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The Physiological Action of Cyanide.—I. *The Effects of Cyanide on the Respiration and Sugar Content of the Potato at* 15° *C.*

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I. Introduction.

The interest which attaches to the physiological action of cyanide arises from the strong inhibitory effect upon the respiratory processes of the cell. This inhibition of cell respiration is interpreted as a specific poisoning of the catalysts responsible for tissue oxidations, since effects of a similar nature are well known in many oxidation systems of both biological and non-biological origin.

Amongst the variety of animal and plant tissues studied, an inhibitory action of cyanide upon respiration has been observed in all but a few cases, and attention has been focussed almost entirely upon this effect. The extent of the inhibition appears to vary considerably according to both the kind and condition of the tissue, and at present the interpretation of these differences remains somewhat controversial. This aspect of the action of cyanide will not, however, be discussed in the present paper since we shall be concerned more particularly with an effect of cyanide which has not been recognised hitherto, although possible indications of its existence are to be found in the observations of other authors.

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In our experiments on the effects of hydrogen cyanide upon the potato tuber at 15° C., it is found that cyanide treatment produces a greatly increased respiration which may be maintained for periods of several weeks. This effect of cyanide stands in striking contrast to the marked inhibition of respiration, which has been observed in so many tissues, and which has come to be regarded as the dominant and usual effect of cyanide upon the metabolism of the cell.

This investigation further establishes that the increased respiration in cyanide is brought about not by a direct effect upon the oxidative system, but rather by a specific effect upon the starch-sugar relationships of the potato, resulting in an increased sugar concentration. In the potato at this temperature the specific poisoning of the respiratory enzymes does not become apparent except after a prolonged and drastic cyanide experience.

Among the observations in the literature a few examples are known in which cyanide produces relatively little or no inhibition upon the respiration, at least initially, *e.g.*, starved yeast, Warburg (1927), and Lathyrus seedlings, Genevois (1929). The only case for which there exists substantial evidence that cyanide causes an increase in respiration is that of *Chlorella* in the absence of added glucose: Warburg and Negelein (1919), Emerson (1927), and here the fact has elicited little attention beyond the suggestion by Warburg and Uyesugi (1924) that the increased respiration may be due to the utilisation of hydrogen cyanide as substrate for respiration. It is impossible to decide from the relatively few observations described in the literature, whether the increased respiration in *Chlorella* is of a similar nature to the effect, which will be described in the potato. This subject will be considered in a later paper.

II. Methods.

In investigating the effects of cyanide upon the potato, continuous determinations were made of the respiration of samples of potatoes during exposures to atmospheres containing low controlled concentrations of hydrogen cyanide gas in air. In one type of experiment only the carbon dioxide production was measured; in a second type both carbon dioxide and oxygen consumption. In both types of experiment readings were continued day after day, usually for periods of several weeks, the aim being to establish the course of respiration during and after experiences in cyanide of different strengths and durations.

Measurement of Carbon Dioxide Production.

The method developed in the Botany School, Cambridge, was employed for measuring the carbon dioxide production. A stream of gas, freed from carbon dioxide, is drawn over the respiring material, and then bubbled through a Pettenkofer tube filled with standard baryta solution. The baryta tubes are replaced at intervals, and the residual baryta is determined by titration with standard acid.

The application of this method to studying the effects of hydrogen cyanide upon carbon dioxide production involved the solution of several technical difficulties. The requirements were :—

(1) A constant known rate of the gas stream.

(2) A device for generating hydrogen cyanide at a constant and controlled rate.

(3) A method of collecting and estimating separately the carbon dioxide and hydrogen cyanide in the gas stream after leaving the respiration chamber.

The following methods were evolved to meet these requirements :—

(1) The gas stream was motivated by a constant negative pressure obtained from a filter pump acting through a mercury valve, and the rate of the stream was regulated by the limiting resistance of a length of capillary tubing. The rate in most experiments was 1500 c.c. per hour.

(2) A form of apparatus was developed which gave a controlled and approximately constant production of hydrogen cyanide. It consists essentially of a small reservoir of 25 per cent. sulphuric acid, into which a solution of potassium cyanide is allowed to drip at a slow uniform rate. The gas stream is bubbled through the acid, and carries out with it hydrogen cyanide gas. The acid in the generating chamber is drained out and replaced each day. The rate of entry of the potassium cyanide solution is kept constant by using a Marriotte's bottle as a reservoir for the solution, and restricting the flow by inserting a length of fine thermometer capillary tubing. By these means a constant rate of dripping was obtained, the rates varying in the different generators used, from 4 to 8 c.c. per day. By adjustment of the concentration of the potassium cyanide solution and the rate of the gas stream, any desired concentration of hydrogen cyanide could be obtained.

(3) A simple method was found for estimating both carbon dioxide and hydrogen cyanide in the gas stream after it left the respiration chamber. The stream was bubbled successively through two Pettenkofer tubes, the first

containing a standard solution of silver nitrate made slightly acid with nitric acid, the second a standard baryta solution. The hydrogen cyanide is absorbed quantitatively in the first tube, forming a precipitate of silver cyanide, and the amount is determined by estimating the residual silver ion by the Volhardt method. The carbon dioxide is absorbed in the baryta tube, and its amount determined in the usual way by titration of the residual alkali with standard acid.

Measurement of Carbon Dioxide Production and Oxygen Uptake.

It will be sufficient to consider only the principle of the somewhat complicated apparatus which was designed to enable the carbon dioxide production and oxygen consumption to be measured continuously during treatment with hydrogen cyanide of known and controlled concentration. The apparatus was developed on a similar principle to that in use by Dr. Franklin Kidd, and modified to suit the requirements. A diagram of the apparatus is shown in fig. 1.

The compensating system, C, and the respiration circuit, R, H, P, N, G, are

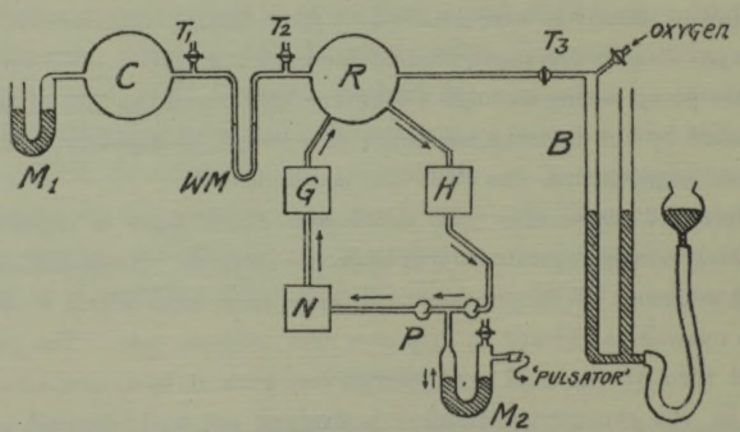

Fig. 1.—Diagram of the apparatus used for measuring the oxygen consumption and carbon dioxide production during treatment with hydrogen cyanide. For description see text.

two closed systems of equal gas volume, which are connected to the two limbs of a water manometer, WM. Each system includes a mercury manometer whose outer limb is open to the air. The two manometers (M_1, M_2) are made of tubing of the same bore. In addition, each circuit may be opened to the atmosphere by the taps, T_1 and T_2.

The respiring material is placed in R, and the atmosphere in this system is circulated in the direction indicated at a constant rate by means of the mercury

pump, P, of which the mercury column of manometer, M_2, constitutes the piston, being made to oscillate by the intermittent suction device described by Blackman and Bolas (1927). When the outer limb is opened to the air the mercury column comes to rest, and M_2 becomes a manometer comparable to M_1.

The hydrogen cyanide generator, G, is of the same general type as described above, but is so constructed that the volume of liquid in the acid reservoir may be drained accurately to a definite volume. The gas passes from G containing a constant concentration of hydrogen cyanide. It enters the respiration chamber, R, and from there is carried to H, a Pettenkofer tube filled with acid silver nitrate, in which the cyanide is absorbed. It then passes through the pump, P, to, C, a baryta tube which absorbs the carbon dioxide.

It will be seen then that hydrogen cyanide does not accumulate, but is being continuously generated in G and absorbed in H. Consequently, the volume of hydrogen cyanide in the system during a given cyanide treatment remains constant (and is actually a very small quantity). The carbon dioxide is also continuously absorbed in C as it is given off by the material, and consequently the only change in the amount of gas in the circuit is due to the oxygen utilised in respiration. This causes a reduction in pressure in the respiration circuit as compared with the compensating circuit, which leads to a difference in level on the two sides of the water manometer, WM.

At intervals the circulation is stopped by opening the outer limb of M_2 to the atmosphere. The various segments of the respiration circuit are then opened together through a system of capillary tubes (which are not shown in the diagram) and thus the pressures equalised throughout the circuit. The acid in the generator, G, is drained to the constant volume, and oxygen is then added to the circuit from the oxygen burette, B, through the tap, T_3, until the pressures of the two systems are equalised as shown on the water manometer. The volumes of oxygen in the burette are measured at the existing atmospheric pressure, both before and after the addition of oxygen. The difference is the equivalent* of the oxygen consumed by the tissues, and its volume, temperature, and pressure are known. Moreover the oxygen concentration of the atmosphere in the respiration circuit is returned to its normal value.

The absorption tubes, C and H, are then removed and replaced, and fresh acid is run into the generator, G. Both the circuits are opened to the air, and then closed, and the circulation is once more started.

* The mercury manometers, M_1 and M_2, being of the same bore, compensate for changes in barometric pressure between successive readings.

H 2

The carbon dioxide and the hydrogen cyanide are then determined as before by titrating the residual baryta and silver in the respective absorption tubes.

*Estimation of Sugar Content of Tissues.**

The samples to be analysed were cut rapidly into thin slices and frozen at —20° C. From this temperature they were removed for analysis as convenient. After grinding, the tissue was first boiled in alcohol, and then extracted in hot alcohol for 4 hours. The extracts were freed from alcohol by distillation under reduced pressure, and the residual liquid was cleared by treatment with basic lead acetate, excess lead being precipitated afterwards with potassium oxalate. The liquid was filtered and the clear solution was made up to a definite volume. The reducing power of this solution was determined by the method of Shaffer and Hartman, both before and after hydrolysis of sucrose, which was effected by boiling for 20 minutes in 10 per cent. citric acid. From these values the concentrations of both reducing sugars and sucrose in the sample of tissue are obtained, both values being expressed as invert sugar in percentages of fresh weight.

III. Experimental Material.

Potatoes of the King Edward variety were used for the experiments. A selected stock of small tubers varying in weight from 20 to 35 gm. was stored at 10° C., and samples were removed to 15° C. as required.

At 15° C. the respiration rate falls slowly for about a month to a level which may be maintained for several months. The material is thus in a steady metabolic state which renders it particularly suitable for experimentation.

The respiration intensity of the potato is extremely low compared with most animal and plant tissues, and notwithstanding its solid appearance the structure is sufficiently porous to enable the whole of the tissue to respire aerobically under normal conditions. This is shown by the fact that the respiratory quotient is unity.

IV. Carbon Dioxide Production during Cyanide Treatment.

Experiment 1.—Samples of four to six potatoes were used to study the effects of different concentrations of hydrogen cyanide in air, and the records of carbon dioxide production† are given in fig. 2.

* We are indebted to the Hon. Mrs. Onslow for the supervision of the sugar analyses. and to Miss N. A. Potter who carried out the estimations.

† The carbon dioxide production is calculated as milligrams per hour per 100 grammes nitial fresh weight of potato tissue.

The control sample, which was kept in air throughout, shows the typical respiration drift of an untreated potato at 15° C.

The four samples, A, B, C and D, after an initial period in air were exposed to atmospheres containing various low concentrations of hydrogen cyanide, during the periods indicated on each record. The concentration used for each sample is given ; it varied from 0·14 c.c. hydrogen cyanide per litre in A, to 10 times that concentration in D.

The striking feature is that the rate of carbon dioxide production increases greatly in each case during the cyanide treatment, and that the increased respiration is not merely ephemeral, but of considerable duration.

The records for each sample show a similarity in form, there being two distinct phases during the cyanide experience, first a rise to a maximum, and then a downward drift. It is also evident that the magnitudes of the effects are closely related to the cyanide concentration ; with increasing cyanide the slope of the phase of rising respiration is greater, the maximal value attained is higher, and the subsequent downward drift is more rapid.

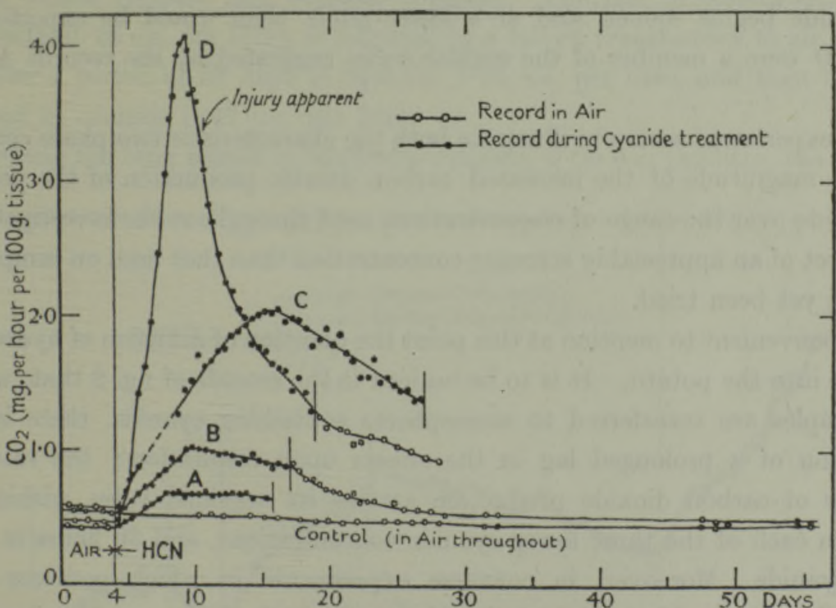

FIG. 2.—*Experiment* 1 (started November, 1929).—Effect on carbon dioxide production of exposure to atmospheres containing different concentrations of hydrogen cyanide. Duration of cyanide treatment is indicated in solid dots. The cyanide concentrations used were :—

Sample A—0·14 c.c. HCN per litre.
" B—0·18 c.c. "
" C—0·30 c.c. "
" D—1·45 c.c. "

A distinction must be drawn, however, between the effects of treatment with the lower cyanide concentrations as in A, B, and C on the one hand, and the relatively much higher concentration of D. In the former the effects appear to be completely reversible, since on removal from cyanide the respiration falls slowly to about the control value and does not fall below it, and the potatoes remain sound for long periods. This is illustrated by the post-cyanide record of sample B (fig. 2). After removal to air the respiration fell gently, reaching the control level some 40 days later. Observations after 80 days in air—record not given—showed that this level was maintained, and the potatoes still appeared sound. At that time a second treatment with cyanide again resulted in an increase in respiration.

In sample D, on the other hand, signs of injury to the potatoes (brown patches and sunken areas) were evident early on the downward phase, *i.e.*, from the sixth day in cyanide. After the sample was removed to air, rapid breakdown occurred, and at the end of the record the potatoes were exuding liquid. Another indication of a difference in the case of D is that the downward drift in cyanide begins sooner, and at a lower pitch, than would be expected if record D were a member of the regular series suggested by the records A, B, and C.

This experiment serves to illustrate both the characteristic two-phase course, and the magnitude of the increased carbon dioxide production of the potato in cyanide over the range of concentrations used throughout the investigation. The effect of an appreciably stronger concentration than that used on sample D has not yet been tried.

It is convenient to mention at this point the question of diffusion of hydrogen cyanide into the potato. It is to be noticed in the records of fig. 2 that, when the samples are transferred to atmospheres containing cyanide, there is no indication of a prolonged lag in the effects upon respiration; the rate of increase of carbon dioxide production attains its maximal value within 24 hours in each of the three lower cyanide concentrations, and 36 hours in the high cyanide. Moreover, in converse experiments in which potatoes are removed from cyanide to air, it is found that the bulk of the cyanide given off is evolved in the first 24 hours. It would appear, therefore, that a state of equilibrium is attained relatively rapidly, and that the forms of the respiration records are not determined by changes in the internal concentration of cyanide.

V. Oxygen Consumption and Carbon Dioxide Production during Cyanide Treatment.

For the following experiments the apparatus previously described (p. 98) was used to obtain continuous measurements of both the oxygen consumption and the carbon dioxide production of samples of potatoes during a variety of cyanide treatments. The data are expressed in cubic centimetres of the gases at 0° C. and 760 mm. pressure, so that the units represent molecular equivalents of oxygen and carbon dioxide (1 c.c. carbon dioxide = 1·96 mg.).

Experiment 2.—The record of this experiment (fig. 3) shows the respiration rate (oxygen uptake and carbon dioxide production) of a sample of potatoes during successive periods in air, and in various cyanide-air atmospheres. In addition there is shown the carbon dioxide production of a control sample which remained in air throughout.

In the initial period in air the oxygen consumed is approximately equal to the carbon dioxide produced, and during the different cyanide experiences both values increase and decrease together. The rise in respiration in low cyanide (0·10 c.c. per litre), is followed by a fall on transference to air. There follows a period of 32 days in cyanide 0·29 c.c. per litre, and then the final period in cyanide 0·47 c.c. per litre.

During the long period in moderate cyanide, 0·29 c.c. per litre, the respira-

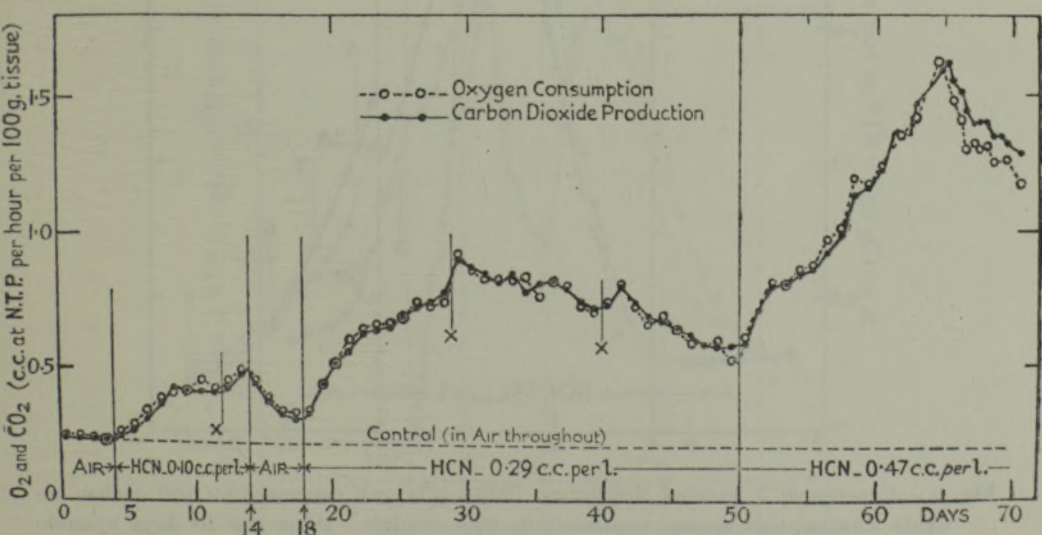

Fig. 3.—*Experiment* 2 (started January, 1930).—Oxygen consumption and carbon dioxide production during a sequence of treatments with various cyanide concentrations At the times marked X there were temporary increases in cyanide concentration due to slowing of circulation.

tion goes through the typical two-phase course, a rise to a maximum and a subsequent slower fall. The record over this period is actually nearly comparable in cyanide strength and in pitch to record C of experiment 1 (fig. 2).

In the final treatment the respiration rises fairly steeply to a value of 1·6, about eight times the control value, and only at this point, after 57 days in cyanide, is there a significant change in the respiratory quotient. It is evident then that profound changes in the respiration rate are induced by cyanide without affecting the respiratory quotient.

After the 65th day of the record, however, the oxygen values lie below the carbon dioxide. This increase in respiratory quotient is further investigated in the next two experiments.

Experiment 3.—A sample was treated with strong cyanide, 1·5 c.c. per litre, a concentration comparable with that of sample D of experiment 1. The oxygen uptake and the carbon dioxide production increase rapidly together

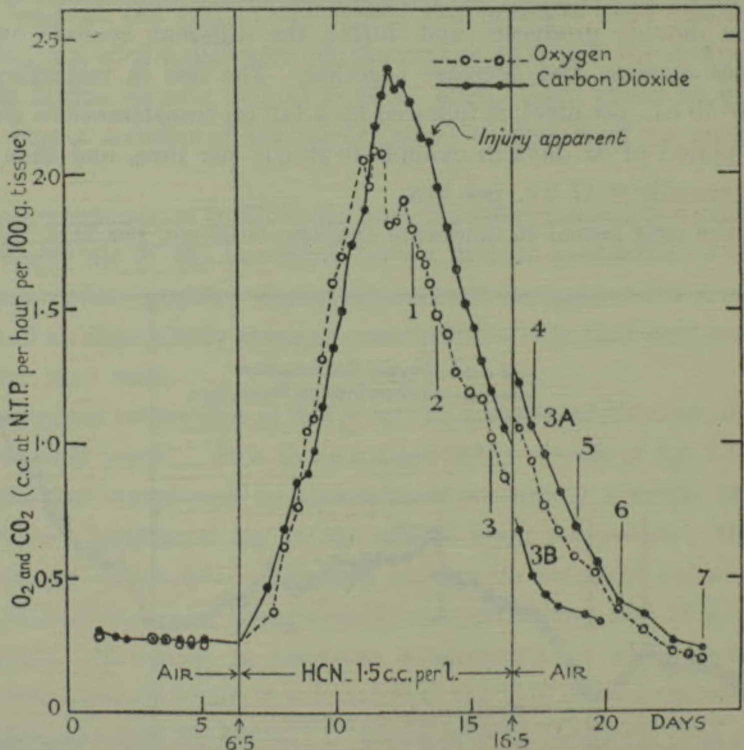

Fig. 4.—*Experiment* 3 (started November, 1929).—Oxygen consumption and carbon dioxide consumption during treatment in high cyanide. After the 10 days' cyanide treatment the sample was divided into two parts, 3A and 3B (see text). The respiratory quotients at the times indicated (Nos. 1, 2, etc.) are :—

No.	1	2	3	4	5	6	7
R.Q.	1·26	1·32	1·17	1·14	1·20	1·06	1·19

to a high value of about 2·1 (fig. 4) from which point onwards a marked change is evident, the oxygen values lying considerably below the carbon dioxide values throughout the subsequent part of the record. Brown patches of injured tissue developed shortly after the change in respiratory quotient.

After 10 days in cyanide the sample was divided into two parts, and returned to air. Readings of both oxygen and carbon dioxide were continued on the less-injured potatoes—3A, and it is seen that the high respiratory quotient was maintained during the subsequent fall in air. Only the carbon dioxide production was followed in the more severely injured potatoes—3B.

Experiment 4.—In order to investigate further the development of the increased respiratory quotient, a sample of potatoes was given alternate exposures to air and to relatively high cyanide in air (0·71 c.c. per litre) for the periods indicated in fig. 5.

A 4-day exposure to the cyanide atmosphere produces a rapid rise in respiration, which continues for 2 days after the sample is replaced in air, until a value of 2·0 is reached. During this rise, and the subsequent fall in air, there is no significant change in the respiratory quotient.

The second treatment in cyanide is of longer duration. A steep rise to approximately the same high level is again produced, but in this case when

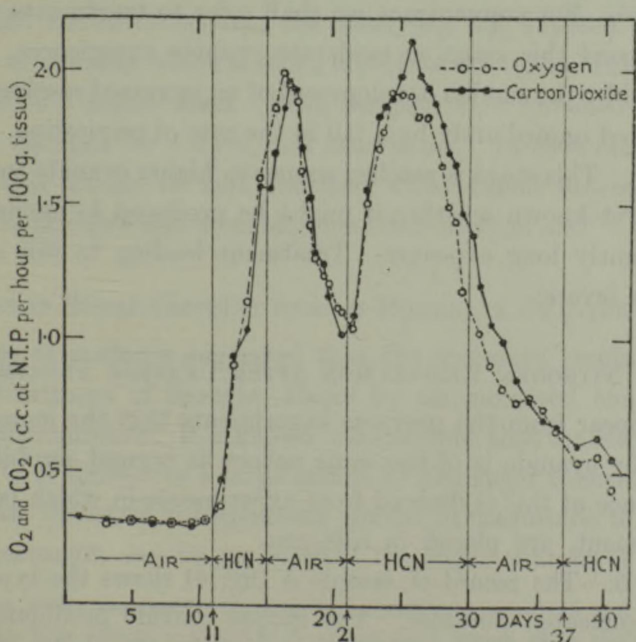

FIG. 5.—*Experiment* 4 (started May, 1930).—Oxygen consumption and carbon dioxide production during alternating periods in air and relatively high cyanide—0·71 c.c. HCN per litre.

the respiration reaches 1·9, the respiratory quotient increases suddenly, and during the subsequent fall in cyanide, the oxygen values lie below the carbon dioxide.

It is evident then that the change in respiratory quotient is not merely a function of respiration intensity, but of duration and strength of the cyanide treatment.

On removal to air after the second cyanide treatment, the respiration continues the downward drift, the respiratory quotient at first remaining high, but later returning to approximately unity. This return to the normal respiratory quotient is in contrast to the effect found in experiment 3, fig. 4, where the cyanide treatment had been more severe before the removal to air, and the respiratory quotient remained high in air.

During the third and final cyanide treatment two points are noteworthy. First, the cyanide no longer causes an increase in the respiration rate, indicating that the previous treatment has produced an irreversible change in the metabolic system; second, the high respiratory quotient immediately returns.

In these three experiments two definite stages in the effects of cyanide upon respiration are distinguishable by the respiratory quotient. In the first stage the respiration may be changed considerably, but the respiratory quotient remains normal. For convenience we shall refer to treatments whose effects do not go beyond this stage as moderate cyanide experiences. The second stage is marked by the sudden development of an increased respiratory quotient which is followed immediately by a fall in the rate of respiration, and definite signs of injury. This stage is reached sooner in higher cyanide concentrations, but it is not yet known whether it might be produced by moderate cyanide after a sufficiently long exposure. Treatment leading to this stage will be referred to as severe.

VI. Nitrogen Respiration after Cyanide Treatment.

It would appear from the previous experiments that the increased respiration induced by cyanide is of the same nature as normal aerobic respiration. Further evidence of this is derived from experiments in which potatoes, after cyanide treatment, are placed in nitrogen.

Experiment 5.—The record of sample A (fig. 6) shows the typical effect of nitrogen on untreated potatoes. The carbon dioxide production falls away in the course of 2 to 3 days to a value which is roughly 0·4 times the air line value. (There is sometimes a slight preliminary increase in carbon dioxide production preceding the fall, as in A, fig. 6.) On replacing in air there is a

striking after-effect, the carbon dioxide production rising for several days considerably above the air line.

The effects of nitrogen on cyanide-treated potatoes is shown in record B of fig. 6. Here a large sample of potatoes was kept in a low cyanide atmosphere

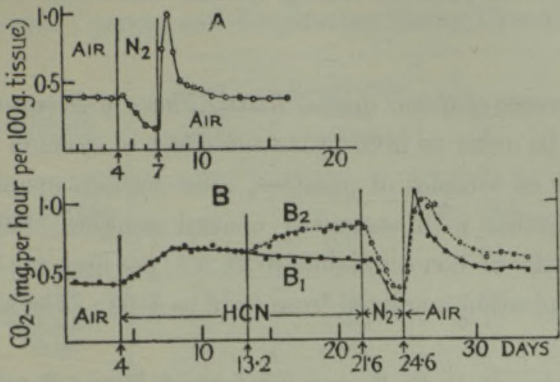

Fig. 6.—*Experiment* 5 (started December, 1929).—Effects of nitrogen on carbon dioxide production, A—untreated potatoes, B—after cyanide treatment. (B in 0·14 c.c. HCN per litre, then divided into two parts, B_1 left in 0·14 c.c. HCN per litre, B_2 given 0·28 c.c.)

for 9 days and then divided into two lots, of which one, B_1, remained in the original cyanide concentration and the other, B_2, was exposed to double this concentration of cyanide, which is still a moderate strength. The respiration of the latter rose to a higher level. Both samples were removed from cyanide and placed in nitrogen for 4 days, and then in air. In each case the effect of this treatment is similar to that obtained with normal untreated potatoes, that is, a fall in nitrogen and a subsequent after-effect in air.*

VII. Changes in Sugar Content during Moderate Cyanide Treatment.

Several lines of evidence suggested that the increased respiration induced by cyanide treatment is brought about by an increased concentration of the substrate of respiration. It is known, for example, that the respiration of the potato at 15° C. is limited to a large extent by the sugar concentration in the tissues, and that increases in respiration, similar in magnitude to those caused by cyanide treatment, can be produced if the sugar content is increased by

* The nitrogen levels reached in B_1 and B_2, respectively, are 0·49 and 0·46 times the corresponding air line values, ratios which are slightly higher than the value with the untreated potatoes. The possible significance of this difference must await further investigation. There is no doubt, meanwhile, of the general similarity of the nitrogen effects.

storage at low temperatures. Moreover, another line of evidence, which will be considered more fully later, suggested that cyanide might have a specific activating effect upon the amylase system, and so possibly bring about an increased production of sugar from reserves.

The sugar content of potatoes during cyanide treatment was, accordingly, investigated.

A.—*Increase of Sugar during Rising Phase of Respiration.*

Experiment 6.—In order to investigate the effect of cyanide upon the sugar content, a number of samples of potatoes, after various cyanide treatments, were analysed, together with untreated control samples. Sample A (fig. 7) was kept in cyanide of concentration 0·33 c.c. per litre for 14 days, during which time the respiration increased from 0·48 to 3·02. The sample was then analysed.

Five other samples, B, C, D, E, and F (of which E and F will be considered in the next section), were treated with a lower cyanide concentration (0·20 c.c. per litre), B being analysed after 7 days, C and D after 14 days in cyanide. The individual respiration records are shown in fig. 7. It will be noticed that there is considerable scatter in the records of these three samples, although the strength of cyanide was identical for each. Such variation is usual with small samples of 3 to 5 potatoes as in the present experiment.

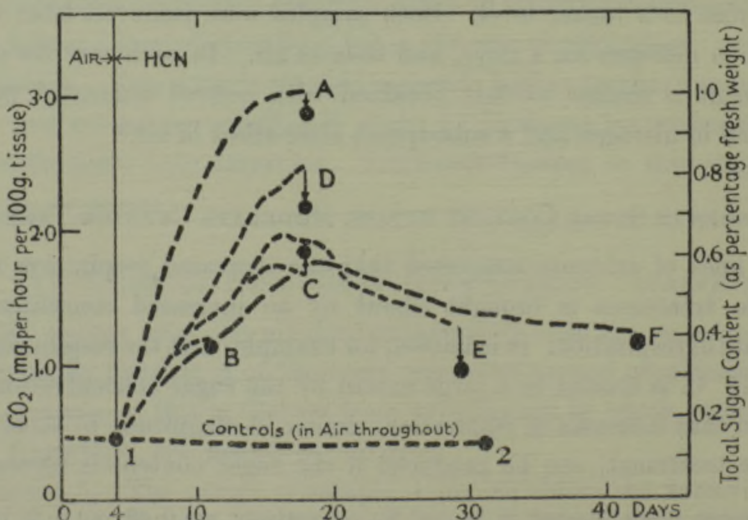

FIG. 7.—*Experiment* 6 (started January, 1930).—Carbon dioxide production and sugar content during cyanide treatment. Sample A in 0·33 c.c. HCN per litre. Samples B, C, D, E, and F in 0·20 c.c. HCN per litre. Sugar content of each sample shown as ● at time of analysis.

The sugar content and the respiration values of samples A, B, C and D at the time of analysis are given in Table I. There are given in addition the values for two untreated samples, control 1, analysed at the beginning of the experiment, and control 2 at the end.

Table I.—Sugar Content and Respiration during Cyanide Treatment.

Sample.	Cyanide strength.	Days in cyanide.	Respiration when analysed.	Hexose.	Sucrose.	Total sugar.
	c.c. per litre			per cent.	per cent.	per cent.
Control 1	—	—	0·50	0·04	0·12	0·16
Control 2	—	—	0·39	0·04	0·09	0·13
Sample B	0·20	7	1·20	0·05	0·33	0·38
Sample C	0·20	14	1·69	0·17	0·45	0·62
Sample D	0·20	14	2·44	0·10	0·62	0·72
Sample A	0·33	14	3·03	0·19	0·77	0·96

The data show a striking increase in the sugar content of the treated potatoes, the amount of the increase depending both upon the cyanide strength and the duration of treatment. The accumulated sugar is largely sucrose, but the reducing sugar (hexoses) also increases.

When the respiration values are plotted against the sugar values as in fig. 8, the data suggest strongly that the respiration is proportional to the sugar

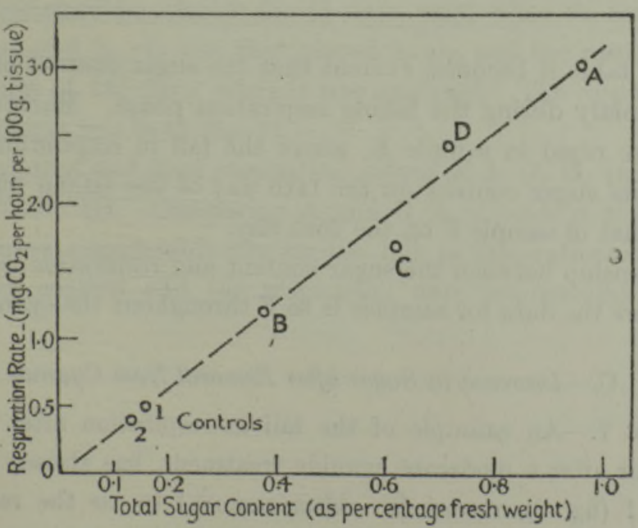

FIG. 8.—*Experiment* 6.—Relationship between respiration rate and sugar content during rising respiration phase.

content, over the range of sugar concerned. We may record here that an almost linear relationship over this range is also found in the case of potatoes whose sugar content is increased by low temperature.

B.—*Decrease in Sugar during Falling Phase of Respiration.*

To investigate the changes in sugar content during the falling respiration phase, the two samples, E and F, were left in the same cyanide strength (0·20 c.c. per litre) for 26 and 39 days, respectively. From fig. 7, in which the respiration records are plotted for the five samples, it can be seen that, whereas samples C and D were analysed approximately at the maximal respiration, the respiration of E and F had fallen considerably by the time of analysis. The divergence in the records of the two samples is again noticeable.

The sugar content and respiration values are given in Table II, together with those of samples C and D, for comparison.

Table II.—Sugar Content and Respiration during Cyanide Treatment.

Sample.	Duration of treatment.	Maximum respiration.	Respiration when analysed.	Hexose.	Sucrose.	Total sugar.
	days			per cent.	per cent.	per cent.
E	26	2·0	1·25	0·08	0·23	0·31
F	39	1·76	1·21	0·11	0·28	0·39
C	14	1·73	1·69	0·17	0·45	0·62
D	14	2·44	2·44	0·10	0·62	0·72

From this table it becomes evident that the sugar content of E and F has decreased rapidly during the falling respiration phase. Moreover the loss of sugar is more rapid in sample E, where the fall in respiration is also more rapid, since its sugar content on the 14th day of the falling phase is already lower than that of sample F on the 25th day.

The relationship between the sugar content and respiration is clearly shown in fig. 7, where the data for samples B to F throughout the cyanide experience are plotted.

C.—*Decrease in Sugar after Removal from Cyanide.*

Experiment 7.—An example of the fall in respiration after removal from cyanide to air after a moderate cyanide treatment, has already been given in experiment 1 (fig. 2), record B. After removal to air the respiration falls slowly to about the control value. The fall in air is more rapid when the cyanide treatment has been such as to induce higher respiration.

To ascertain the change in sugar content during the downward drift of respiration in air, two samples, G and H, were treated with the same cyanide concentration as sample A, experiment 6, *i.e.*, 0·33 c.c. per litre.

The respiration record of sample G was very similar to that of A (fig. 7). At the time when A was analysed, after 14 days in cyanide, G was transferred to air. The respiration fell rapidly in the course of 4 days from 2·8 to 1·6, at which time the sample was analysed (fig. 9).

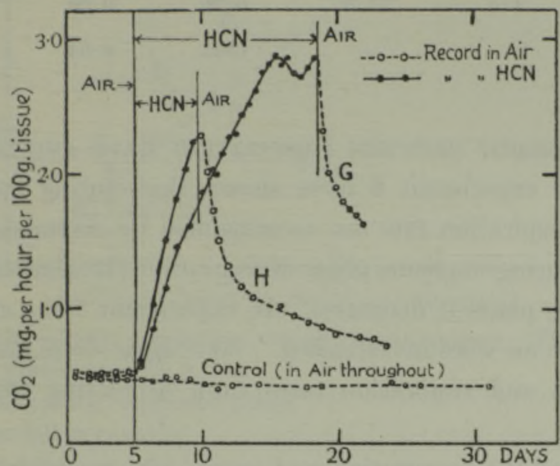

Fig. 9.—*Experiment* 7 (started January, 1930).—Carbon dioxide production during cyanide treatment (0·33 HCN per litre) and after removal to air. Samples were analysed at end of records.

Sample H remained in cyanide for 4½ days only, when its respiration had reached a value of 2·2. It was then placed in air, and the respiration fell to 0·7 in the course of 14½ days, when it was analysed. The respiration of the control at this time was 0·42 (fig. 9).

The sugar content and final respiration values of A, G, H, and the control are given in Table III. Comparing A and G it is evident that the sugar content decreased considerably during the fall in respiration in air. In H both the sugar content and the respiration were approaching the control level.

Table III.—Sugar Content and Respiration after Cyanide Treatment.

Sample.	Days in cyanide.	Days in air.	Respiration when analysed.	Hexose.	Sucrose.	Total sugar.
				per cent.	per cent.	per cent.
A	14	—	3·03	0·19	0·77	0·96
G	14	4	1·60	0·09	0·32	0·41
H	4·5	14·5	0·70	0·08	0·11	0·19
Control	—	—	0·42	0·04	0·09	0·13

VIII.—Relationship between Respiration Rate and Sugar Content.

The results of experiment 6 have shown that during cyanide treatment changes in the respiration rate are accompanied by corresponding changes in sugar content; during the rising phase of respiration the sugar content increases, during the falling phase it decreases. In experiment 7 the effects of removal from cyanide into air were investigated. Here again there are parallel changes in sugar content and respiration rates, both decreasing toward the normal value.

On the basis of this respiration and sugar data we may survey the ratio respiration/sugar content to see whether the respiratory efficiency is changed by the different cyanide experiences. In the first part of experiment 6 the relationship between respiration and sugar content during the rising phase in cyanide was shown to be approximately linear, as is indicated by the broken line of fig. 8. There is no evidence then of a changing respiratory efficiency during the rising phase in cyanide.

For purposes of comparison fig. 8 has been reproduced in fig. 10, where are plotted in addition the sugar and respiration values of the samples which were analysed (*a*) during the fall in cyanide—samples E and F, plotted as +, (*b*) during the fall in air after removal from cyanide—samples G and H, plotted as ●.

It will be seen that the points lie sufficiently close to the broken line to suggest that the respiratory efficiency is not appreciably altered during moderate cyanide experiences of this sort.

After more severe cyanide treatment, however, this relationship no longer holds. For example, the sugar and respiration values of sample 3B of experiment 3 are also plotted. In this case, the potatoes had undergone a prolonged treatment in a relatively high cyanide concentration ; the respiratory quotient

had increased, and breakdown of the tissues had begun (fig. 4). The position of the point (3B in fig. 10) indicates a considerably diminished respiratory

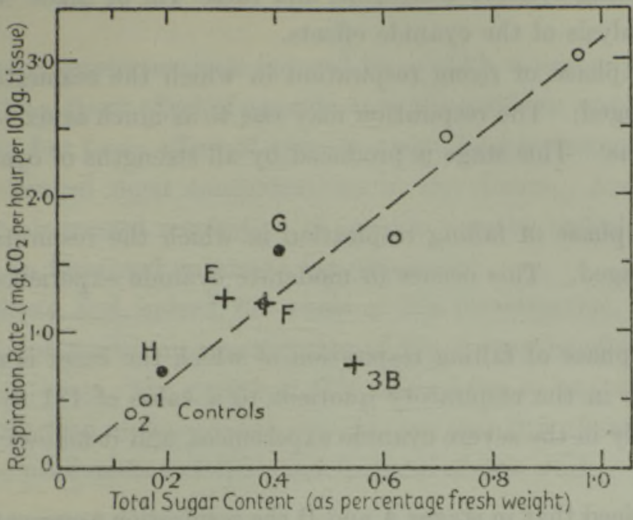

Fig. 10.—Relationship between respiration rate and sugar content. The broken line and circles—○, are plotted from fig. 8.

○ during rising respiration phase.

+ after fall in cyanide.

● after fall in air.

efficiency since its respiration rate is only about one-third that of potatoes with the same sugar content on the rising respiration phase, as shown by the broken line in the graph.

IX. Interpretation of Results.

A.—*Respiration during Cyanide Treatment.*

In the foregoing experiments a striking and hitherto unrecorded effect has been clearly defined. It has been shown that when potato tubers at 15° C. are exposed continuously to atmospheres containing low concentrations of hydrogen cyanide, the respiration increases considerably and may be maintained at a high level for periods of several weeks.

The respiration during a cyanide experience goes through a typical two-phase course, first a period in which the respiration rises to a maximum, and then a slower fall. Over the range of cyanide concentration investigated, the slopes of the two phases and the peak respiration values attained are greater with increasing cyanide.

The effects of moderate cyanide experiences, on the one hand, and relatively

severe cyanide experiences, on the other, exhibit important differences. In the experiments which have been described three metabolic states are distinguishable during cyanide treatment, and these will be made the basis of a preliminary analysis of the cyanide effects.

Stage A.—A phase of rising respiration in which the respiratory quotient remains unchanged. The respiration may rise to as much as six to eight times the normal value. This stage is produced by all strengths of cyanide investigated.

Stage B.—A phase of falling respiration in which the respiratory quotient remains unchanged. This occurs in moderate cyanide experiences, following stage A.

Stage C.—A phase of falling respiration of which the onset is marked by a sudden increase in the respiratory quotient to a value of 1·1 to 1·25. This stage occurs only in the severe cyanide experiences, and it follows immediately after stage A.

It will be noticed that in stages A and B the respiration appears to be similar in nature to normal aerobic respiration, since the respiratory quotient remains unchanged. These stages, moreover, seem to be completely reversible. In stage C, on the other hand, not only is the respiratory quotient increased, but permanent injury to the tissues develops, and subsequent cyanide treatment after removal to air no longer induces increased respiration. With moderate cyanide treatment the sequence of effects is stage A–stage B ; with high cyanide stage A–stage C. It is not yet clear whether a sufficiently prolonged experience in moderate cyanide might induce the sequence stage A–B–C, but so far this has not been observed.

B.—*Sugar Changes in Moderate Cyanide.*

The experiments, in which sugar analyses were made, have shown that the drifts in respiration during both the rising phase (stage A) and the falling phase in cyanide (stage B) (that is during the two phases of unchanged respiratory quotient) are accompanied by parallel drifts in the sugar content of the potatoes. Moreover it has been established that the respiratory efficiency (the ratio respiration/sugar concentration) is not appreciably changed throughout such an experience, showing that there is no apparent effect upon the respiratory enzymes.

As already mentioned, there is good reason to believe that sugar concentration forms a limiting factor in the normal respiration of the potato at 15° C.—a large body of evidence in favour of this view has arisen in a detailed study

of the effects upon respiration of increasing the sugar concentration by storage at low temperatures. The results of the present investigation also support this view that the rate of respiration at 15° C. is conditioned by the sugar concentration.

The changes in respiration rate induced by cyanide would appear, therefore, to be due, not to a direct effect of cyanide upon the catalytic systems responsible for respiration, but to an effect of cyanide upon the starch-sugar relationships resulting in changed sugar concentrations in the tissues. According to this view, then, the increased respiration in cyanide is to be regarded as an effect produced by an increased substrate for respiration.

This hypothesis, and, indeed, the whole of this investigation, was developed in close relationship with an investigation of the properties of certain amylase preparations *in vitro*. The results of this study have so important a bearing on the interpretation of the present experiments that it is thought essential to include here a brief outline of the main features of this work.

C.—*Cyanide Effects on Barley Amylase* in vitro.

In an investigation of the effects of cyanide upon a large number of preparations of barley amylase, several interesting phenomena have come to light. It has been found that under certain conditions cyanide exerts a dual action upon the enzyme.

There is first a rapid activation, after which a slower inactivating process becomes apparent, which obscures the activation and leads eventually to the partial, or in some cases, total destruction of the enzyme. This inactivating process is greatly retarded by the presence of the substrate, starch, or by low temperature.

The magnitude of the activation varies from one preparation to another,* and also according to the previous history of the enzyme. For a particular preparation the degree of activation depends upon the cyanide concentration, and the relationship appears to be hyperbolic.

An activated amylase, under conditions where the inactivating process is negligible, becomes deactivated by the removal of the cyanide as hydrogen cyanide under reduced pressure.

* Some preparations have been found which are not activated by cyanide; in other cases, the activation may be extremely great, as much, for example, as 25 times the control value. A full discussion of the problems connected with the incidence and character of the activation will be given in a subsequent paper.

D.—*Cyanide Effects on Starch-Sugar Relationships of the Potato.*

The above effects of cyanide upon certain amylase preparations *in vitro* suggest a basis for interpreting the changes in sugar content in the potato during cyanide treatment.

When potatoes are treated with cyanide at 15° C. the sugar concentration (and the respiration) increase progressively for a time and at a rate which depends upon the cyanide concentration (stage A). It is evident that during this phase there is an increased hydrolysis of reserve carbohydrates. It is suggested that this is due to an activation of the starch-hydrolysing system of the potato, corresponding to the activation of amylase *in vitro* by cyanide.

Eventually this period of increased sugar production comes to an end, and the metabolic system settles down to a new drift (stage B) in which the sugar content and respiration are falling. During this phase the rate of production of sugar from reserves is no longer sufficient to maintain the former high sugar level. It would appear that a second effect of cyanide has now come into play, an inactivation of the system which was originally activated. This would correspond to the second effect observed *in vitro*, the inactivation.

Removal into air after a moderate cyanide experience causes a drift of the sugar content, and respiration, back toward the normal state. This suggests that the cyanide-activated mechanism becomes " deactivated " by the loss of hydrogen cyanide, an effect which again has a strict parallel in the case of amylase preparations *in vitro*.

It will be seen, then, that the effects of cyanide upon the starch-sugar relationships in the living potato suggest a close analogy with the effects, which have been demonstrated on amylase preparations *in vitro*.

The close analogy between these effects tends to support the hypothesis that cyanide produces changes in the starch-hydrolytic system similar to those observed *in vitro* with preparations of barley amylase. According to this view it would seem that the rate of production of sugar in the potato is determined by the activity of the amylase system.

The further development of this hypothesis must await an extension of the study on amylase extracts, and, in particular, an investigation of the effects of cyanide upon the amylase of the potato.* This work, and also further experi-

* We note in the 'Amer. J. Bot.,' vol. **17**, p. 1050, Dec., 1930, that F. E. Denny, with whom we discussed the progress of our work at the International Botanical Congress, 1930, has since then investigated this particular point. Denny confirms our expectation, namely, that extracts of potato amylase can be activated by cyanide *in vitro*.

ments on changes in sugar content in potatoes treated with cyanide, are in progress.

E.—*Inhibition of the Respiratory Enzymes.*

We have seen that in stage B, the falling phase in moderate cyanide, the decreasing respiration is due to a falling sugar concentration and not to an inhibition of the respiratory enzymes. During the rapid fall in high cyanide (stage C), however, in addition to the changed respiratory quotient, we find a marked decrease in the respiratory efficiency. In this stage of cyanide treatment the inhibition of the respiratory enzymes becomes apparent, that is, the effect which is usually associated with the physiological action of cyanide. Further investigation of this aspect of cyanide action is in progress.

F.—*Cyanide Effects upon Cell Mechanism—Limiting and "Non-limiting" Factors.*

We have seen that the various effects of cyanide upon the metabolic system become apparent in a definite sequence. It is evident that the order of appearance, and the magnitudes, of the different effects will depend upon the relationships of the individual processes affected to the system as a whole. It seems clear that where the rate of respiration is largely limited by one particular part of the metabolic system, then any effect of cyanide upon this "limiting factor" will preponderate over effects upon other cell mechanisms which under the particular conditions are "non-limiting." In such a system cyanide may act upon several cell mechanisms, but specific effects on "non-limiting factors" may be almost completely obscured, until these in turn become "limiting."

Consequently, in the case of the potato, although there is no direct evidence that the oxidative systems of the cells are being inactivated during a moderate cyanide experience, yet it cannot be concluded that this effect is absent, since under the conditions of our experiments the rate of respiration is limited by the sugar concentration. Under such conditions relatively large changes in the effective amount of respiratory enzyme might result in negligible changes in respiration rate.

It is felt necessary to emphasise this point of view since it has been found in the case of the potato, that the nature of the cyanide effect depends upon the metabolic state of the tissues. The present investigation has established that at 15° C. cyanide induces an increase in the respiration rate, and that this is due to a specific effect upon the starch-sugar relationships. We shall show in a subsequent paper that, in other metabolic states, cyanide effects upon other cell mechanisms become apparent.

X. Summary.

(1) The respiration of potato tubers at 15° C. increases when they are exposed to atmospheres containing low concentrations of hydrogen cyanide.

(2) The respiration rate goes through a typical two-phase course, first a rise to a maximum, and then a slower fall.

(3) During moderate cyanide treatment the respiratory quotient remains unchanged and the effects are reversible. During severe treatment, however, the onset of the falling phase is marked by an increased respiratory quotient, and irreversible injury occurs.

(4) During the rising phase of respiration in cyanide the sugar content increases. In moderate cyanide the falling respiration phase is accompanied by a parallel fall in sugar content, and there is no evidence of inactivation of respiratory enzymes.

(5) The changes in respiration rate in a moderate cyanide experience are due to changes in the concentration of sugar.

(6) The changes in sugar concentration are interpreted as a direct effect of cyanide upon the starch-hydrolysing system of the potato, since analogous effects of cyanide have been observed with certain amylase extracts *in vitro*.

(7) During the falling respiration phase in high cyanide, in addition to the development of an increased respiratory quotient, inactivation of the respiratory enzymes is shown by the decreased respiratory efficiency (the ratio respiration rate/sugar concentration).

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