On the Effect of Chloroform on the Respiratory Exchanges of Leaves.¹

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With fifteen Figures in the Text.

THAT the carbon dioxide evolved in the respiration of plants is of complex origin is now a generally accepted view. It is recognized that enzymes play a large part in the processes leading to its evolution and also to the absorption of oxygen, which is normally concurrent with it. The available evidence seems to show that a close correlation is maintained between the rates at which oxygen is absorbed and carbon dioxide produced in normal respiration, but the chain of processes is still incompletely known and the regulating mechanism a matter for conjecture. It is to be expected that a careful quantitative investigation of the temporary increase in the intensity of respiration produced by various chemical and other agencies may throw light upon the factors which are concerned in keeping the balance between the respiratory processes.

One interesting aspect of the problem that has received little attention is whether or how far a close quantitative relation continues to exist between the evolution of carbon dioxide and the absorption of oxygen under the influence of stimulating agencies. The work of which an account is given here was undertaken at Dr. F. F. Blackman's suggestion with this aspect in view.

There is little doubt that the augmentation produced by different agencies is not necessarily of the same nature, even if, as Palladin holds,² the primary effect is always protoplasmic. Müller-Thurgau and Schneider-Orelli³ found that stimulation due to exposure to a high temperature and stimulation following upon injury, in Potato tubers, were antagonistic. In the case of an anaesthetic such as chloroform, it may be inferred with probability from its chemical inactivity and high degree of saturation, that

¹ This paper forms Part XI of 'Experimental Researches upon Vegetable Assimilation and Respiration', carried out in the Botany School, Cambridge. A preliminary account was given at the Sheffield meeting of the British Association in 1910. See Report, p. 765.

² Jahrb. f. wiss. Bot., xlvii, 1910.

³ Flora, 1910.

its direct effect will be of a relatively simple physical nature.¹ It is with the changes which follow the exposure of leaves to chloroform vapour that this paper is concerned. Miss Irving² has given numerous data for the rate of production of carbon dioxide by Barley shoots, and by leaves of Cherry Laurel under the influence of chloroform. In the experiments described here, in which leaves of Cherry Laurel and certain other plants received similar treatment, the relation which the absorption of oxygen bears to the concurrent evolution of carbon dioxide was investigated. As the chemical nature of the material used in respiration alters the respiratory quotient, some leaves were first starved in the dark and then chloroformed.

The work was begun during my tenure of a Mackinnon Research Studentship of the Royal Society in 1909–10, and carried out at the Cambridge Botany School. I desire to express my thanks to Dr. Blackman for his kind and helpful interest in the work.

METHOD.

In order to investigate absorption of oxygen as well as evolution of carbon dioxide, the procedure adopted was to analyse samples of the air of a closed chamber in which leaves were contained. It appeared desirable to be able to examine the initial period of stimulation very closely, and for this purpose to take samples at relatively short intervals. As the change in the composition of the air in the respiration chamber during any given interval is smaller the shorter the interval, but depends also upon the total volume of air and on the quantity of respiring material, these factors had to be adjusted so that the changes could be measured with sufficient accuracy.

The analyses were made with the capillary eudiometric apparatus of Bonnier and Mangin, of the form described by Aubert.³ This has the two advantages that analyses can be made quickly, a valued feature when samples were to be taken at short intervals, and also that very small volumes can be analysed, and thus the diminution of the total volume of gas can without serious error be ignored. An account of the way in which this apparatus was used (which differed in some respects from Aubert's instructions), with a discussion of the sources of error, has been given separately.⁴

As the limit of error was found to be about 0.1 per cent. of the total volume of air analysed,⁵ in determining the percentages of CO₂ and O₂ in

¹ Cf. H. E. and E. F. Armstrong, Roy. Soc. Proc., B, 82, 1910, pp. 588–602; Annals of Botany, xxv, 1911, p. 508; Bechold, Die Kolloide in Biologie u. Medizin, 1912, p. 32.

² Annals of Botany, xxv, 1911, p. 1077.

³ Rev. gén. de Bot., iii, 1891.

⁴ Annals of Botany, xxvii, 1913, p. 565.

⁵ In addition to a variation from the mean up to about ± 0.1 % among a series of analyses of the same sample of air, the percentage of O₂ was on the average 0.2 below the correct value, 20.9. This error has since been traced to its sources; it does not vitiate the results, as it enters into all the analyses equally, and is eliminated when two results are subtracted in calculating the *change* of

a sample, it was necessary to ensure that the changes of composition to be measured should be relatively large. For this reason several leaves were enclosed together in a chamber of minimal volume specially constructed for the purpose. This chamber (shown in plan and sectional elevation in Fig. 1) was designed in collaboration with Dr. F. F. Blackman and made by the Cambridge Scientific Instrument Company. It consisted of two parts, a heavy circular base of brass (A), and the flat chamber itself (B), carried by a brass disc (C) which was accurately ground into the base, and so when greased made an air-tight junction with it. There was a small tubulure (D) at the top of the chamber to which a manometer could be attached, and another (E) at the side of the base, leading into the small space between the disc and the base. This latter tubulure could be connected to an apparatus for withdrawing samples of the enclosed air, which was similar to that used by Aubert.¹ This chamber had the special advantage that it could readily be opened to renew the air or examine the condition of the leaves enclosed in it, and as readily closed again perfectly air-tight, working, when greased, like a well-ground stopper.

No attempt was made to investigate respiratory exchanges *during* exposure to chloroform vapour, as its presence in the air would have introduced errors into the analyses, and there appears to be no satisfactory method of removing it before analysis. Leaves were therefore chloroformed in a separate vessel, with a capacity of about a litre, by adding a measured volume of liquid chloroform on a piece of cotton-wool. The duration of the exposure varied from 2 to 25 minutes in different experiments, and the dose from 0.05 c.c. to 1.0 c.c. (0.075 to 1.5 grm.) of liquid chloroform to the litre of air.

The procedure here outlined has not proved in all cases sufficiently sensitive for the satisfactory investigation of changes in the respiratory

composition which has occurred during a given interval. Improvements have been introduced into the technique by which the range of difference between analyses of the same sample as well as the absolute error are now very much reduced. See *loc. cit.*

¹ *Rev. gén. de Bot.*, iv, 1892.

FIG. 1. Plan and sectional elevation of leaf-chamber.

quotient when these are small. When a number of leaves are packed together in a small space it is difficult to ensure the uniform distribution of the gases before withdrawing a sample for analysis; and also during a period of several hours' enclosure the large increase in the concentration of carbon dioxide which takes place results in a storage of carbon dioxide in the tissues ready to reappear as an apparent acceleration of the respiration as soon as the air has been renovated. Modifications are in progress by which it is hoped to push the investigation further.

EXPERIMENTS WITH CHERRY LAUREL.

Six leaves were enclosed together in the respiration chamber for each experiment. Before treatment with chloroform, determinations of the normal rate of respiration were made.

The results are given, usually in graphic form, in cubic centimetres of oxygen or CO_2 per hour *per leaf*. The continuous line gives the rate of absorption of oxygen, the broken line the rate of production of CO_2 .

All the experiments were carried out at the temperature prevailing in the laboratory.

The experiments first given show the effect of exposure to the vapour of chloroform in relatively low concentration, which produced no visible change in the leaves.

EXPERIMENT I. July 14, 1911. Dose, 0.2 c.c. liquid chloroform per litre of air for 15 minutes. Fresh Cherry Laurel leaves.

Six leaves of the current season, weighing 11.0 grammes, were gathered

FIG. 2.

the previous evening after a bright sunny day, and left in the dark with their stalks in water under an inverted beaker. Next morning they were enclosed for three hours to determine their normal rate of respiration, and then chloroformed. The rates of absorption of oxygen and evolution of CO_2 per hour per leaf are shown graphically in Fig. 2. The temperature rose gradually from 19.4°C . at the beginning of the preliminary three hours to 22.8°C ., nine hours after the chloroforming.

The O_2 intake and the CO_2 output show a similar increase, the curves for both corresponding with type B of Miss Irving's *schema*,¹ in which, after the initial stimulation, the respiration diminishes as starvation proceeds. Seventy hours after removal from the chloroforming vessel the respiration had still further diminished, the O_2 intake being 0.16 c.c., and the CO_2 output 0.14 c.c. per hour per leaf. The leaves were then still green.

EXPERIMENT II. July 11, 1911. Dose, 0.2 c.c. per litre for 15 minutes. Temperature, 19–22° C. Six leaves of the current season, weighing 9.6 grammes, starved for four days in the dark.

Owing to starvation the leaves used in this experiment were respiring at a very low rate. In Fig. 3 are plotted the results obtained during the first thirty-four hours, after treatment with chloroform exactly as in Experiment I.

FIG. 3.

The respiration was in this case greatly augmented, and remained at the high level for a considerable time. The leaves were kept under observation for fifteen days, and their rate of respiration determined at intervals. Comparison with results obtained with other lots of leaves of similar age which had been in the dark for the same time but had not been chloroformed, shows that the respiration of the chloroformed leaves had fallen to the normal level on the sixth, but not on the third day. The parallel results in c.c. per gramme of fresh weight, and reduced to 22° C., are given in the following table :

Day.	Chloroformed leaves c.c. per hr. per grm. fresh weight.		Leaves not chloroformed c.c. per hr. per grm. fresh weight.	
	CO_2	O_2	CO_2	O_2
3	{ 0.083 0.086 }	{ 0.105 0.109 }	0.068	0.082
5			0.049	0.075
6	0.059	0.080		
7			0.066	0.082

In these two experiments the stimulatory effect of chloroform is plainly visible, and the augmentation affects the absorption of oxygen and production of CO_2 nearly equally. The recovery which follows is apparently

¹ Loc. cit., p. 1083.

complete. The fresh leaves soon regained their normal intensity of respiration. In the starved leaves of Experiment II, on the other hand, respiration was maintained at the high level for several days. Other similar experiments with starved leaves, though not all, showed a similar relatively persistent augmentation of the respiration; what the exact conditions may be which determine this interesting effect is not yet clear.

EXPERIMENT III. July 16, 1909. Dose, 0.2 c.c. for sixteen minutes. Temperature, 18–20° C.

The six leaves used had been gathered two days previously. The rate of respiration before chloroforming had fallen a little below that of other similar leaves newly gathered.

In this experiment the dose was the same as in the previous experiments, but more chloroform appears somehow to have penetrated into the

FIG. 4.

leaves during exposure; for, although no change was visible before they were put into the respiration chamber, at the end of six hours small chocolate-coloured spots were found on them, showing that in these places the cells were completely disorganized. As before, the respiration was augmented; it reached a greater maximum intensity than in Experiment I, and remained at a high level

much longer, though not so long as in Experiment II. It appears that the rate of production of CO_2 rose slightly above the rate of absorption of oxygen, and was so maintained for about six hours; the respiratory quotient thus rose from about 0.9 to about 1.03. In the subsequent slow fall the O_2 intake fell more slowly than the CO_2 output, so that eventually the original respiratory quotient was reached again.

In the next two experiments the dose of chloroform was large enough to be followed by complete disorganization. The first sign of the change was already visible as a uniform faint brown tinge when the leaves were removed from the chloroforming vessel, and the brown coloration rapidly deepened during the period of enclosure in the respiration chamber to a uniform chocolate brown. The first faint tinge of brown is always, as far as my observations go, a sign that irreversible changes have begun from

which there is no recovery, for it is always quickly followed by the condition of complete disorganization associated with the chocolate colour.¹

EXPERIMENT IV. July 14, 1909. Dose, 0.5 c.c. for twenty minutes. Temperature, 18.4–18.7°C. Fresh leaves. The leaves were of the current season, and were cut a few hours before they were used.

The absorption of oxygen proceeded much more quickly during the first half-hour after treatment with chloroform, but rapidly diminished to a low level, from which it slowly sank through a period of thirty-six hours. The CO₂ output, on the other hand, had fallen even in the first half-hour to a very low level, and fourteen hours later was inappreciable.

FIG. 5.

EXPERIMENT V. July 10, 1909. Dose, 0.5 c.c. for fifteen minutes. Temperature, 14–15°C. Starved leaves.

The leaves had been in the dark for several weeks, and were yellow to the extent of about a third of their area. Here the O₂ intake was still more markedly increased. In fact, during the first half-hour, the six leaves had absorbed together more than 3 c.c. of oxygen. This rapid absorption of oxygen is still more striking when compared with the low rate of respiration previous to chloroforming. It is to be correlated with the change of colour to chocolate, due to the oxidation of substances of the nature of tannins under the influence of oxidases.

In both these experiments it is probable that much oxygen had already been absorbed in the chloroforming vessel. This applies especially to Experiment IV, where the fresh leaves would still have their stomata open, and so present little resistance to the inrush of oxygen, which is the probable explanation of the lower rate of absorption detected in this experiment. In both, the rate of absorption appeared already to be rapidly diminishing. This point was studied further in the case of *Helianthus*.

¹ If the first symptom appears locally, the rapid complete disorganization is similarly localized, and only spreads slowly to neighbouring parts of the leaf.

There is evidence, however, that starved leaves are more susceptible to the influence of chloroform, and this factor may have contributed to the greater absorption of O_2 observed in Experiment V. Experiments with larger doses of chloroform have not been made using Cherry Laurel leaves; but in a few experiments with fresh leaves of Portugal Laurel, oxygen was absorbed more rapidly after bigger doses.¹

When leaves of Cherry Laurel were treated with doses intermediate between 0.2 and 0.5 c.c. of liquid chloroform per litre of air, they were in part rapidly disorganized, but in part green; the results were therefore more complex, and need not be considered here as no new point arises from them.²

FIG. 6.

The experiments which have so far been described fall obviously into two classes, the first dealing with the gaseous exchanges during so-called 'stimulation', the second with the gaseous exchanges accompanying disorganization.

In experiments of the former class absorption of oxygen and production of CO_2 are similarly affected, suggesting that they are still closely correlated.

In the latter class treatment with bigger doses of chloroform led to profound disorganization, accompanied by a rapid inrush of oxygen, and

¹ See also experiments with *Helianthus*. It is probable that such results represent the immediate fatal disorganization of a larger proportion of the cells of a leaf by bigger doses.

² Cf., however, p. 714.

a greatly diminished production of CO_2 . Miss Irving's experiments, in which the CO_2 production was determined from the first moment of the introduction of chloroform, show that the first effect here also is to increase the production of carbon dioxide; but the stimulation is of short duration. How the rate of absorption of oxygen is affected in this very transient initial phase has as yet not been determined, nor the course of the change from stimulation to the onset of irreversible changes: in my experiments these stages were passed through in the chloroforming vessel.

In order to determine whether the marked absorption of oxygen which accompanies disorganization in leaves of Cherry Laurel be a general phenomenon, similar experiments were made with other leaves. Some experiments with leaves of Portugal Laurel showing a similar inrush of oxygen have already been referred to. These leaves change at the same time to a very dark chocolate colour. Experiments were also made with leaves of *Helianthus tuberosus* and *Tropaeolum majus*.

EXPERIMENTS WITH HELIANTHUS TUBEROSUS.

In the leaves of *Helianthus tuberosus* disorganization is accompanied by a blackening of the leaf, and if the dose of chloroform is sufficiently large, by the exudation of water and marked flaccidity.

In the first two of the following experiments the dose was small and the leaves showed none of these signs of disorganization.

EXPERIMENT VI. August 24, 1910. Dose, 0.05 c.c. for ten minutes. Temperature, $17.6-19^\circ \text{C}$. Eight starved leaves weighing 12.6 grammes.

The leaves were respiring at a low rate, having been in the dark for

FIG. 7.

seven days. Here, as in the case of Cherry Laurel, a small dose of chloroform augmented the respiration, affecting both the production of CO_2 and the absorption of oxygen. The respiratory quotient, however, appeared to be distinctly lower, the absorption of oxygen having been the more affected. Here, too, the maximum rates were attained at once, and the curves fell from the beginning, instead of rising to a later maximum as in Experiments

I and II with Cherry Laurel, which suggests that all the effects take place, or show themselves, much more rapidly in *Helianthus*, where stomata are present on both sides of the leaves and exchange of gases is normally rapid. A determination made after twenty-three hours showed a further slight fall in the rates; the CO_2 output still bore a smaller proportion to the O_2 intake than before chloroform. The leaves remained quite green to the end.

EXPERIMENT VII. July 7, 1911. Dose, 0.1 c.c. for five minutes. Temperature, 22.2–24.7° C. Five leaves weighing 7.5 grammes.

The leaves used had been gathered the day before and left in the dark for twenty hours. Immediately after treatment with chloroform they were

FIG. 8.

still turgid and green. After five and a half hours the leaf-chamber was opened and signs of a small amount of local disorganization were then visible. Twenty-two hours after chloroform respiration had fallen approximately to its original level, but disorganization was slowly spreading.

Stimulation was here relatively greater than in the previous experiment, in which the dose was only half as big, though applied for double the time. The absorption of oxygen was again affected more than the production of CO_2 , and the respiratory quotient remained at the lower level.

In the following experiments disorganization had already begun when the leaves were enclosed in the respiration chamber.

EXPERIMENT VIII. August 25, 1910. Dose, 0.1 c.c. for ten minutes. Temperature, 17.4–18.2° C. Eight leaves freshly gathered, weighing 12.8 grammes.

When removed from the chloroforming vessel, the leaves had already begun to show signs of disorganization over the greater part of their surface. The first sample of air for analysis after chloroform was taken when the leaves had been enclosed for a quarter of an hour. Analysis gave as the composition of this sample 0.6% CO_2 and 19.1% O_2 . Thus in this short time the leaves had absorbed more than 2 cubic centimetres of oxygen

(the volume of the chamber being 150 c.c.). As shown in Fig. 9, a stimulation of the CO_2 output is also indicated, although the quantity concerned was small.

The very rapid absorption of oxygen only lasted a short time, then dropped to nearly the normal level and fell further slowly along with the

FIG. 9.

CO_2 output. After twenty hours both had fallen considerably, but the CO_2 output more than the O_2 intake, the respiratory quotient being 0.4.

EXPERIMENT IX. September 22, 1909. Dose, 0.1 c.c. for fifteen minutes. Temperature, $14.3\text{--}15.8^\circ\text{C}$. Six leaves in the dark for four days previously.

FIG. 10.

The rate of respiration before treatment with chloroform was low owing to starvation; the values given in the diagram are, however, rather too low, as they represent the average for nineteen hours.

As in Experiment VIII there was an immediate acceleration of the O_2 intake, and at the same time a very unmistakable increase in the CO_2 output. The latter fell off, however, to a very low rate by the fourth hour, the O_2 intake falling meanwhile to near its previous level. Compared with the low CO_2 output before chloroform, the relatively great increase after chloroform suggests comparison with the experiments with starved leaves of Cherry Laurel in which relatively great and persistent stimulation was shown.

After five hours the dose was repeated, and the results indicate a repetition on a small scale of the effects produced by the first dose. This may mean that cells hitherto but little affected were by the second dose strongly affected.

It is probable that only the outer cells are effectively exposed to the action of chloroform vapour unless its concentration is much greater, so that while the outer cells are killed, the inner remain alive. This would explain the fact that the leaves remained, as a whole, turgid. Microscopic examination of the distribution of the brown coloration supports this interpretation, as at first only the outermost layers of cells are affected.

On the other hand, repeated stimulation of a given cell is possible (cf. Expt. XII, with *Tropaeolum*, on p. 711), though it is uncertain whether the oxidation of tannin, once begun, can be further accelerated by exposing the cell a second time to chloroform.

EXPERIMENT X. August 19, 1910. Dose 0.3 c.c. for five minutes. Temperature $18.7-19.2^\circ C$. Eight leaves freshly gathered, weighing 9.2 grammes.

FIG. 11.

In this experiment a greater initial maximum rate of O_2 intake (as in Experiment VIII) would have been revealed if a sample for analysis had been taken earlier than an hour after the leaves were enclosed. The total amount of oxygen absorbed was much greater than in Experiment VIII, where disorganization was initiated by treatment with a smaller dose of chloroform.

EXPERIMENT XI. August 27, 1910. Dose, 1.0 c.c. for five minutes. Temperature, $16-17^\circ C$. Leaves freshly gathered.

The first sample was taken after the leaves had been enclosed for seventeen minutes, and two others after further

intervals of an hour each. Here the result corresponded with that in Experiment VIII, since the very high rate of absorption of oxygen, the highest obtained, was confined to the first short interval. The results were as follows:

		c.c. per hour per leaf.	
		CO ₂	O ₂
After chloroform,	17 minutes	0.20	1.80
next 63	„	0.21	0.45
„ 60	„	0.14	0.31

Doubtless the brevity and rapidity of the inrush of oxygen revealed in this experiment and in Experiment VIII always characterizes the absorption of oxygen which accompanies the beginning of disorganization in leaves of *Helianthus*: in the other experiments the first sample of air was not taken soon enough to show this feature so clearly.

Treatment of leaves of *Helianthus tuberosus* with chloroform to the point of disorganization resulted, therefore, in a marked absorption of oxygen very similar to that observed in the case of Cherry Laurel. Here, again, it was correlated with the change of colour due to oxidation of tannin. Small doses, too, evoked a temporary augmentation of both production of CO₂ and absorption of oxygen.

It is clear, however, that chloroform penetrates the more delicate leaves of *Helianthus* much more readily than the better protected leathery leaves of Cherry Laurel. Whereas in the case of the latter a dose of 0.2 c.c. in the litre vessel usually left the leaves green for at any rate a long time, this was not always so in the case of *Helianthus*, even after a dose a quarter as big (0.05 c.c. per litre).¹ It is not unlikely, as a further consequence of this difference, that in some of the experiments with doses a little larger, especially where the leaves were exposed but for a short time to the chloroform vapour, most of it was absorbed by the outer layers of cells.² The results, some of which show stimulation of the CO₂ output as well as the much accelerated O₂ intake associated with disorganization, may therefore be complex, like those with Cherry Laurel, in which different parts of the leaves were differently affected.

The most striking feature of the results is the sharpness and relative brevity of the acceleration in the absorption of oxygen. It has already been remarked that the curve of O₂ absorption in the case of leaves of Cherry Laurel exposed to a fatal dose falls very rapidly at first and slowly later. The same is more distinctly shown by the experiments with *Helianthus*, where a first short period of enhanced O₂ absorption appears to be quite sharply distinguishable. This is illustrated in Fig. 12, in which the

¹ Cf. Weevers: Betrachtungen und Untersuchungen über die Nekrobiose und die letale Chloroformeinwirkung. *Recueil des travaux bot. néerland.*, ix, 1912, p. 255, &c.

² Weevers records the coloration of the epidermis alone in petals of *Magnolia*. *Loc. cit.*, p. 252.

total amount of oxygen that has been absorbed *per gramme* is plotted against the time as abscissa, the data being taken from two experiments in which freshly gathered leaves were exposed to the vapour of 0.1 and 0.3 c.c. respectively, for ten minutes. For comparison with these a third curve is added, from another experiment, in which treatment with 0.05 c.c. chloroform was followed by a typical stimulation effect. The broken lines represent the corresponding curves of total production of CO_2 .

The contrast between the typical stimulation curves and those resulting from more drastic treatment is obvious. The former show no such sharp change in the rate of O_2 absorption as is shown in the curves for the other

FIG. 12. Curves showing total O_2 absorbed and CO_2 produced after chloroform by leaves of *Helianthus tuberosus* in c.c. per gramme of fresh weight.

hour after treatment with chloroform, and a steady fall from this initial maximum followed.

EXPERIMENTS WITH *TROPAEOLUM MAJUS*.

Leaves of *Tropaeolum majus* do not darken during disorganization, and the plant was chosen for this reason, as contrasting with the Laurels and *Helianthus*. The symptom most readily observed is flaccidity, accompanied by exudation of water into the air-spaces, and from the water-pores and the cut end of the stalk.

For each experiment the stalks were removed from sixteen small leaves, gathered usually a few minutes before use, and the laminae were

two experiments. In those, the later parts of the oxygen curves are practically parallel, but at different levels corresponding to the different quantities of oxygen absorbed in the initial period. These quantities appear roughly proportional to the dose of chloroform received.

While the amount of oxygen absorbed is at first far greater than in the typical stimulation curve, the amount of CO_2 produced is smaller, and the difference increases the more rapidly the bigger the dose.

With regard to the character of the augmentation produced by small doses, the respiratory quotient appeared to be less during stimulation than before treatment, even when the leaves remained fresh and green. The highest rate of respiration was observed as a rule within the first

floated on water. They were then placed between a folded sheet of moistened paper, and so inserted into the leaf-chamber.

The first experiment is chosen as an example of the effect of a small dose of chloroform, insufficient to produce disorganization.

FIG. 13.

EXPERIMENT XII. September 24, 1909. Dose, 0.1 c.c. for ten minutes, repeated after three hours. Temperature, 16–17° C.

In this experiment the effect of the first dose of chloroform was a marked though relatively brief stimulation of both CO₂ output and O₂ intake, more especially of the latter (Fig. 13). The second dose produced a similar though rather smaller effect.

It is interesting to notice that the changes in the respiratory coefficient following the two doses are concordant, rising gradually from 0.7—after the first dose in three hours to 0.8, after the second reaching eventually 0.9. The same change was shown by other experiments.

The next two experiments are examples of cases in which the dose of chloroform was sufficient to produce disorganization.

EXPERIMENT XIII. October 15, 1909. Dose, 0.3 c.c., administered in six doses of 0.05 c.c. each at intervals of two and a half minutes; duration of exposure, fifteen minutes. Temperature, 15.7–17° C.

After chloroform the leaves were curled and rather flaccid, and their rate of respiration had greatly diminished; it fell still further to a very low level within six hours. In this case, however, unlike *Helianthus* and Cherry

Laurel, no inrush of oxygen accompanied disorganization; on the contrary, the O_2 intake diminished more than the CO_2 output, the ratio of the latter to the former rising to 2.0. Similar results were obtained in other experiments in which a similar dose of chloroform was employed.

FIG. 14.

EXPERIMENT XIV. September 28, 1909. Dose, 0.5 c.c. or ten minutes. Temperature, 14–15° C.

Here for the first three hours after chloroform, the O_2 intake remained greater than the CO_2 output (Fig. 15), but this may be connected with the abnormally low respiratory coefficient during the hour preceding exposure

FIG. 15.

to chloroform. The depression after this large dose continued to a level still lower than in the previous experiments, and again the residual O_2 was less than the CO_2 output.

Thus in the leaves of *Tropaeolum*, which contain no tannin, the absorption of oxygen is affected by strong doses as well as weak in a similar way to the production of CO_2 , and there is no such marked absorption of oxygen after strong doses as occurs in the other kinds of leaves.

The temporary augmentation of the respiration produced by small

doses was like that produced in the other leaves. As in the case of *Helianthus* the absorption of oxygen was more affected than the production of CO_2 ; when the dose was big enough to cause a marked stimulation, the respiratory quotient fell from about 0.9 before exposure to 0.7 after chloroform; but after a few hours the original ratio was reached again. Also, as in the case of *Helianthus*, the maximum rates were observed in most experiments in the first hour, the rates then falling quickly to the normal level. A repetition of the same dose after three hours produced a second stimulation, the maxima being, however, rather less than after the first dose.

After exposure to doses of 0.2 c.c. or more no stimulatory effect was detected; but both CO_2 production and O_2 absorption diminished rapidly to a low level. Here again the absorption of oxygen was the more affected, and its rate fell below the rate of production of CO_2 , the ratio $\frac{\text{CO}_2}{\text{O}_2}$ rising much above unity, instead of falling far below unity as it did in the other leaves.

It is to be noticed that *Tropaeolum* lies midway between Cherry Laurel and *Helianthus* in the degree of susceptibility, or (more probably) penetrability, of its leaves to chloroform vapour. The stimulation produced by ten minutes' exposure to 0.1 c.c. chloroform per litre is similar to that after a dose of 0.05 c.c. for ten minutes in the case of *Helianthus*, and 0.2 c.c. for twenty minutes in the case of Cherry Laurel. Gaseous exchanges take place normally less rapidly in *Tropaeolum* than in *Helianthus*, and the distribution and character of the stomata (including, for instance, their very ready tendency to close) may in part account for the less rapid penetration of chloroform. It is probable, however, that the wax which covers the surface of the leaf takes up the chloroform and protects the epidermal cells from being affected so soon as in leaves of *Helianthus*, where they have neither the waxy covering of *Tropaeolum* nor the thick cuticle of Cherry Laurel.¹

DISCUSSION.

The fact which stands out most prominently in the foregoing experiments is the large absorption of oxygen which accompanies the disorganization of leaves of Cherry Laurel, Portugal Laurel, and *Helianthus* exposed to chloroform vapour in sufficient concentration. This result was only observed when visible signs of disorganization appeared, and only when one of these signs was the appearance of a brown or black coloration; in leaves of *Tropaeolum* which do not show any such marked change of colour during

¹ Weevers (loc. cit., p. 255) attributes the differences which he observed in the minimum time of exposure that was followed by fatal results in great part to differences of water content. This view will clearly not explain the different behaviour of leaves of *Helianthus* and *Tropaeolum*.

disorganization, the rate of absorption of oxygen was, on the contrary, greatly depressed. This change of colour is attributed to the oxidation of substances of the nature of tannins,¹ present in the Laurels and *Helianthus*, but not in *Tropaeolum*, owing to the activity of oxidases which follows as a result of the fatal influence of the chloroform. As is already well known, the breaking down of the organization of the leaf-cells of the Cherry Laurel is also followed by the production of hydrocyanic acid, owing to the hydrolysis of the cyanogenetic glucoside prulaurasin by an emulsin.²

The other fact of general importance is that during the temporary augmentation, or stimulation, of the respiration which follows less drastic treatment in all the leaves studied, the absorption of oxygen and production of CO₂ apparently still remain co-ordinated. The ratio between the quantities of oxygen and CO₂ concurrently absorbed and evolved shows indeed small changes; but such changes were not always observed, and they appear to differ in different kinds of leaves, though within the same kind some degree of concordance is shown.

A very interesting point is suggested by the character of the stimulation curves obtained in most of those experiments in which the leaves had previously been starved in the dark. These curves indicate a relatively greater and much more prolonged augmentation of the respiration, in comparison with the low rate of respiration characteristic of their starved condition. The same effect was not, however, obtained in all the experiments, and further investigation is necessary before a discussion of its significance and the conditions on which it depends would be profitable.

The transition from stimulation, with the production of CO₂ and absorption of oxygen still closely correlated, to disorganization and the complete breakdown of this correlation would appear to be sharply marked; for not only is the difference between doses which merely stimulate and doses which initiate disorganization very small, but intermediate doses may initiate disorganization in one part of a leaf and not in another, this part remaining green for a long time. It is interesting that even in leaves of Cherry Laurel disorganization only slowly spreads to parts which have been left green, notwithstanding the evolution of prussic acid from the disorganized areas.³

¹ In the case of *Aucuba*, the blackening of the leaves is said to be independent of oxygen. See Maquenne and Demoussy, *Comptes rend.*, cxlix, 1909, p. 957.

² According to Weevers (*loc. cit.*, p. 254) the HCN is detected, even by its odour, later than the beginning of the brown coloration, as it has first to diffuse out of the leaf.

³ Weevers has found that the darkening of leaves may only begin some time after exposure to chloroform. He supposes (*loc. cit.*, p. 261) that where this happens death has already taken place during exposure. The other alternative would appear, however, to be conceivable: even if, as Weevers holds, necrobiosis and stimulation be sharply distinct from each other, necrobiosis might still be preceded by stimulation and, in cases of deferred coloration, set in after exposure, not as a direct effect of the chloroform but through excessive stimulation of the normal respiratory processes. Prolonged starvation alone leads eventually to increase of permeability and necrobiosis.

Miss Irving, who determined the rate of production of CO_2 from the first moment of exposure, has shown that the first effect, even of large concentrations of vapour, is to stimulate the production of CO_2 ; this effect becomes more intense and more shortlived the more the dose is increased, until it may be described as a transient outburst of CO_2 . In my experiments this brief stimulation was seldom observed, as it took place in the chloroforming vessel before the measurement of the gaseous exchange could be begun. When a detailed collation is attempted, however, it appears probable that the intensity and duration of the augmented output of CO_2 shown in Miss Irving's experiments, depends in some degree upon the continuance of the exposure to chloroform vapour. This is well brought out if Miss Irving's Experiment XIII¹ and my Experiment IV² are compared. In her experiment leaves of Cherry Laurel were exposed continuously to the vapour of 0.63 c.c. of chloroform per litre of air; in mine, similar leaves were exposed for twenty minutes to the vapour of 0.5 c.c. per litre. The dose was rather smaller in my experiment, yet whereas in it the production of CO_2 was far below normal from the first (i. e. from less than half an hour after the first moment of exposure, onwards), in Miss Irving's experiment it was increased nearly threefold, and was still above normal in the fourth hour, though rapidly diminishing. Only with much larger doses was the outburst over in half an hour under continued exposure to chloroform. It seems improbable that the difference of temperature (18.5° in mine, and 25° in Miss Irving's) could account for such a difference, and the inference is suggested that this exaggerated production of CO_2 only lasts so long as the leaf remains exposed to chloroform vapour, or dies away with great rapidity as soon as no more chloroform is administered.

When, on the other hand, the concentration of chloroform is low, the smaller acceleration then produced dies away more slowly after exposure to chloroform ceases: this more persistent stimulation appears, therefore, also as an after effect. Here again, however, if exposure is repeated, a similar stimulation is again produced, as in Experiment XII with *Tropaeolum*; ³ while continuous exposure intensifies and prolongs the stimulation, or may lead to disorganization.

There are many points which still require elucidation. One of the most interesting is the relation between the respiratory exchanges and changes of permeability. Lepeschkin⁴ showed that the exudation of water from the sporangiophore of *Pilobolus* can be diminished by a small dose of chloroform gradually applied, indicating a decrease of permeability, whereas a large dose increased the permeability. Recently Osterhout⁵ has found that 1 per cent. ether or 0.05 per cent. chloroform in sea-water pro-

¹ Loc. cit., p. 1089, Fig. 15.

² p. 703.

³ p. 711.

⁴ Beihefte z. Bot. Centralbl., xix, Abt. I, 1906, pp. 416-17.

⁵ Science, N. S., xxxvii, 1913, p. 111.

duces a reversible increase of the resistance of the living thallus of *Laminaria* to the passage of ions in an electric current ; whereas three times the concentration of anaesthetic produces a brief reversible increase of resistance followed by a progressive decrease which is irreversible, and always ends in the thallus becoming as good a conductor as the sea-water itself, i. e. completely permeable.

The increase of permeability shown by the exudation of fluid in leaves exposed to chloroform vapour has been recorded by Miss Irving and others, and already remarked on here ; it is especially obvious in leaves of *Tropaeolum*. Miss Irving¹ observed the exudation of water and flaccidity in Barley leaves, in experiments in which the respiration did not indicate fatal disorganization, and it must still be held an open question whether a slight increase of permeability is always irreversible, as might be inferred from Osterhout's experiments. According to Lepeschkin the increased exudation of water which follows a moderate dose of chloroform, due to an increase of permeability, only lasts for a time when the chloroform is removed.

In leaves the evidence seems to point to the possibility of recovery so long as visible disorganization has not begun, even though some increase of permeability has resulted. Whether the recovery is complete is, however, another question. There is evidence that starvation is hastened ; but this might be due merely to the depletion of reserves during stimulation. Müller-Thurgau and Schneider-Orelli² conclude, on the other hand, that etherized potatoes are prematurely aged, in the sense that the balance between starch formation and dissolution is altered in the same direction as during the normal ageing process which precedes sprouting, the concentration of sugar in the sap increasing.

Another important question is the nature of the augmentation of the respiratory exchanges spoken of as stimulation. H. E. and E. F. Armstrong have shown that after drastic treatment with chloroform leaves of Cherry Laurel contain more sugar ; but this may be a degenerative change, due perhaps to hydrolysis of the glucoside, and associated with disorganization. It is true that where a distinct change in the respiratory quotient was observed in leaves of Cherry Laurel, it was an increase to approximately unity. On the other hand, the decrease in the respiratory quotient observed in leaves of *Helianthus* and *Tropaeolum* during stimulation suggests a temporary change in the nature of the respiratory material of quite a different kind. The data are not yet sufficiently numerous to allow of generalization.

¹ Loc. cit., p. 1079.

² Loc. cit., pp. 368-9. See also review in *New Phytologist*, ix, 1910, p. 337.

SUMMARY.

In conclusion, the following facts appear to be established :

1. In all the leaves examined, treatment with a small dose of chloroform results in stimulation of the respiration, the absorption of oxygen and production of CO_2 increasing in like proportion, and therefore probably remaining co-ordinated.

In starved leaves the effect of stimulation was usually prolonged.

2. When the concentration of chloroform vapour was large enough to initiate visible disorganization, the production of CO_2 after treatment was always diminished, the outburst of CO_2 demonstrated by Miss Irving having already occurred, and it quickly fell to a very low level. At the same time the absorption of oxygen was no longer closely correlated with the production of CO_2 .

In leaves of *Tropaeolum*, which contain no tannin, the absorption of oxygen was depressed still more than the production of CO_2 .

On the contrary, in leaves of Cherry (and Portugal) Laurel, and *Helianthus*, which contain tannins, the oxidation of which imparts a brown or black colour to the disorganized leaves, the absorption of oxygen was very rapid for a short time, and, though quickly falling, remained at a much higher level than the production of CO_2 .

Thoday, David. 1913. "On the effect of chloroform on the respiratory exchanges of leaves." *Annals of botany* 27, 697-717.

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