out in which the bulb with its membranes intact was irritated by tapping it with a blunt object. A rise in respiration proved to be the result (see Fig. 20). In analogous experiments symptoms of damage were not observed. A checking experiment in which the bulbs were only put into the baskets and taken from them again gave a negative result. When the bulbs are tapped more energetically with a blunt instrument the respiratory effect is proportionally greater (Fig. 20). Afterwards obvious damage was proved to have occurred. This effect can probably be compared with the effect occurring after the removal of the shoot from the bulb, which caused considerable damage (see Fig. 27). However, we have to take into account that when tapping energetically part of the effect may be caused by loosening of the tunics.

As far as could be ascertained no essential difference was found to exist between the tunic effect and the wound effect after mechanical

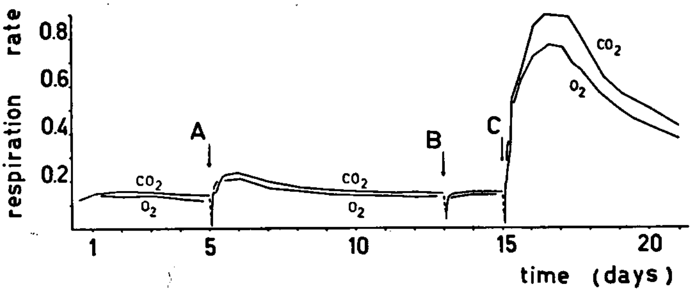

Fig. 20. Changes in the respiration rate after mechanical treatments of the 'van Vliet' bulbs. $CO_2$ production and $O_2$ consumption were measured simultaneously. Respiration rate in °/o of the gas in an air current of 6 l/h. A. bulbs tapped with a blunt object. B. bulbs removed and again brought in the baskets. C. bulbs tapped violently (damages).

damage. In both cases the effect is connected with the aerobic phase of respiration, for in a nitrogen atmosphere there are no effects at all. The tunic effect may also be caused by a mechanical irritation of the outer tissue without any damage. The experiments suggest this, but they provide no conclusive evidence.

### V.3.3. *Influence of micro-organisms*

It is most difficult to prove that respiratory effects that have been observed are not the result of the activities of micro-organisms that have developed. In experiments with the iris bulbs it is not very probable that organisms have developed, since dry air was led across the bulbs all the time, and furthermore no indications of their presence have been discovered during microscopic observation. Nevertheless more proof will be furnished by two more experiments. For these the respiratory rise at 40° was chosen, because under these circumstances the chance for micro-organisms to develop seemed greatest.

In the first experiment the bulbs were taken out of the respiration container during the respiratory rise and for an hour they were put in a small closed container above a 30 per cent formalin solution, also at a temperature of 40°. The formalin vapour acts as a disinfectant, in case any organism has developed on the surface of the bulb. This method was used by DOLK & VAN SLOGTEREN (1930) and by ALGERA (1947). The experiment indicated that the treatment had no effect.

In a second experiment a more direct method of decontamination was used. Before the 40° treatment the bulbs were immersed in a sublimate solution of 0.1 per cent for half an hour. After being dried they were restored to the respiration container and the measuring was continued. Though an obvious stimulating effect of the sublimate treatment was observed, the 40° effect could nevertheless develop undisturbedly afterwards (Fig. 21).

In view of the results of these experiments it is highly improbable that micro-organisms can be held responsible for the respiratory effects.

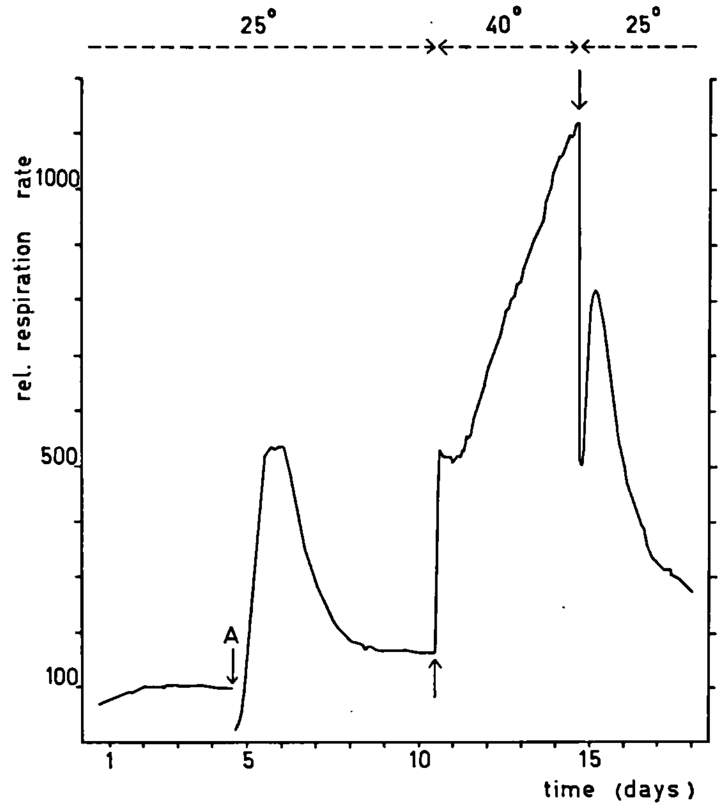

Fig. 21. The occurrence of the 40° effect after a sublimate disinfection of the bulbs. At A the 'Wedgwood' bulbs were removed from the respiration vessel for a short period for the disinfection treatment. Respiration rate in terms of $CO_2$ production.

### V.3.4. *Influence of the air-humidity*

As has been mentioned in Chapter II.2, the air that was led across the bulbs in the respiration container was first desiccated with silica gel. The question arises whether humidified air would have any influence on the respiration. At 25° the respiration of the bulb is practically stable, as was proved by the experiments. In the experiment the air was strongly humidified before it reached the respiration container: it was led through an erlenmeyer flask with humidified filter-paper (temperature 25°). Since the air supply was only 3 liters per hour, the air had a high humidity.

The experiment showed that not the slightest effect occurred.

### V.3.5. *Conclusions regarding the specificity of the temperature effects*

In the beginning of Chapter V respiratory effects have been described that were the results of temperature treatment. In the following sections the influence of various factors on the respiration was investigated, in order to get some insight into the specificity of the temperature effects. These investigations have shown that the temperature effects are not related to other factors mentioned and are most probably the result of changes in the respiratory system, changes which were only caused by the temperature. In the next sections further experiments with temperature will be described and discussed.

## V.4. RISES IN RESPIRATION AT 15° AND 40° (THE 15° AND 40° EFFECT)

In this chapter we shall further discuss some qualities of the 15° and 40° effect. The question to be answered is whether they are two separate effects, or one and the same effect caused by different temperatures.

The phenomena occurring during the temperature change and the phase when the respiration of the bulb adapts itself will be discussed in section V.5. Here we confine ourselves to the secondary rises which last longer.

### V.4.1. *Mutual dependence of the 15° and 40° effect*

The experiment was conducted with the following intention. If the 15° effect is given the opportunity to complete its development until a new condition of steady state is reached, there are two possibilities when the temperature is brought up to 40°. Should the causes of the respiratory rises that develop at 15° and 40° be mutually dependent or identical to each other, then the level of the respiration would only rise according to the temperature coefficient and a new secondary rise would not occur. However, if the two causes work independently, then an additional secondary rise of the respiration would be observed after the temperature change.

From the result of the experiments can be deduced that there is an addition of the two effects (Fig. 22). This supports the theory that the respiratory rises at 15° and at 40° have different causes.

The sharp peaks and the less sharp drops in the curves, occurring with a temperature rise or drop resp., should be considered to be unconnected with the secondary rises (see V.5.3.).

Another approach we are dealing with could be first to bring about a steady state respiration at a temperature of 40°, after which the bulbs are brought to a temperature of 15°. The drawback of this procedure is that other experiments have shown that the lethal limit may be passed after a 4-day period at 40°. For this reason it would be better to do the experiment at a temperature of 39°, of which it is known that it can be carried out without injury for much longer. It was however impossible to bring about a steady state condition at 39°, because in three days' time the sharp rise in the respiration changed rather suddenly into a very clear, sharp drop of the respiration without a change of temperature. These observations also indicate a difference between the nature of the 40° effect and that of the 15° effect.

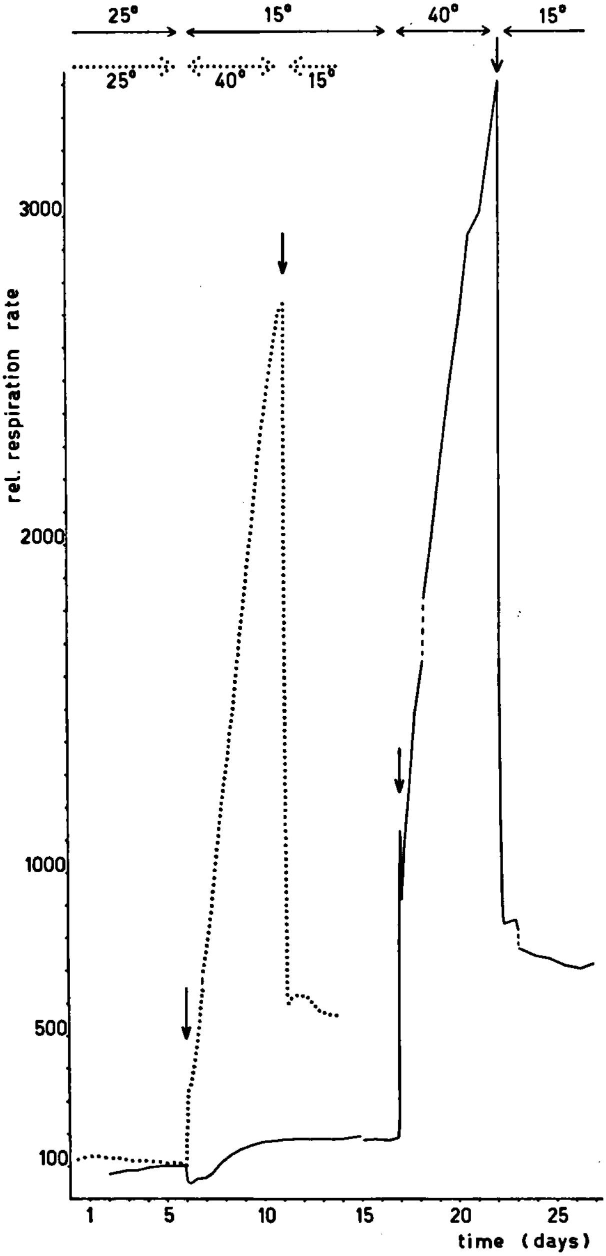

Fig. 22. The 40° effect before and after the development of the 15° effect. Respiration rate of the 'van Vliet' bulbs in terms of $CO_2$ production.

Thus the experiments show that the 15° effect has an independent existence from the 40° effect.

### V.4.2. *Influence of oxygen on the 15° and 40° effects*

A few experiments were made to determine the influence of oxygen on the effects. After the 15° effect had developed to its maximum in air, the bulbs were transferred to pure oxygen, whereas in a similar experiment nitrogen was led through (see Fig. 23A). In an oxygen atmosphere the effect is fully maintained but there is no additional effect. In a nitrogen atmosphere a sharp drop in carbon dioxide production occurs. When air or pure oxygen is added to the nitrogen after the drop, the effect is only slightly restored. The rise is much less pronounced than in the normal development of the effect. We see that a nitrogen atmosphere has largely destroyed the effect. As other experiments have shown that the comparatively short period in nitrogen does not noticeably damage the bulb, we may regard the phenomenon as a normal reaction of the living bulb. Fig. 23B shows an experiment in which the bulbs were taken into an anaerobic atmosphere before transferring the bulbs to a temperature of 15°. This experiment shows that at 15° no effect occurs during the anaerobic phase and that there is no preparation for a later rise in respiration in air either, because the rise in respiration during the

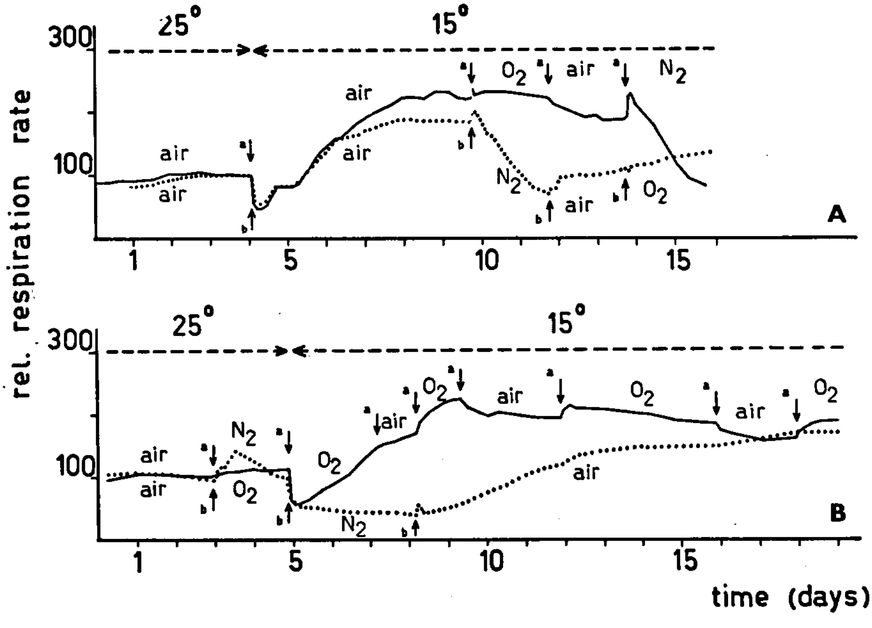

Fig. 23. A and B. Influence of air, oxygen and nitrogen atmospheres on the occurrence and the development of the 15° effect of 'van Vliet' bulbs. Respiration rate in terms of $CO_2$ production. The solid and dotted lines in each figure refer to the different experiments, a and b.

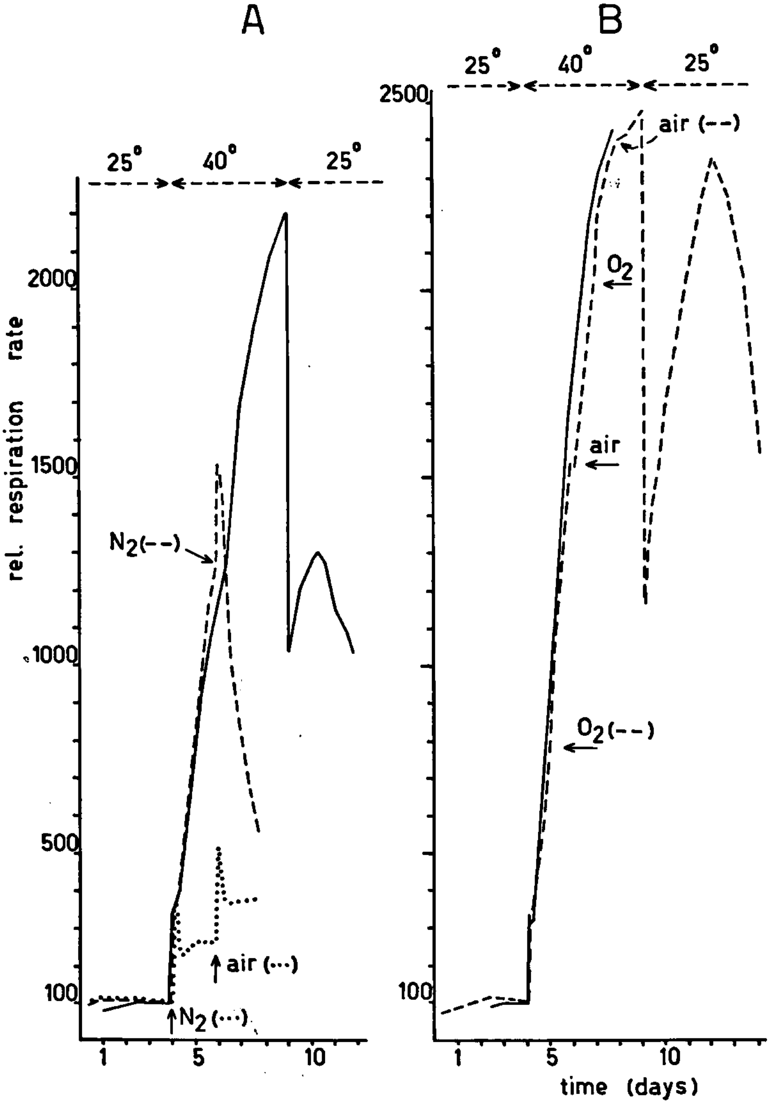

Fig. 24. A and B. Influence of air, oxygen and nitrogen atmospheres on the occurrence and the development of the 40° effect of 'van Vliet' bulbs. Respiration rate in terms of $CO_2$ production. The solid and broken lines in each figure refer to different experiments.

following air-phase is slower than was observed previously in a parallel experiment.

We may draw the following conclusion from these experiments:

1°. That the rise in respiration at 15° only occurs on the condition that there is oxygen present. (This is only to be expected; the following conclusion is more important).

2°. That under anaerobic conditions at 15° no effect is prepared either and any existing effect is destroyed. Probably this means that the process (or processes) causing the development of the secondary respiration is also dependent on oxygen.
A third conclusion can be drawn from the fact that pure oxygen has practically the same result as air with an oxygen concentration that is 5 times lower.

3°. This means that under normal conditions another factor than oxygen limited the development of the 15° effect.

The influence of an oxygen and nitrogen atmosphere on the 40° effect has been investigated as well. These experiments are illustrated in the Figs. 24A and B. The same conclusion can be drawn from these experiments as were given under 1°, 2°, 3° for the 15° effect. It is remarkable that, under conditions of very high oxygen consumption, no effect is observed if air is replaced by oxygen.

### V.4.3. *The oxygen uptake during the development of the two effects*

In some experiments the oxygen consumption and the carbon dioxide production were measured simultaneously during the development of the secondary respiration rises at 15° and 40°. As has been mentioned respiration chambers of a larger size, with a greater number of bulbs were used in these experiments. See Figs. 13 and 14.

They show that the carbon dioxide production runs completely parallel to the oxygen consumption. The respiratory quotient remains practically the same (see V.7). If fermentation had taken place, one would have expected the R.Q. to change. We therefore conclude that an aerobic condition exists in the bulb even during the development of the respiratory rises under conditions of a higher oxygen consumption.

This conclusion is valid for the bulb as a whole and within the limits of accuracy. The possibility remains that small localized parts with an anaerobic condition, which may have escaped our observation, occur in the centre of the bulb.

In very small bulbs (circumference 3 to 4 cm) the 15° and 40° effect proved to be quantitatively of the same order as in bulbs of a normal size (9 to 10 cm), in spite of the fact that the diffusion distances for the oxygen must have been much shorter. This confirms the supposition that under normal circumstances an aerobic condition exists in the bulb.

## V.5. INDUCTION PHENOMENA AT CERTAIN TEMPERATURE CHANGES OF THE BULB

With regard to the carbon dioxide production and the oxygen consumption, peculiar effects were observed during the period when temperature of the bulbs was changed, and a few hours afterwards.

These effects are most apparent at the temperature change from 25° to 40° and vice versa. At a temperature change from 25° to 35° and vice versa reactions were observed as well, but their nature was not very clear. We shall restrict ourselves in our discussion to the observations during the former change.

The period needed by the gas exchange for its adaptation to the new temperature might be called an induction period. This period lasts somewhat longer than the time the bulb needs to adapt to the new temperature (see also V.1 and II.2). It is impossible to state the length of the induction period accurately, because at 40° the earlier discussed secondary rises occur quite soon.

### V.5.1. *The peak effect in whole bulbs*

What is actually observed at the temperature change from 25° to 40° and vice versa? First attention should be paid to the carbon dioxide production of 'Wedgwood' bulbs with a circumference of 9 to 10 cm. After the temperature change the carbon dioxide production increases strongly and reaches a maximum in about three hours. Figure 2 shows that it can be safely assumed that the whole bulb has reached the new temperature. Nevertheless, according to the respiration curve, the carbon dioxide production is seen to decrease, which causes a slight peak (see Fig. 25). The decrease, however, is a temporary one, for when in 8 to 12 hours a minimum is reached, the carbon dioxide production rises again. This is a secondary rise and continues for days. The first effect is called the peak effect. The size of the peak effect cannot be accurately determined, because after the first decrease no new steady state occurs, but another rise in the respiration. As a result, the temperature coefficient of the primary respiration at 25° and 40° cannot be accurately determined either. The $Q\,\frac{25}{40}$ of the primary respiration is approximately 2.68.

In bulbs of the cultivar 'van Vliet' (size 8 to 9 cm) the peak in the course of the carbon dioxide production was less easy to observe. The secondary rise following the peak was so immediate that the peak effect was almost completely lost (see Fig. 9). The average height of the top of the little peak in 'van Vliet' was of the same order as in 'Wedgwood'.

Though the peak effect did not always manifest itself very clearly, a number of experiments showed that there is a real effect that can be reproduced. There are no indications that it may be a result of technical defects in the experimental arrangement or registration. In small bulbs, with a circumference of 3 to 4 cm the effect could be observed as well. The top of the peak was again proportional to the respiratory intensity at the starting-point. The level of the trough after the peak was slightly lower than in bulbs of a larger size, and this corresponds with a $Q_{10}$ of about 1.62.

### V.5.2. *The trough effect in whole bulbs*

With a temperature change of 40° to 25° the opposite of the peak effect occurred (see Fig. 10). We have called this negative effect, a temporary decrease of carbon dioxide production, the trough effect. This effect was always easier to observe than the peak effect, possibly because the starting point of the respiration was much higher. To indicate the size of the effect we may mention that in 'Wedgwood'

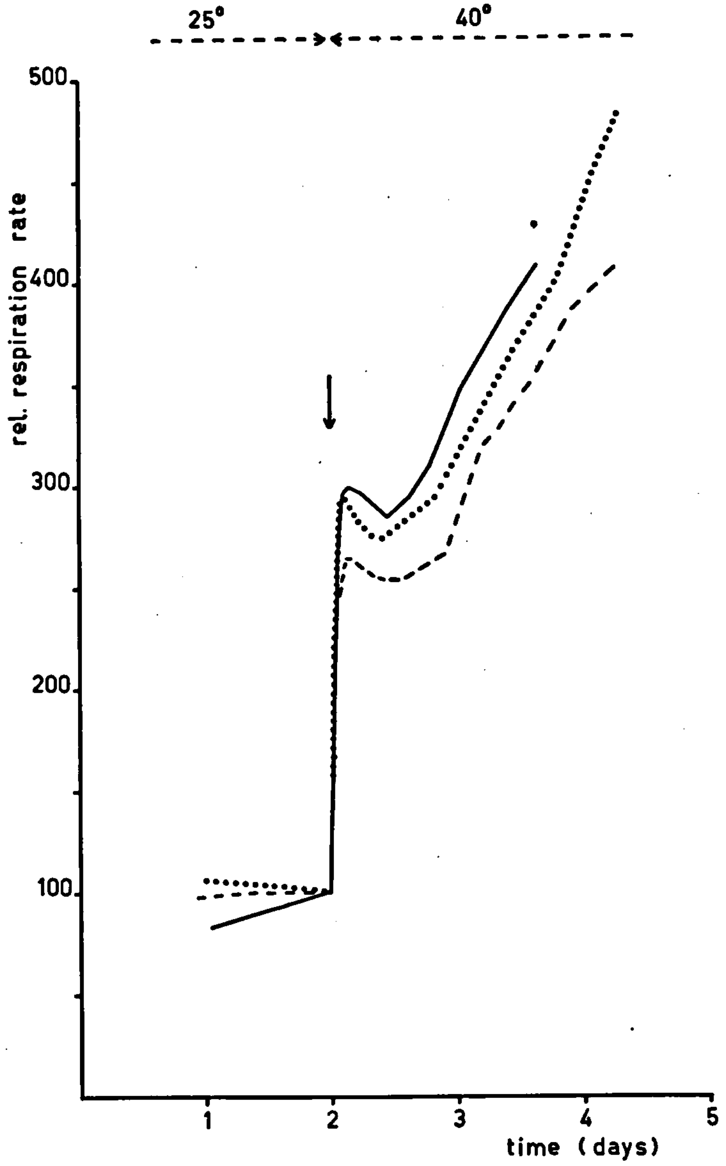

Fig. 25. The peak effect of three different experiments with 'Wedgwood' bulbs at temperature changes from 25° to 40°. Respiration rate in terms of $CO_2$ production.

bulbs (9 to 10 cm) the lowest point of the trough was reached after about two hours and that it was at approximately 50 per cent of the respiration value at the starting point before the change of the temperature from 40° to 25°. The top after the trough was reached in about 13 hours and occurred at a level of approximately 80 per cent of the starting point already mentioned. With 'Wedgwood' the effect was less pronounced than with 'van Vliet'.

### V.5.3. *Relation between the peak effect and the trough effect on the one hand and the secondary respiratory rises on the other*

That the peak effect is fundamentally different from the effects of the secondary rises could be proved by chance. For some experiments small 'Wedgwood' bulbs (3 to 4 cm) were used, whose condition proved to be not very good and whose tissue was no longer quite turguscent. With these bulbs the peak effect could be registered, but no secondary rise was observed (see Fig. 26). This led to the conclusion mentioned above. The trough effect was also observed, without any secondary rises occurring before. The matter is, however, far more complicated, because the observations indicate that the trough effect in a quantitative sense is possibly related and proportional to the level of the respiratory intensity before the change of temperature.

### V.5.4. *The peak effect and the trough effect in shootless bulbs and loose scales*

When the shoot with a part of the base disc and the socle was excised from the bulb by means of a cork-borer the peak effect as

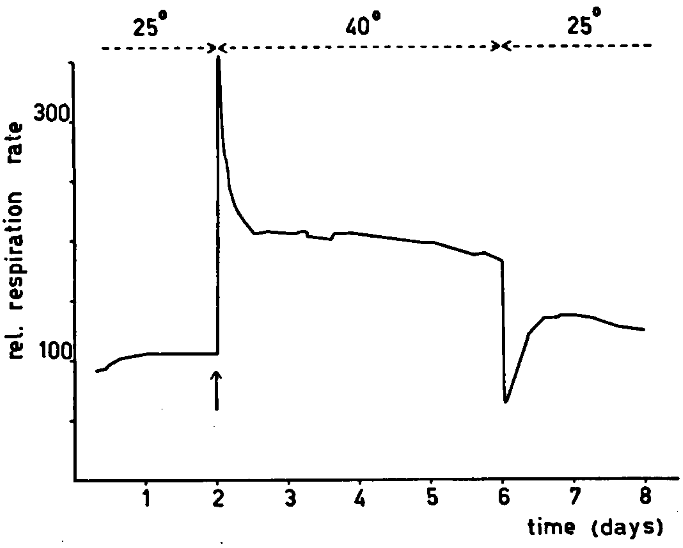

Fig. 26. The non-occurrence of the 40° effect in small 'Wedgwood' bulbs in non-optimal condition. Respiration rate in terms of CO2 production. Only the peak effect and the trough effect are observed.

well as the trough effect in the shootless bulb can be demonstrated (Fig. 28).

In scales that were cut loose, the effects were also noticeable (Fig. 29). There is reason to suppose that the effects can also be studied with the tissue parts.

### V.5.5. *The two effects and the oxygen consumption*

So far the description of the effects was based on observations of the carbon dioxide production. With the oxygen consumption, however, similar phenomena were noticed, though they were less pronounced (see Fig. 14). For the oxygen measurements a larger respiration container was used, which possibly was the cause that the effects were levelled out. It is improbable that it is a result of a changed ratio between the number of bulbs and the capacity of the respiration container, because this ratio remains about the same when the large and the small container is used. In spite of the greater volume of the respiration vessel, the rate of gas-flow does not change. The difference in the size of the effects may be caused by a difference in the carbon dioxide concentration.

It is important for the discussion in Chapter VII.7 to stress that the effects on the carbon dioxide production and the oxygen consumption run parallel. This means that a decrease in carbon dioxide production occurs simultaneously with a decrease in oxygen consumption and vice versa. For a discussion on the nature of the peak effect and the trough effect we refer to Chapter VII.7.

## V.6. LOCALIZATION OF THE 15° AND 40° EFFECT

With the exception of the experiments mentioned in V.5.4. and V.6. whole bulbs were used for all the experiments. We may therefore be certain that so far we only discussed effects "in vivo", without considering their nature. In view of future investigation of the biochemical aspects of the observed effects in tissue parts, the question was put where in the bulb these effects were localized. A closely connected problem is whether the observed respiratory reactions can be recognized in parts of the bulbs.

It should be stated in advance that the problems concerned with the separating of bulb parts and with the study of the respiration of these parts were most difficult. An important difficulty lies in the fact that in proportion to the original respiration a marked wound respiration occurs and that it is only after a long time that a steady state is reached. In addition to this the question arises whether the same respiration mechanism as in the entire bulb is studied now. Moreover, the bulbs without shoots (and certainly the shoots themselves too, if they are not transferred to some liquid) will lose so much water that the scales will be flaccid at the end of the experiment. This implies that it is doubtful whether a normal respiration in that case may be expected. This will be discussed briefly because as yet the results do not provide a clearly outlined picture. Only those experiments that were necessary for the interpretation of the rest of the experiments are mentioned.

In view of the natural structure of the bulb a separation has been made between the bulb scales and the centrally situated shoot: these are the principal parts of the bulb. Technically the separation was easy to accomplish. The shoot can be bored out from below with a cork borer of a suitable size, together with the central part of the living base disc and the socle (the remnant of the base discs from two previous years). In this way a certain organic entity remained, but many fundamental physiological changes certainly have occurred, because many of the vascular bundles have been cut. The shootless bulb and the shoot were used separately for the investigation.

### V.6.1. *Respiration of shootless bulbs and of excised shoots*

In a preliminary experiment with 'Wedgwood' bulbs (9 to 10 cm) the carbon dioxide production of the two separated parts has been investigated. The shootless bulb showed a respiration that rose sharply, then reached a maximum, after which it dropped again. The separated shoot (average length 3.8 cm) did not show this clearly, because secondary infections soon occurred, with all the complications they imply. After the separation both bulb parts, taken together, showed a maximum respiration after 1 day, amounting to about 500 per cent of the respiration of the undamaged bulb at the starting point. Generally one third was ascribed to the shoot and two thirds to the shootless bulb. If we express the respiratory

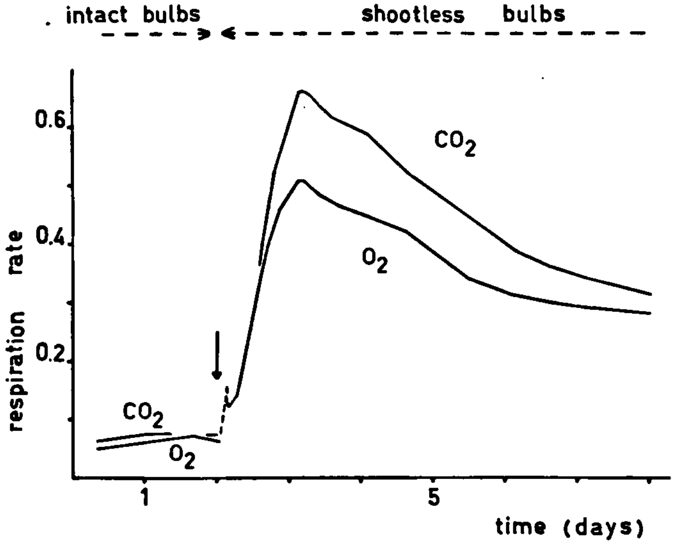

Fig. 27. The respiration rate of 'van Vliet' bulbs before and after the removal of the shoots. Wound effect. $CO_2$ production and $O_2$ consumption were measured simultaneously. Temperature 25°.

intensity in terms of the fresh weight of the separated parts then the ratio of the respiration of the shoot to the shootless bulb is 5 : 1. Strictly speaking these figures tell us very little about the ratio of the respiration of the parts in the undamaged bulb, but the figures do suggest that the share of the shoot may be considerable.

In the cultivar 'van Vliet' (8 to 9 cm) the respiration maximum of the shootless bulb after separation was 15 times higher than the starting point in the undamaged bulb. The shoot respiration was not determined in this case.

In 'van Vliet' the oxygen consumption was measured simultaneously with the carbon dioxide production of the undamaged bulb and of the shootless bulb. See Fig. 27. Both the carbon dioxide production and the oxygen consumption proved to rise sharply after the separation, after which they gradually dropped again. The R. Q. may have risen slightly after the wounding, but it is doubtful whether the differences are significant when compared with normally existing values (see table 7).

Great difficulties with excised shoots were experienced. Because they dried very fast it was necessary to put the shoots in water. As could be seen in analogous experiments with small bulbs this probably entailed changes in the gas exchange, the cause of which we do not know enough about. Moreover, complications occurred because bacteria developed in experiments lasting longer than a day. So far no satisfactory remedy has been found that did not itself influence the respiration. For this reason the experiments with shoots were somewhat doubtful.

### V.6.2. *The 40° effect and the 15° effect in shootless bulbs*

In Fig. 28 three experiments with the 40° effect which were conducted shortly after each other, are illustrated. In the first experiment (solid line) the effect of undamaged bulbs was investigated, in the second (broken line) the 40° effect of bulbs from which the shoots were removed and the cavity filled with norit and sealed with plasticine. In the third experiment (dotted line) the second was repeated, but the cavity was left empty and open. At the result of the wounds the respiration rises about 15 times. In the second and third experiments we waited 5 days before changing the temperature to 40°, although the respiration had not yet reached a steady state. The bulb scales now became so flaccid that it was not desirable to wait any longer.

From the reproducable results we may draw the following conclusions:

1°. That the 40° effect remains after the removal of the shoot.
2°. That in spite of the wound respiration the 40° effect in the three experiments generally is of the same magnitude.

Here it should be noted that the absolute rise of the secondary respiration was taken into consideration and that the first rise according to the temperature coefficient was not taken into account. The secondary rise of 40° is therefore an additional effect and is independent of the intensity of the wound respiration before the temperature change. It is evident that the secondary rise occurs independently.

If we compare the quantity of different 40° effects more accurately, we see that the effect proves to be smallest in the third experiment, where the cavity of the wound was left open. This was not a

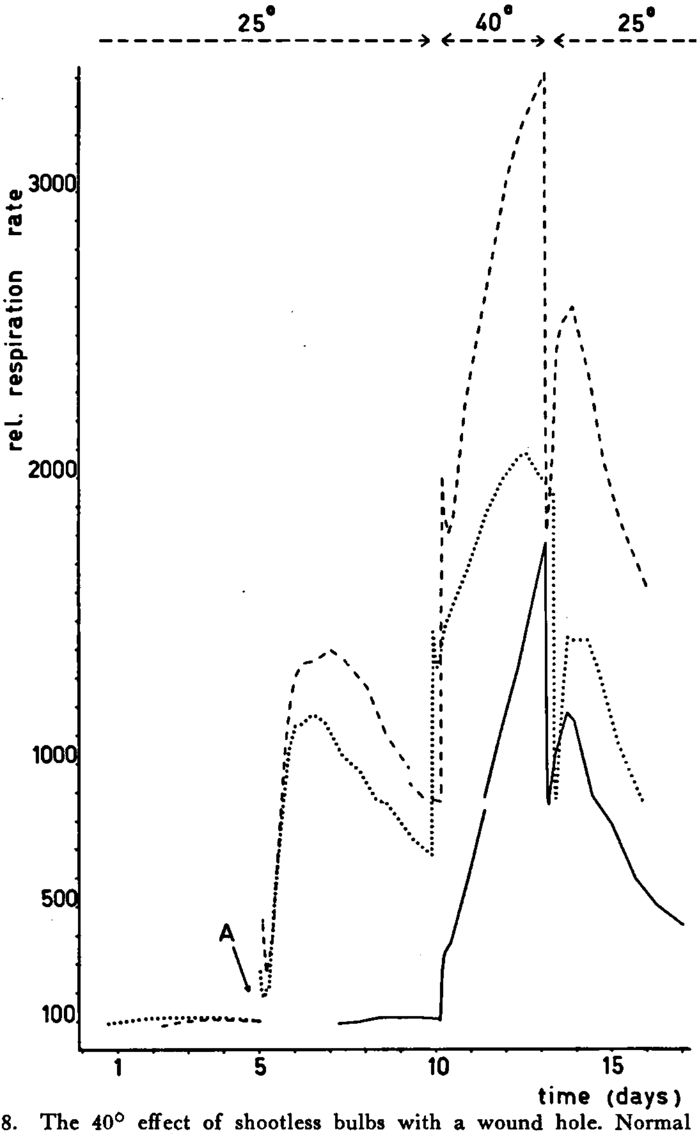

Fig. 28. The 40° effect of shootless bulbs with a wound hole. Normal effect of intact bulbs (solid line). In the other two experiments the shoots were removed at the moment indicated by A. Wound hole filled with norit and sealed with wax (broken line). Wound hole open (dotted line).

chance observation, for the effect was reproducable. This is not surprising if one knows that the 40° effect cannot be demonstrated any more in scales that are cut loose. This important phenomenon is illustrated in Fig. 29. We conclude that the 40° effect is lost as the connection of the scales is broken.

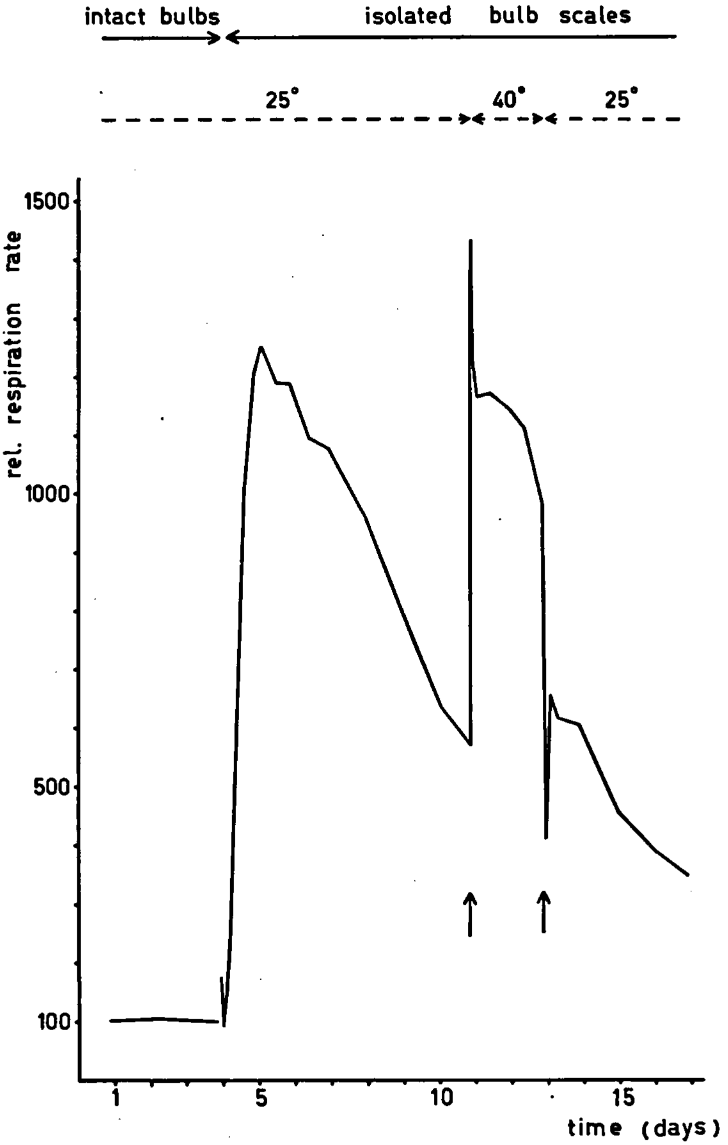

Fig. 29. Non-occurrence of the 40° effect in isolated bulb scales. Respiration rate in terms of $CO_2$ production.

Of the several experiments made with the 15° effect in shootless bulbs only the experiment in Fig. 30 is given. In 11 days' time the wound respiration reached a steady state condition, but the level was much higher than in the beginning of the experiment. If we consider how large the 15° effect is normally, we would certainly have noticed it in the above-mentioned experiments after the eleventh day. Thus the result gives no indication of the existence of the 15° effect in shootless bulbs.

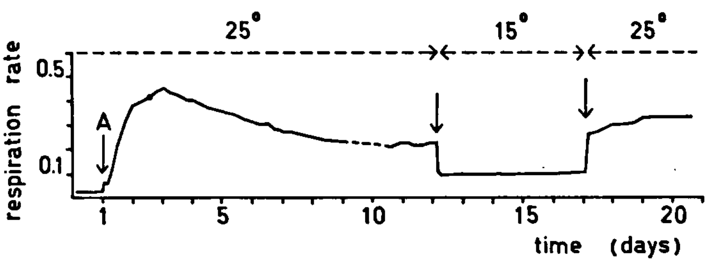

Fig. 30. Non-occurrence of the 15° effect in shootless bulbs. At the moment indicated by A the shoots were removed. Respiration rate in terms of $CO_2$ production.

For the time being we may conclude that in separate bulb scales neither the 15° effect not the 40° effect can be traced. In shootless bulbs the 40° effect was indeed found, but not the 15° effect. However, the results are not entirely conclusive, and should be confirmed. From the experiments with excised shoots no conclusion at all could as yet be drawn.

## V.7. THE RESPIRATORY QUOTIENT

Since in some experiments the carbon dioxide production and the oxygen consumption were measured simultaneously by two independent methods on the same biological material, the possibility was created to calculate respiratory quotients. It is important that in the method used, the respiratory quotient can be continuously followed under conditions that cause a change in the rate of respiration. The arrangement used has the advantage that the bulbs are under normal gas conditions during measuring. It may therefore be assumed that the respiratory quotient is a reflection of normally existing circumstances in the bulb.

The accuracy of the R.Q. is directly determined by the absolute accuracy of the methods of analysis that are used. Although every precaution was taken to ensure the greatest possible accuracy, it is advisable to check the measurements with other methods of gas analysis. In this case it was not done.

From the experimental series we abstracted data about the course of the respiratory quotient, in particular during the changes in the respiratory intensity. These data were summarized in table 7. From the figures obtained during continuous registration of the carbon dioxide production and the oxygen consumption an R.Q. was calculated twice a day and they were put in the vertical columns of table 7. In the table the respiratory quotient of respiration measurements can be followed at 25° and 30° (column 1), in dry and in humid air (column 2), during the respiratory rise at 15° (3 and 4) and at 40° (5 and 6), after the removal of the tunics (7), after the inflicting of wounds (8), and after treating the bulb with a blunt instrument (9). We refer to the figure only when the experiment

TABLE 7

Respiratory quotients in the time-course of the respiration of different experiments (each half day an R.Q. is given)

| 25° and 30° | Dried and humidified air | 15° effect | 15° effect (exp. of fig. 13) | 40° effect | 40° effect (exp. of fig. 14) | Tunic effect (exp. of fig. 15) | Wound effect (exp. of fig. 27) | Mechanical treatment (exp. of fig. 20) |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | 1.14 | | | | | | | |
| 0.97 | 1.16 | | | | | | | |
| 1.00 | 1.19 | | | | | | | 1.00 |
| 1.05 | 1.17 | | | | | | | 1.06 |
| 1.06 | 1.15 25° dried air | | | | | 25° bulbs with tunics | 25° intact bulbs | 1.12 before mech. treatment |
| 1.06 25° | 1.15 | | | 0.94 | 1.03 | | | 1.15 |
| 1.05 | 1.16 | | | 1.00 | 1.20 | | 1.23 | 1.12 |
| 1.09 | 1.13 | 1.06 25° | 1.19 25° | 1.00 25° | 1.29 25° | 1.06 | 1.20 | 1.13 |
| 1.13 | 1.13 | 1.06 | 1.18 | 1.02 | 1.38 | 1.13 | 1.06 | 1.17 |
| 1.14 ▬ | 1.17 ▬ | 1.04 ▬ | 1.18 ▬ | 1.07 ▬ | 1.22 ▬ | 1.13 ▬ | 1.13 ▬ | 1.21 ▬ |
| 1.10 | 1.09 | 0.92 | 1.28 | 1.17 | 1.47 | 1.63 | 1.06 | 0.88 |
| 1.16 | 1.10 | 1.14 | 1.27 | 0.93 | 0.78 | 1.02 | 1.27 | 1.12 |
| 1.14 | 1.10 | 1.12 | 1.11 | 0.99 | 0.98 | 1.40 | 1.32 | 1.12 |
| 1.14 | 1.10 | 1.08 | 1.14 | 0.99 | 1.06 | 0.73 | 1.31 | 1.16 |
| 1.11 | 1.10 | 1.09 | 1.16 | 0.98 | 1.04 40° | 1.26 | 1.26 | 1.15 |
| 1.08 30° | 1.06 25° humidified air | 1.11 15° | 1.16 15° | 0.98 40° | 1.02 | 1.23 | 1.27 | 1.10 |
| 1.08 | 1.03 | 1.07 | 1.21 | 1.00 | 0.99 | 1.23 | 1.32 25° shootless bulbs | 1.11 |
| 1.06 | 1.03 | 1.08 | 1.18 | 1.03 | 0.99 ▬ | 1.22 25° without tunics | 1.27 | 1.14 after mechanical treatment |
| 1.08 | 1.06 | 1.08 | 1.15 | 1.03 | 0.72 | 1.20 | 1.23 | 1.14 |
| 1.08 ▬ | 1.06 | 1.06 | 1.15 | 1.03 ▬ | 1.05 | 1.16 | 1.15 | 1.15 |
| 0.97 | 1.06 | 1.10 | 1.13 | 0.79 | 1.05 | 1.15 | 1.15 | 1.12 |
| 1.07 | 1.06 | 1.10 ▬ | 1.13 | 1.14 | 1.04 | 1.14 | 1.11 | 1.09 |
| 1.07 | | 1.06 | 1.14 | 1.11 25° | 1.08 | 1.10 | | 1.12 |
| 1.10 | | 1.04 | 1.22 ▬ | 1.11 | 1.10 25° | 1.04 | | 1.16 |
| 1.10 | | 1.03 | 1.03 | | 1.10 | | | 1.16 |
| 1.10 25° | | 1.04 | 1.04 | | 1.11 | | | 1.09 |
| 1.10 | | 1.03 25° | 1.04 | | 1.09 | | | |
| 1.14 | | 1.03 | 1.05 25° | | | | | |
| 1.14 | | 1.02 | 1.07 | | | | | |
| 1.14 | | 1.05 | 1.06 | | | | | |
| | | | 1.05 | | | | | |
| | | | 1.06 | | | | | |

from which the data were taken is illustrated. The columns also feature the bulb treatments.

The measurements show that the respiratory quotient of undamaged bulbs at 25° is 1.11 on the average. No clear trend in the changes of the respiratory quotient can be distinguished during the rises and drops of the respiration. The changes are no greater than in the respiration at 25° in a condition of steady state. Other authors also mention the fluctuating R.Q. values (VERLEUR, 1960; MÜLLER, 1958; BIALE, 1946). Whether the fluctuations of the R.Q. should be ascribed to real changes of the respiratory mechanism or to unforeseen experimental conditions cannot be decided without further investigation. If an obvious fermentation had occurred in the experimental series, this would certainly have resulted in higher R.Q. values.

## CHAPTER VI

# THE NATURE OF THE SUGARS AND THE INFLUENCE OF THE TEMPERATURE ON THE SUGAR CONTENT OF THE IRIS BULB

### VI.1. THE NATURE OF THE SUGARS

By means of the technique described in Chapter II.4. the nature of the soluble sugars in the bulb was investigated. The chromatograms showed that sucrose, glucose and fructose were present. The sucrose content was considerably greater than the glucose or fructose content. The identity of the sugars was deduced from the fact that the unknown substances on the chromatogram showed $R_F$ values similar to those of reagent grade sugars. Apart from the three sugars mentioned the extract of the iris bulb produced a number of more or less clear spots with an $R_F$ value less than that of sucrose. These spots were also identified by means of spray-solutions containing benzidine and para-anisidine. It was only after long flowing periods that it was possible to separate these spots from each other. Three of them were clearly distinguishable, but two others could be made visible only with difficulty. The position of these substances, probably all sugars or sugar containing compounds, has been approximately determined with respect to the position of the sucrose and expressed in $R_S$ values. For the three clear spots the $R_S$ values were 0.09, 0.17, and 0.44 and for the two faint spots 0.24 and 0.34. Hydrolysis with HCl or with invertase of an extract of these spots from the paper was made. After hydrolysis of the substances with $R_S$ value 0.09 and 0.17, glucose and fructose could be detected, whereas in the substance with $R_S$ value of 0.44 (hydrolyzed with HCl only) a small amount of glucose was found.

At first sight there seems to be some resemblance between these fractions and the oligosaccharides, separated by BACON (1957) from extracts of onions (*Allium cepa* L.). A further investigation would be required to verify this, but none was carried out.

The result of this experiment was that the oligosaccharides (?) may have caused inaccuracies in the sugar determinations that were carried out on the crude extract, because after hydrolysis they have caused reducing products to be formed. The quantity of disturbing compounds was, however, small in comparison with the quantity of sucrose.

## VI.2. INFLUENCE OF THE TEMPERATURE ON THE SUGAR CONTENT

It is important to determine whether any correlation between the sugar content and the respiration activity exists. Several authors have pointed out that in their experiments the sugar limited the respiration rate and that by means of a temperature treatment changes in the sugar content which directly affected the respiratory intensity, could be brought about. The experiments with various objects however gave contradictory results (see Chapter VII.4.). We have investigated the effect of temperature changes on the iris bulb. A number of bulbs are given the same temperature treatment as in respiration experiments, where the reactions on the temperature were known. It was investigated whether the sugar content changed under the same conditions.

In the first series of experiments 'van Vliet' bulbs were taken from the 25° storage and transferred in batches to temperatures of 0, 5, 10, 20, 30, 35 and 40° C. The sugar content of the bulb, determined after 6 days, can be found in table 8. The reducing power before and after hydrolysis is shown in columns 2 and 3. The difference that

TABLE 8

Sugar content in % of fresh weight of the entire iris bulb ('van Vliet') after a six days' treatment at different temperatures

| Temperature treatment °C | Reducing power before hydrolysis (calculated as % glucose) | Reducing power after hydrolysis | % sucrose (deduced from difference between red. power before and after hydrolysis) | % sucrose (directly determined after a chromatographic separation) |
|---|---|---|---|---|
| 0 | 0.21 | 3.44 | 3.23 | 2.6 |
| 5 | 0.17 | 3.01 | 2.84 | 2.3 |
| 10 | 0.15 | 2.78 | 2.63 | 2.4 |
| 15 | 0.19 | 3.15 | 2.96 | 2.4 |
| 20 | 0.20 | 2.95 | 2.75 | 2.2 |
| 25 | 0.18 | 2.92 | 2.74 | 2.0 |
| 30 | 0.15 | 3.22 | 3.07 | 2.2 |
| 35 | 0.18 | 3.17 | 2.99 | 2.5 |
| 40 | 0.19 | 2.68 | 2.49 | 2.0 |
| mean: | 0.18 | 3.04 | 2.85 | 2.3 |

is found (column 4) must be ascribed mainly to the sucrose present, as is shown in column 5, in which the chromatographically determined sucrose content is given. If columns 4 and 5 are compared, other compounds are seen to have been submitted to hydrolysis and to have had their share in the increased reducing power. The qualitative analysis (see Chapter VI.1) also proved this.

On closer inspection of the figures there proves to be no ground for the supposition that the temperature treatment of the bulb has resulted in clearly definable changes of the sugar content after 6 days. The slight changes to be found in the figures are probably the effect of a normal variation and are not in any respect proportional with the changes the respiratory intensity has been subjected to.

The same conclusions can be deduced from the experiments of Rodrigues Pereira, when we restrict ourselves to a few days of his observations, which continued for a much longer time than ours. The normal concentrations of both the reducing sugars and of the sucrose in the bulbs of our experiments are in accordance with those found by RODRIGUES PEREIRA (1962).

A second series of experiments indicated the same conclusions. In this series batches of 'Wedgwood' bulbs were taken from the 25° storage and transferred to 15°. At various times after the transfer the sugar content was determined. After a 6 day period of 15° the bulbs were again returned to 25°. Here the temperature has also had no effect on the sugar content, in spite of the fact that the respiration must have changed considerably.

We therefore conclude that these preliminary experiments do not indicate a coincidence of an increase in respiration and the sugar content.

## CHAPTER VII

## DISCUSSION

The most important facts which have come to light in the course of the experiments will now be discussed. One should constantly keep in mind that the bulb has a heterogeneous structure and that the effects observed may occur only in certain parts of the bulb and not in the whole. Our point of departure is that the iris bulb at 25° and 30° is in a stationary phase, which in a certain sense may be called a rest phase. This applies to both the morphological development and the respiratory activity. At these temperatures the initiation of new primordia of the growing-point goes exceedingly slowly and there is practically no elongation-growth of any importance (so-called retarded bulbs). The respiratory intensity is also almost stationary and is in a steady state. As appeared from the investigations the lowest respiratory activity is practically always registered at the temperatures mentioned, so that we can speak of a rest metabolism or basal metabolism. In this phase the respiratory process consumes glucose of the order of about 0.008 mg/h/g fresh weight.

If we take respiratory activity as a standard for metabolic activity, we see that the latter is enhanced by changes in temperature. Both at temperatures lower and higher than 25° and 30° the respiratory activity increases, i.e. the carbon dioxide output and the oxygen uptake rise. If measured after six days' treatment the activity at 15° and 40° has increased most of all. Especially at 40° we observe a pronounced increase. At 15° another steady state is reached after six days. At 40° this is not the case, as the activity still increases after six days. When after this period the bulbs are restored again to temperatures of 25° and 30° a slow regression of the increased activity takes place. It can be deduced from this that the processes connected with the increases in activity are fundamentally reversible. Most probably a certain time-limit exists for this reversibility. The respiratory quotient (R.Q.) remains almost unchanged at 15° and 40° during the respiratory increments. If oxygen is excluded the two effects do not occur. Both the 15° and the 40° effect is therefore obligatorily aerobe. If oxygen is excluded increases in respiration naturally do not occur, and moreover no preparations whatsoever take place, which when oxygen is admitted later, could lead to a greater than normal rate of increase. The respiratory stability can also be interrupted by means of wounding or by the removal of the dead tunics round the bulb. If this is done very pronounced increases in the respiratory rates occur.

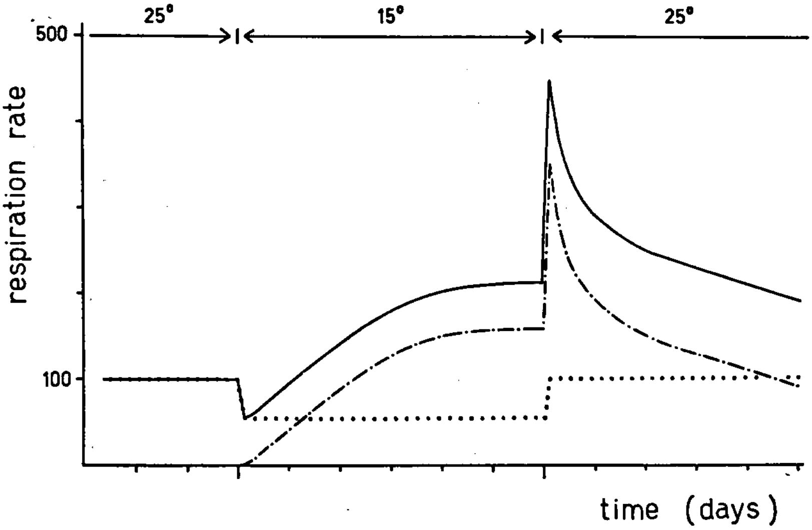

Fig. 31. Analysis of the respiratory curve of the 15° effect. The solid line, dotted line, and alternately broken and dotted line represent the observed respiration, the basic respiration, and the secondary of developed respiration.

## VII.1. ANALYSIS OF THE RESPIRATORY CURVES AT 15° AND 40°

In Figs. 31 and 32 analyses of the time-course of respiration at 15° and 40° are given. Before and after these temperatures a temperature of 25° has been given. The curves shown here are slightly styled representations of the real observations. In the 15° curve the time-course of respiration may be regarded as being constructed by adding a "basic respiration" (dotted line) to a "developed respiration" (alternately broken and dotted line). At 15° a secondary respiratory increase develops, which — as will be discussed in VII.2. — is related to growth activity. This "developed respiration" is

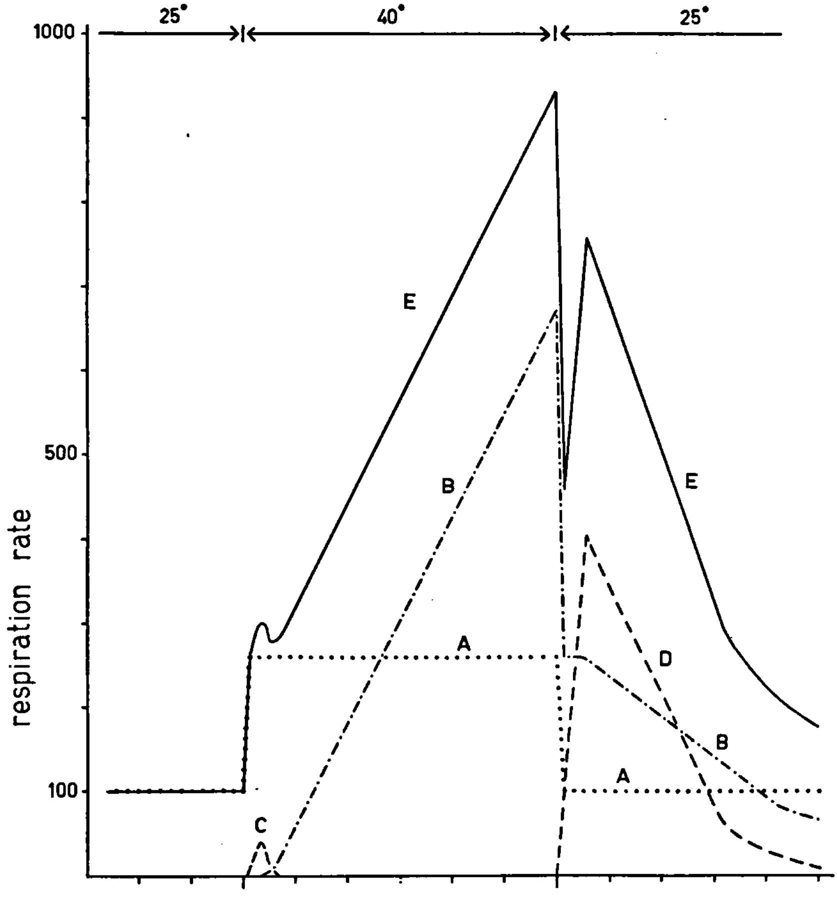

Fig. 32. Analysis of the respiratory curve of the 40° effect. The solid line, dotted line, and alternately broken and dotted line represent the observed respiration (E), the basic respiration (A), and the secondary or developed respiration (B). Broken line C represents the peak effect, and broken line D represents a hypothetical reaction which leads to the trough effect.

reversible as is evident when the bulbs are exposed again to a temperature of 25°. This temperature also retards the growth of the shoot in the bulb again. Allowing for the temperature coefficient of the respiratory process, the interpretation of the curve did not give us any difficulties.

The interpretation of the curve at 40° (25° before and after) is more complicated. A "basic respiration" and a "developed respiration" can likewise be distinguished (dotted line, and alternately broken and dotted line), but some complications arise in the 40° effect which have not been observed in the 15° effect. As may be seen from Fig. 32 special effects occur, if an abrupt increase of temperature takes place. These have been called the "peak effect" and the "trough effect". These effects have been described in Chapter V.5. and will be discussed in greater detail in Chapter VII.7.

## VII.2. RELATIONSHIP BETWEEN THE RESPIRATORY EFFECT AT 15° AND THE GROWTH OF THE SHOOT

In the activity development of the respiration an optimum of 15° has been observed in the iris bulb, at least during a six days' observation. From other experiments it was concluded that in the same range of temperature an optimum activity develops in the growth of the parts of the shoot which had been retarded in growth at temperatures of 25° and 30°. In as far as the accuracy of the measurements of the growth makes it possible to reproduce the real incipient activities of the growth, an increasing activity of the respiration is observed already after some hours, but only after some days an elongation of the parts of the shoot becomes noticeable. The question arises whether there is a causal relationship between the two activities. In addition to the iris bulb, increases in respiratory activity and incipient growing activity have also been observed in other objects, if they are taken from storage temperature to lower temperatures (garlic bulbs: MANN & LEWIS, 1956; bulbils of *Ranunculus ficaria* L.: BORRISS & SCHMIDT, 1957). In the iris bulb the problem of the relation between rest and growth and temperature shows a certain parallelism with the same problem in these objects. Mann & Lewis associate the respiratory activity in garlic bulbs with the "rest-breaking process". BORRISS & SCHMIDT also investigate the influence of temperature on the germination and the respiratory activity, but in their case these activities do not seem to run quite parallel.

In dealing with the iris bulb, we shall examine whether arguments exist which point to a relationship between iets growth and its respiration. In the first place it has been established that the development of activity both as regards respiration and growth takes place optimally at a temperature of about 15°. Moreover the two activities are fundamentally reversible and subject to retardation by restoring the bulbs again to 25° or 30°. The restriction has to be made here that after a prolonged 15° treatment the reversibility can fade away. This phenomenon is inherent to the resting-phase of the bulb. To what extent this reversibility disappears with the duration of the treatment at the low temperature has not been further examined.

When the bulbs are stored for a prolonged time at retarding temperatures of 25° or 30°, the parallelism between growth and respiratory activities is maintained, i.e. if a temperature of 30° is compared with 25°, the lower respiratory activity coincides with a lower activity of the growth of the shoot (Figs. 5, 6A and table 4).

In addition it has been observed that at a lower temperature than the retarding temperature, e.g. at 20°, a clear growing activity keeps pace with a pronounced increment of the respiratory activity (see Fig. 6A). A further argument is provided by experiments with bulbs from which the shoots have been excised and where it was observed that no other secondary increase than the wound respiration occurred at 15° (see Fig. 30).

Furthermore it may be asked to what extent the respiration and the growth react similarly to other environmental factors. Both processes have proved to be dependent on oxygen. Whether the quantitative relations between the growth of the shoot and the oxygen concentration on the one hand and the same relations between the respiratory activity and oxygen concentration on the other hand correspond, cannot yet be said. The influence of the $pO_2$ on the respiratory activity at 15° has not yet been examined, but we did examine the influence at 25° (see Fig. 7). In pure oxygen the respiration at 25° is higher than in air (10 to 20 %). If an oxygen concentration of 10 % is provided, the respiration is lower than in air (10 to 20 %). The relationship between the respiration rate and the $pO_2$ at 25° is therefore not likely to give an optimum curve. However, this seems to be the case in the relationship between the incipient growth of the shoot and the $pO_2$ at least at 15° (see table 3). In pure oxygen the growth is significantly retarded. In this case malformations in the growing-point have been observed. We have reason to assume that the incipient growth of the growing-point is stimulated by a reduced $pO_2$. The relation between the $pO_2$ and the growth possibly gives an optimum curve. Similar observations were made by RANSON & PARIJA (1955) in coleoptiles, hypocotyls and epicotyls. The concept of growth, however, will have to be defined by means of further investigations, for it is not at all certain that the increase in length that we have measured is solely the result of elongation growth, as was the case with coleoptiles, epicotyls and hypocotyls in the experiments of Ranson & Parija. In order to ascertain the quantitative relationship of the growth and the respiration in the iris bulb accurately, it is necessary to make further observations and to study the influence of the $pO_2$ on the respiration at 15°.

The above data at any rate point to a correlation between the growth and the respiration at 15°. Similar relations were mentioned by a number of authors: BONNER, 1933, 1949; COMMONER & THIMANN, 1941; KANDLER, 1950, 1953; ZIEGLER, 1951; RAMSHORN & KOENIG, 1959; RAMSHORN, 1959.

However, on closer examination it often appears, that in a quantitative sense the growth and respiratory activity do not completely correspond. The exact relationship is not at all clear. This discrepancy may be explained by supposing that respiration at a higher $pO_2$ is little or not influenced, while the growth is strongly reduced owing to the oxydative destruction of auxin (Ranson & Parija). KANDLER (1953) points out that under condition of a higher $pO_2$ the "degree of synthesizing intensity" may be lower, even if the respiration is higher.

From the experiments in a very recent publication of RODRIGUES PEREIRA (1962) a relationship between the respiration activity of the shoot and a temperature of about 15° may also be deduced. He came to the conclusion that this respiration activity is localized in the central shoots and not in the bulb scales. Moreover his experiments gave indications that this activity may be localized in the basal part of the shoot in the central disc (meristematic activity of a basal growth?).

## VII.3. RELATIONSHIP BETWEEN THE RESPIRATORY EFFECT AT 40° AND OTHER PROCESSES

In the 15° effect interrelations with the growth-process of the shoot are probable, but for the 40° effect there are no clear indications entitling us to associate this process with another. Therefore we shall specify some properties of the 40° effect and discuss the reason why it cannot be associated with some processes to be mentioned. The effect is probably of a more general nature, for in the case of potatoes we have diagnosed an analogous phenomenon. It appeared from experiments with iris bulbs that the 40° effect is independent from the 15° effect. This has been demonstrated clearly in Chapter V.4.1., where an addition of the 15° and 40° effect proved to be possible. If the 15° effect develops to its maximum, the 40° effect can develop additionally (Fig. 22). This implies that either another part of the respiratory mechanism is activated or that the effects have been localized in different parts. We regard the latter as more probable. This localization will be further dealt with in the final discussion (VII.9.). The effect is independent of the wound respiration which occurs when removing the shoot from the bulb (Fig. 28), but it is possible that it is related to the increase in respiration which initiates after the removal of the tunics (Fig. 17). It seems improbable that the increase in respiration is a result of a change of the amount of the respiratory substrate, because of an autolysis process which could occur at high temperatures. As has been said, the R.Q. remains the same. An additional reason to exclude the possibility of an autolysis is that the 40° effect could not be reproduced with separated bulb scales. Neither could the effect be observed with bulbs in a bad condition, in which an autolysis can rather be expected. A temperature of 39°, which can be endured by the bulb for a long time without any damage, causes a similarly pronounced increase as at 40°. For this reason, too, an autolysis is not likely to cause the 40° effect.

It could be thought that an increase of the $CO_2$ production is the result of anaerobic conditions in the bulb. The 40° effect, however, is strictly aerobe. In a nitrogen atmosphere there is not only no effect whatsoever, but when we exclude oxygen there is no preparation for a later rapid increase, when oxygen is admitted. The opposite will rather occur. This is an important argument against the idea that the effect is a result of anaerobiosis. The increase during the development of the 40° effect is not influenced by an oxygen concentration that is higher than that of air. Thus oxygen is not a limiting factor under these conditions.

Up till now no relationships of the 40° effect with other processes have been demonstrated. We shall have to keep in mind that there is a possible relationship of the 40° effect and the tunic effect. In the discussion under VII.8. this will be mentioned again. Conclusions about this problem, however, must be suspended until a further investigation.

## VII.4. SUBSTRATE AS A FACTOR LIMITING RESPIRATION

Before proceeding to a discussion of the collateral effects of the respiratory activity, it will be useful to keep in mind to what extent the investigation has given indications on the contingent limiting factors in the regulation of the respiratory mechanism. As regards its numerous possibilities, the investigation has only given some information on two of these factors. We shall restrict ourselves to the two outer factors of the respiration chain, that is to say substrate (VII.4.) and oxygen (VII.5.) and we shall state to what extent they have limited the respiration at a temperature of 25° in a relative minimum of activity.

A substrate limit of respiration has mostly been examined in tissue parts and micro-organisms under conditions of a low substrate level and of a supply of substrate in the environmental solution. Very much less research on intact organs with internal substrate sources has been made. We restrict ourselves to this subject. It was BARKER (1936) who in his investigations on potato tubers clearly pointed out that the curve of the relation between the sugar concentration and the respiratory rate had the character of a rectangular hyperbole and that in fact we had to do with an enzyme-substrate curve. GANE (1936) mentioned a similar relation between the sugar concentration and the respiratory activity in bananas. In other objects a similar relation is not found. BORRISS & SCHMIDT (1957) reject a similar relation in bulbils of *Ranunculus ficaria* L.. The results of HASSELBRING & HAWKINS (1915) with sweet-potatoes do not point in this direction. Without discussing these results any further, we pass on to the result found in introductory determinations of sugars in the iris bulb.

In Chapter VI.2. it appeared that in the short period of six days neither the sucrose content nor the reducing-sugars content is subject to changes by temperature treatments. Considering the great changes in the respiratory activity called forth by the same temperature treatment, a complete discrepancy can be observed between the two phenomena. We must stress that it was only a six days' period, for it is not at all impossible that prolonged temperature treatment would lead to observable changes in the content of soluble sugars. Fundamentally it is possible that changes in the sugar content did take place but escaped our observation, because of the fact that the additionally formed sugar is immediately consumed by the increased respiration. In order to get a better insight into this question, it has been calculated how many sugars are present in the total "pool" at a fixed moment and how much disappears per time-unit from the pool as a result of the respiration. Calculations have been made for the respiratory activity at 25° and afterwards for the development of the 40° effect. The experiment at 40° ('van Vliet'), mentioned above and illustrated in Fig. 10 has served as an example. The calculations of the amount of sugars available in the total pool is based on the figures in table 8. From the calculations it appeared that about 28 mg soluble sugar (expressed in glucose units per g. fresh weight) is present and that at 25° the respiration process daily consumes about 0.18 mg glucose/g fresh weight. During the six days of the development of the 40° effect the total consumption amounts to about 9.7 mg sugar/g fresh weight. Considering the ratio of the sugar present in the total pool and the quantity that is consumed, it is not very probably that at 25° respiration is limited by the substrate concentration. Calculations like the above are of a speculative nature because it always remains doubtful whether the sugars are in fact available in the very centres of respiration, i.e. the mitochondria. The sugars in the cell may be spatially separated from the centres of oxydative activity and may for instance be stored in the vacuoles of the cell (see: LATIES, 1961). This heterrogenity need not be regarded as confined to the cell; it is likewise conceivable that no transport of sugars takes place.

## VII.5. INFLUENCE OF OXYGEN ON RESPIRATION

It will briefly be discussed why the respiration in the iris bulb is probably not limited by the oxygen content in the bulb under normal circumstances. The quantity of oxygen soluble in water and in cell-sap must be called small. Hence the oxygen supply in plant tissue practically entirely depends on a slow process like oxygen diffusion through the tissue, especially through its inter-cellular spaces. Thick bulky parts of the bulb will certainly impede gas diffusion. Therefore it is supposed that oxygen-tension in thick organs, consequently in bulbs as well, must be lower than the tension in the air surrounding the organ. Certainly, when oxygen consumption in the bulb is large, the question arises whether no anaerobic conditions will arise and whether the respiratory intensity is not limited by the oxygen supply (see JAMES, 1953).

We shall also examine what has been the practical result of the investigations in the iris bulb as regards the influence of oxygen-tension on respiration (Fig. 7). In 10 % oxygen respiration is lower than in air. In pure oxygen respiration is higher than in air, but the reaction on a five times higher oxygen concentration must be called relatively small. The facts do not point to an oxygen shortage under normal circumstances of the bulb. It was established that the R.Q. is approx. 1.1. When the respiratory intensity is strongly increased the R.Q. remains almost unchanged. Though a fermentation process is possible, it is not very likely that it plays a part here, because of the unchanged R.Q.. An atmosphere of pure oxygen does not accelerate the normal development of the 40° effect and the 15° effect in air, so we conclude that enough $O_2$ is available under normal circumstances. There are no indications of an oxygen shortage either. Though it is not quite impossible that locally anaerobic conditions are present in small parts in the centre of the bulb, it may be asserted that oxygen is not limiting to respiration, not even when the consumption of oxygen becomes much larger than is the case in storage conditions of the bulb.

Are these facts in accordance with more detailed studies of other objects concerning this problem? BURTON (1950) devoted a detailed study to the question of the oxygen content in the tissue of the potato tuber. By means of these data we can calculate (see JAMES, 1953) that under normal circumstances in the surrounding air the oxygen content of the tuber with a diameter of 28 cm (!) can still be 2 %. This supply of oxygen is thought to be possible only because of the intercellular system of the tissue. In temperatures higher than 25° the oxygen diffusion seems to cause difficulties soon, because of a rapid increase in oxygen consumption and a decrease in solubility of the oxygen in the cell sap. In view of its having a much smaller diameter than a potato, the supply of oxygen in the iris bulb is probably not unfavourable, but no exact data to base calculations on exist. This would require a special investigation. However, Burton's work indicates, that anaerobic conditions, even in thick, fleshy organs, need not occur, on condition that a diffusion of oxygen can take place through the capillary spaces.

## VII.6. THE RESPIRATORY QUOTIENT

The experiments have shown that the R.Q. at 25° is higher than 1. It is true that the average deviation is only approximately 10 %, but it exists. When interpreting this subject, we must constantly keep in mind that part of the bulb can be responsible for an increase in R.Q., because the value found is always a resultant. It is not likely for an organ containing so many accumulated sugars and reserve carbohydrates to use any other substrate than sugars for its respiratory process. We therefore have to determine the cause of the deviation. Of the many possibilities, we shall only mention those that we regard as the most obvious.

In the first place our starting-point has been that there are no longer any defects in the two different methods of absolute determination of carbon dioxide and oxygen. For the accuracy of the R.Q. is wholly dependent on this. In order to be certain the results would have to be checked with quite a different method.

A second explanation for extra emission of carbon dioxide could be found in the existence of a small anaerobic fermentation. Though it has been proved that the bulb-tissues have no difficulty in obtaining oxygen, the supply of oxygen in the centre of the bulb, e.g. in the shoot, may perhaps be suboptimal, so that respiration is not diminished, but a small fermentation takes place simultaneously. If this is true, we might certainly expect an increased R.Q. if the respiratory activity is very high, e.g. at 40°. This, however, is not the case.

A third explanation is the existence of an aerobic fermentation process independent of the oxygen-tension, which has often been found in meristemic tissues (RUHLAND & ULLRICH, 1936; RUHLAND & RAMSHORN, 1938). In the iris bulb we do in fact find a meristemic part of the tissue in the slowly developing shoot. (The problem of aerobic fermentation in meristemic tissues has not at all been solved. See BETZ, 1960). These authors deal with active meristems, and this does not occur in the iris bulb at 25°. At 15°, where we do think that the meristem is active, the R.Q. is not different from that at 25°. So the supposition that there exists an aerobic fermentation in the meristemic tissue does not seem to be very justifiable. In the development of buds, a problem showing possible analogies with the development of the growing-point in the iris bulb, an increased R.Q. can also be found (see ZIEGLER, 1961). On this problem, too, knowledge seems to be very limited. No chemical analyses effected on the iris bulb yielded arguments which enable us to conclude whether, in addition to carbohydrate, any other compounds serving as a substrate are involved in the oxydative catabolism leading to an increase of the R.Q. So it is of little use to deal with these possibilities here. In the first place we shall have to think of sugars as substrates. It must be stated as our conclusion that without further experiments it is impossible to give an explanation for the small increase of the R.Q. in the iris bulb.

## VII.7. RESPIRATORY EFFECTS AT THE TEMPERATURE TRANSITIONS

In Chapter V.5. two effects have been described, which were called the peak effect and the trough effect. The former occurs at a sudden increase of temperature and the latter at a decrease of temperature. These effects have also been registered without a secondary rise in temperature at 40° occurring. Therefore they are not the result of transitions from the "basic respiration" to the "developed respiration" (see Fig. 26). The effect could be demonstrated with bulb scales that were cut loose and isolated. Most probably these effects can also be studied with tissue parts, which is very doubtful for the other effects described. For these reasons the peak effect and the trough effect have a character of their own. The phenomena observed in the iris bulb resemble the reactions described by ROSENSTOCK (1955b) in tissue slices of potato tubers, but he gives no explanation for them.

We might suppose that in the event of a sudden fall or rise in temperature a quantity of gas is released from or dissolved in the cell sap as a result of a decrease or increase of the solubility of the carbon dioxide gas. The effect would then be of a merely physical nature. The interpretation of probably analogous phenomena described by Willaman & Brown (1930), Singh, Seshagiri & Gupta (1937) and by Algera (1947) points in that direction. This explanation was rightly rejected by Blackman & Parija as early as in 1928. They do so on the ground of the fact that, though in case of a rise in temperature the absorption coefficient for the solubility of the gas in the liquid phase diminishes, the internal tension of carbon dioxide on the other hand proportionally gets much higher, owing to the increased respiratory intensity, so that there can be no question of a release of gas. When dealing with the iris bulb we should like to reject the above-mentioned physical explanation for an empiric reason. The fact is that it appeared from our experiments that a temporary extra output of carbon dioxide coincides with a temporary extra consumption of oxygen (the reverse is also the case). Assuming that a temporary extra output of the solved oxygen from the bulbs exists, one would expect a temporary fall in the registration of the oxygen uptake. The opposite is the case, however. Moreover it is questionable whether in view of the low solubility of oxygen in water, there could be a release of much oxygen. Although other physical causes are not excluded, (we could for instance think of a change in a diffusion barrier for the gas; cell-membranes?) we prefer to point out that Blackman & Parija (1928) stated that these are metabolic reactions. For instance we may assume that some process in the chain of the numerous respiratory-reactions temporarily gets out of pace, owing to its higher temperature coefficient.

The experiments carried out with iris bulbs, however, do not afford a further explanation of the peak effect. The peak effect, shown in Fig. 32 in the diagram as an independent effect, always remains of modest proportions. Much more pronounced is the trough effect (Fig. 32). This effect has been observed by us not only in the iris bulb, but also in the intact potato tuber. The size of the trough effect as given for the iris bulb in the figure mentioned above is the same as is usually observed. In certain cases we observed that the peak occurring after the trough was obviously higher than the respiratory intensity at 40° just before the decrease in temperature. During the experiments (Fig. 22) it appeared that the trough in the curve hardly appears if a temperature of 15° instead of 25° is provided after the 40° treatment. For the time being we would prefer to adhere to the hypothesis that the trough effect is an expression of an extra respiratory reaction after the decrease of temperature to 25°. This hypothesis is illustrated in reaction D in Fig. 32. Line D of the figure is the difference between the respiration as recorded in practise on the one hand and the basal respiration together with the developed respiration on the other hand. A few hypotheses have been incorporated here, namely: 1. The basal respiration (A) is supposed to be influenced in accordance with the temperature coefficient of the process only. 2. The developed respiration (B) can be reversed. 3. Both the respiration process (B) as such and the regression rate of the process are influenced by temperature according to the usual temperature coefficient. So the existence as well as the form of process D remains disputable as long as no further data are available. It is possible, for instance, that process D is mediated by the extra consumption of special substrates which are accumulated during the 40° treatment. It would be interesting to know whether the intensity of this effect correlates with the duration of the 40° treatment. An explanation, both of the peak effect and of the trough effect, is not possible at the present state of knowledge.

We shall not discuss the effects occurring in the transition of an aerobic to an anaerobic phase and vice versa.

## VII.8. THE EFFECT OF MECHANICAL TREATMENT, WOUNDING AND PEELING OF THE TUNICS ON THE RESPIRATION OF BULBS

Slight mechanical manipulations of the bulb without visible damage may lead to increases of the respiration rate. Rough mechanical manipulations, accompanied by injuries show quantitatively larger increases in respiration. With EBERHARDT (1960) we are inclined, at least for the time being, to consider these effects as essentially similar and only quantitatively different. MULDER's observations (1955) on the "bruising effect" in potatoes indicate the same.

In the iris bulb the wound effect appears to be restricted to the oxydative phase of the respiratory process, i.e. there is no other output of carbon dioxide in a nitrogen atmosphere than the normal one after transference of the bulb in a new gas atmosphere. Of the other investigators who have tested the dependence of analogous effects on oxygen, AUDUS (1940), MULDER (1955) and HACKETT (1956) came to a same conclusion. In the iris bulb the additional output of carbon dioxide, whether or not there is visible wounding, appears to coincide in all cases with a practically equal quantity of oxygen consumed. So the R.Q. does not change (table 7). This also points to the absolutely aerobic nature of the effects. It has as yet not been investigated whether an increased oxygen-tension causes a quantitative change in these effects in the iris bulb. MULDER (1955) found that in potatoes an increase to 60 % in the oxygen concentration promotes the wound effect in a quantitative sense. The R.Q. is not always constant during the development of the wound effect. In this respect MARKS & VARNER (1957) showed that in the bruising effect of cherries the respiratory quotient increases owing to an output of carbon dioxide that is larger than the consumption of oxygen. POLLACK & HILLS (1956) (cited by EBERHARDT, 1960) have also observed an increased R.Q. after wounding of fruits.

It seems that we have to distinguish between two problems. In case of increases of the respiratory rate owing to wounding, bruising or the appliance of mechanical pressure on the tissue, we have first of all to deal with the oxygen dependence of the process called forth by the mechanical action. In addition to this there is the question of whether in that special case sufficient oxygen can be supplied by means of the diffusion process. A non-constant R.Q. need not lead to the conclusion that we are not dealing with the same process. A number of investigators take it that the traumatic increases of the respiratory rate are the result of a better supply of oxygen caused by the intervention (ROSENSTOCK, 1955 a; STEWARD, WRIGHT & BERRY, 1932; ZIEGLER, 1958). This is denied, e.g. by MULDER (1955). The oxygen dependence of these effects has never been investigated in a quantitative sense. This would be exceedingly useful. Difficulties in the supply of oxygen may differ from case to case and this may be the reason that in a certain case the R.Q. changes whereas in another case it does not. In the iris bulb it is not likely that oxygen diffusion is limiting because the R.Q. does not change, but in this case it would also be useful to study the oxygen dependence of the effect. The wound effect caused when excising the shoot is not diminished when the gap of the wound is sealed with wax. Therefore it is not likely that the increase of respiration can be attributed to a better supply of oxygen.

It is possible that the increase in respiration owing to wounding or bruising is the result of the existence of another pathway of the respiratory mechanism. The work of MAC DONALD & DE KOCK (1958) gave strong support to the idea that internal changes cause an increase of the effective substrate.

It is also possible that under normal circumstances there is a retardation of respiration owing to an accumulation of carbon dioxide. After an injury this gas could possibly be set free and then the retardation factor would be ineffective or diminished. The quantitative difference of wound respiration detected in tests with the iris bulb under a higher (Fig. 27) or lower pressure (Figs. 28 and 30) of carbon dioxide may point to this. Therefore it would be interesting to study the influence of the pressure of carbon dioxide on the respiratory intensity as well.

The tunic effect described in Chapter V.3.1., was already established earlier in tulip bulbs (ALGERA, 1947), in iris bulbs (RODRIGUES PEREIRA, 1962), and probably in onions too (SMIRNOFF, 1903). We can consider the tunic effect as a bruising effect without visible injuries. It remains remarkable that such a small intervention as the peeling of the tunics, which are anatomically quite separate from the bulb scales, involves such a quantitative effect. The effect appears to depend on oxygen just like the wound effect and the bruising effect, i.e. it does not occur in a nitrogen atmosphere. Here too the fluctuation of the R.Q. during the increase of respiration is of little importance. Is the effect a result of a better supply of oxygen after the removal of the tunics? If this is the case, it is inexplicable why a large concentration of oxygen (pure oxygen, not air) does not have a clear effect on the respiration of the intact bulb. The decline of the respiratory intensity — after the increase — which appears some time after the removal of the tunics, does not seem to be the result of anatomic impediments induced by peeling the tunics (see p. 372). The phenomena do not fit the theory that the effect is caused merely by a better supply of oxygen. RODRIGUES PEREIRA (1962) came to the same conclusion.

The only difference between the tunic effect and the wound effect is that the former possibly has a relationship with the 40° effect, while the wound effect has none. This was deduced from the following facts. It was observed that after wounding the size of the 40° effect remained the same, so here we may speak of an addition of two independent effects (V.6.2.). The 40° effect, however, seems to be proportionally related to a preceding tunic effect (V.3.1.). We think it justifiable to conclude from this that there is a relationship between the tunic effect and the 40° effect, and that both result from the same process. It seems plausible that this tunic effect is a reaction of cells at or near the surface of the bulb and the question arises whether this is not the case in the 40° effect as well. This hypothesis would make it very understandable why there is no difficulty in the oxygen diffusion during the development of the 40° process. However, the matter is not quite clear, because one would be inclined to deduce from similar experiments, that a relationship between the 15° effect and the tunic effect exists. Somewhere else we mentioned that the 15° effect is related to the respiration of the shoot and this is not quite in agreement with the relationship mentioned here. A solution of this question must therefore be postponed until after further investigation.

The experiments of Rodrigues Pereira, who measured the respiration rate in each separate bulb scale, also lead to the conclusion that the effect must be localized in the outer bulb scale.

To summarize: the observations support the hypothesis that increases in the rate of respiration — caused by mechanical treatment, by wounding or by peeling the tunics — are the result of changes in the structural organisation in the cell which result in oxydative enzymatic reactions, rather than the hypothesis that a better supply of oxygen stimulates the respiratory process.

## VII.9. FINAL DISCUSSION

The purpose of the investigation was to establish correlations between morphological development and respiratory activity. From the investigation it appeared that the growing-point in the bulb in storage condition at 25° and 30° is in a state of comparative rest and that at a temperature of 15° it shows an initial growth. This growth is correlated with an increase of the respiratory activity (VII.2.). There is no question of a perceptable activity of the root primordia under those circumstances (III.4.). In the experiments an increased respiratory activity was found to be primary and an elongation of the primordia to be secondary. Though the way of measuring the growth could be refined, it is unlikely that an improvement would cancel the difference in time of the two reactions. According to the prevailing opinions the respiratory activity is considered as a result of a requirement for energy, which in our case would be necessary for the growth. We would then have to suppose that in the case of the iris bulb the phase of measurable growth is preceded by a period of preparatory processes for which energy is needed. A closer examination must decide how far the parallelism between the growth and the respiratory reaction applies. Considering the indications — brought to light by the experiments — that a decrease of oxygen tension causes a better cell extension in the first growing phase, it is important to make a further study of the influence of the oxygen-tension on the initial growth and on the respiratory activity at 15°.

The respiratory activity in retarded bulbs (25° and 30°) is at a low level and in a steady state. The increase of the respiration rate at 15°, a temperature at which growth starts, probably has the nature of a controlled respiration in the sense of CHANCE & WILLIAMS (1956) and LARDY(1956). The investigation has proved that probably neither the oxygen concentration, nor the substrate concentration, nor the percentage of water limits the respiration under these conditions. It would be interesting to investigate whether the adenylic system or any other regulation system regulates the respiration under these circumstances. In this connection it would be advisable to measure the free and bound phosphate first.

For the problem we are dealing with, the effects other than the 15° effect are less important. These are the 40° effect, the wound effect, the effect of mechinical treatment of the bulb and the tunic effect. It was not possible to relate the 40° effect to morphological changes. From the investigation it appeared more or less clearly that respiratory effects are the result of local reactions in the bulb. This is shown by the large reaction which follows when the tunics are removed from the bulb. Probably this can be explained as a reaction of the exterior cell layers of the first bulb scale. There are indications suggesting that the 40° effect is also localized here. Therefore it would be worth-while to investigate to what extent there is a relationship between the tunic effect and the 40° effect. It is remarkable that the 40° effect could no longer be observed when bulb tunics which had been cut loose were used for the tests. A bulb from which the shoot had been excised but without severing the connection between the bulb scales showed the 40° effect. The difficulties arising from wound respiration when working with separated bulb parts have been sufficiently indicated.

It appeared from the experiments that the steady-state respiration is labile at 25° and that apart from by changing the temperature the labile respiratory intensity could also be disturbed by peeling of the tunics or by wounding or by mechanical treatment of the bulb. The question arises whether in these cases there is a shift in the metabolic pathway when respiration switched from the rest phase at 25° to an increased activity. Is the pathway in the various respiratory activities always the same? The notion of different pathways emphasized especially by LATIES (1957) on biochemical grounds is an interesting question, which as yet is supported by few experiments. In all kinds of investigations this question is put (auxine-induced respiration:

COMMONER & THIMANN, 1941; climacteric respiration: TAGER, 1956; wound respiration: ROMBERGER & NORTON, 1961; anion-respiration: LUNDEGÅRDH, 1962). To answer these questions the physiological investigation should be supported by biochemical investigations. The study of the gas exchange of the bulb is not the proper method to solve the questions respecting different pathways. It should be stated here that the step to a biochemical investigation is greater than one would suppose. It is not only a question of wound respiration that confronts us with difficulties, but in addition certain effects, which have been discussed above and observed in vivo, can be lost when one works with parts of the bulb.

The time factor is of special importance when metabolic drifts are investigated. This has been stressed sufficiently by Blackman & coworkers. As regards the iris bulb we have arrived at some conclusions about respiratory drifts.

## SUMMARY

The temperature is a very important factor in the development of the growing-point in the iris bulb. Many publications deal with these experiments, but little research on the relationship between the metabolism of the bulb and temperature has been done. For that reason the relation between the respiratory activity and the temperature factor has been studied closely. The carbon dioxide output and the oxygen uptake of the bulbs have been measured by self-registering gas analysers. The environmental circumstances during the measurements were in most cases similar to normal conditions of storage.

After lifting, the respiration rate of bulbs decreased sharply at high temperatures such as 25° C and 30° C. After a relatively short period of about 3 weeks a steady state respiration is reached at a low level of activity. Only very slowly does the respiration rate increase during the rest of a year's storage period at 25° C or 30° C. The 'van Vliet' and 'Wedgwood' bulbs can be stored at these temperatures for a year without losing their viability. The development of the central growing-point in the bulb is highly retarded under these circumstances, and these retarded bulbs are particularly suitable for physiological research.

During a period of 2 or 3 weeks the respiration rate of the bulbs remains in a steady state at a temperature of 25° C (storage condition). If the bulbs — coming from the storage conditions — are transferred to lower temperatures, an increase of the respiration rate is observed within a day. At 15° the increase of the activity is greatest and after a few days a new steady state is reached. This effect is called the 15° effect. There is a relation between the increase of the respiratory activity and an incipient growth of the central growing-point. The optimum temperature of the incipient growth likewise lies at a temperature of 15°. Both the developed respiration and the growth activity can be reversed by transferring the bulbs to the retarding temperatures of 25° and 30° again.

If the retarded bulbs are transferred to higher temperatures than the storage temperatures, for instance to 40°, then a steady increase of the respiration rate is also observed, but there is no question of a steady state. The increase at 40° is very decided and potentially reversible under certain conditions. This effect is called the 40° effect. Shootless bulbs also show this phenomenon, but the effect is lost when experiments with bulb scales that are cut loose are made. If the dry membraneous tunics, which surround the bulbs, are peeled a pronounced rise in the respiration rate of the bulb occurs (the tunic effect). The hypothesis is put forward that this activity is similar to the 40° effect. It was tentatively deduced from the experiments that this activity is located in cell-layers near the convex side of the outer (first) bulb scales.

The low respiratory activity of retarded bulbs at 25° is neither a result of a low oxygen tension normally existing inside the bulb, nor the result of a water

loss of the tissues. Nor is it likely that the sugar as a substrate limits the respiration process. No changes of the sugar content in the bulbs were observed after some days' temperature treatments. The controlling mechanism of the respiration rate is as yet unknown.

The data give some indications that the incipient growth of the primordia inside the bulbs is stimulated by an oxygen concentration that is lower than in air.

One would expect an oxygen shortage to occur in bulky tissues like in bulbs, but we did not observe a lack of oxygen under normal circumstances in any experiment. It is true that the respiration quotient fluctuates to some extent; the R.Q., however, practically remains unchanged, even under conditions of a high respiration activity.

The 15° effect, the 40° effect, the tunic effect, as well as the wound effect appeared to be obviously dependent on oxygen.

Transition effects at sudden temperature changes from 25° to 40° and vice versa are described. These effects were termed the peak effect and the trough effect.

## ACKNOWLEDGEMENT

The author is greatly indebted to Professor A. Quispel for his valuable criticism and advice during the preparation of the manuscript. It is with pleasure that I thank Miss A. Verlind and Miss H. Veldhorst for their assistance with the experiments. My sincere thanks are also due to Drs. Frederika A. Falken and Mr. L. F. van der Heyde for preparing the English text and to Mr. P. H. Vermeulen, M. A. for the final correction. Dr. J. M. Tager gave suggestions and comment. Moreover the author wants to express his gratitude to the "Centraal Bloembollen Comité" for giving the opportunity to work the more fundamental problems of flower bulbs.

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