

I

THE RESPIRATION OF A POPULATION OF
 SENESCENT RIPENING APPLES*

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INTRODUCTION

Of all protoplasmic functions, the one which is, by tradition, most closely linked with our conception of vitality is the function for which the name of respiration has been accepted. It might, therefore, well be expected that every variation of the intensity of the metabolic activity of a cell would be correlated with some change in the respiration of that cell. Before we can decide whether respiration really holds this position as an index of the integrated activities of the cell, we need to accumulate respiration data for different types of plant organs throughout their life history of development, maturity and senescence. These data must then be examined critically with the hope of establishing the nature of the major and minor determinants of the variations of intensity of respiration. No such collection of data has yet been published. The present paper aims at making a contribution to this collection and other contributions should follow.

A good deal of work has already been carried out in the Cambridge Botany School upon respiration of evergreen leaves, which continue to exist in a state of maturity for very long periods of time. In striking contrast with this type of organ is the type of the ripening fruit. The natural biology of the two is so different that it is obviously of importance to establish whether the same fundamental principles are manifest in both. In the ripening fleshy fruit, senescence is the dominant stage of ontogeny. The fruit of the apple, which possesses such striking keeping properties, is most suitable for investigation, since it runs through its ripening senescence at a slow rate.

An opportunity of taking up such work was provided by the beginning of the experimental work of the Food Investigation Board, at Cambridge, under Sir William Hardy. The apples made available for us were those that were kept in cool storage at about 2.5° C. for the investigations of Dr F. Kidd and Dr C. West.

* This paper was published in 1928 as the first paper in the series 'Analytic Studies in Plant Respiration'. The authors were F. F. Blackman and P. Parija, the reference being *Proc. Roy. Soc. B*, **103**, 412, 1928.

We are indebted to the Board for a subsidy to enable the junior author to devote a year to the investigation presented in this and the following papers.

The outlook and the problems. Our outlook upon these apples has been to regard them as a population of individuals slowly progressing in cool storage through the metabolic drift which constitutes the senescent and penultimate stage of ontogeny, popularly spoken of as ripening. Some biological truths of this drift can be brought out by statistical treatment of the population as a whole, others only by intensive study of individual behaviour. It is the latter aspect that we wished to explore, in order that we might find out which features of individual respiration can be held to be capable of physiological interpretation and which must, at present, be regarded as indeterminable chance happenings.

The nature of the test applied to the respiration of the apple population was to take out of store, throughout a period of eight months, individual apples, one by one, and examine the intensity and course of their respiration in air; and also, as part of the same inquiry, to subject them to a variety of oxygen mixtures ranging from zero to 100%. The survey of the results in air is given in the present paper, while those in the oxygen mixtures are brought together in the following papers.

The previous history of the population was that they were Bramley's Seedling apples grown on fen soil, gathered at the beginning of October 1920, and maintained in cool storage between 2 and 2.5° C. for the investigations of Dr Kidd and Dr West, to whom our thanks are due for this essential assistance. The apples were picked from one orchard at one time and believed to be a homogeneous population, though they had not been gathered or graded under scientific supervision. When once brought into store the population is, of course, exposed to an extraordinary and quite unnatural uniformity of environment—no change of temperature or humidity, and no alternation of light and dark, for months in succession. One interest of our investigation would be to find out how far the population declared itself homogeneous under our physiological tests.

The individual apples were brought to the laboratory under standardized conditions and investigated at one temperature only, namely, 22° C. No conscious selection was exercised in taking individuals from store, except the avoidance of any that were bruised or showed traces of brownness. The average condition of the population was, of course, changing with the progress of the metabolic drift, and this revealed itself by the gradual colour change from full green through yellow-green to golden yellow and finally brown.

Allowing for all this drift, the conclusion was yet forced upon us by the results of our work that the population could not be described as homogeneous. Clearly, the apples continued to be distinguishable by physiological characteristics that differentiated them on the day that they were picked and put into store. The fact that recent differences of environment were negligible as a contributory factor to the observed differences of behaviour encouraged us to persevere in the endeavour to explain observed differences in terms of initial inherent qualities and temporal physiological drift.

Our observations of their respiration at 22° C. were continuous and revealed many minutiae of difference in behaviour, all of which we have endeavoured to

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bring to account, and either interpret them or formulate problems as to their determination. It is at least made clear, that had three or four apples instead of one been employed in each experiment, yielding merely average results of behaviour, then it would not have been possible to push our analysis very far. It is an essential consequence of the metabolic drift in storage that results obtained in one month are not repeated exactly in the next. When any problem arises in this type of work, it is not possible to go back and repeat an observation on identical material once more.

Experimental methods and procedure. The apples were brought from the cool store to the laboratory, weighed, and placed singly in a glass respiration chamber of a spherical form, which consisted of two hemispherical domes with a wide equatorial flange and two polar tubes as inlet and outlet for the constant current of gas, maintained by aspirators through the chambers, at a rate of about 1500 c.c. per hr. The chamber halves were waxed together, fixed in a weighted frame and lowered into a large thermostat bath kept at 22° C. The whole of this occupied about 30 min.; the air currents were then started at their proper rate and run for about 90 min. as a preliminary before estimations were started. There is therefore about 2 hr. of respiration in all before the point of time that figures as zero time in the records. The bath temperature generally kept constant within 0.2° C.; there were a few misadventures during the nine months' work, which are noted in the records of the individual cases. The current of air passes from the chambers through Pettenkofer tubes of standardized baryta, which are arranged as a parallel set, and the current is shifted on automatically by clockwork from one tube to the next at intervals of 3 hr. In this way continuous records of the production of CO₂ can be obtained for an indefinite period of time. In the present work some records continue for 16 days without a break.

Each day the used Pettenkofer tubes are lifted out one by one, washed into a beaker and titrated with decinormal HCl and phenolphthalein. They are then refilled and replaced in their frame, ready for the air current to come round again. The CO₂ production is expressed in mg. CO₂ per 300 g.hr. Medium-sized apples were selected from the store, averaging 140 g.; they were weighed again at the end of the experiment; the loss of weight averaged 1.5 % per 10 days, the minimum being 0.77 % and the maximum 2.09 %. The individual cases will be found detailed in Appendix I.*

The respiration records. In all, twenty-one experiments, numbered V–XXV, were carried out, in that sequence, on single apples brought from cool store, from the middle of November 1920 to the end of June 1921. The CO₂ production of some was examined in air only, but most were exposed as well to the effects of one or other of the following concentrations of oxygen: 0 (nitrogen), 3, 5, 7, 9 and 100% O₂.

The work involved nearly 2000 estimates of the CO₂ of respiration; the numbers are not tabulated in this paper but presented in graphic form throughout. The graphic records of the respiration values of the twenty-one experiments will be found set out in Appendix I*; these records will be referred to as the 'General

* Appendix I will be found at the end of this book; it was originally published as an appendix to 'Analytic Studies in Plant Respiration. II' (*Proc. Roy. Soc. B*, **103**, 446, 1928).

Charts' of the results. Mostly two experiments were carried on concurrently; and where this was so, the two records are grouped together in the same chart, one often serving as control to the other.

In the various sections of this paper, discussing special points, excerpts from the general charts bearing on the problem are brought together and correlated. In all the charts the ordinates express mg. CO₂ per 300 g.hr. for the fresh weight of the apple when taken out of cool storage. The abscissae are hours of time from the beginning of the respiration measurements. Each 3 hr. measurement is represented graphically by a single heavy line, or by three consecutive dots, covering the period of 3 hr. duration.

The long series of continuous estimations show a constant tendency to fluctuate up and down. We have thought that the general drift of the respiration is brought out more clearly in our graphic records when we represent it, not by a single median line, but by two 'contour lines' which are drawn parallel, one above and the other below the range of the fluctuation. When a definite numerical value is needed for respiration, it is, of course, the value midway between the contour lines that is adopted.

The fluctuations. It is not to be expected that under constant conditions the sequence of estimations would give values lying on one steady line, but it is clear that the fluctuations that actually occur are much greater than those that can be attributed to small random errors of titration, tube-washing and manipulation.

As a striking example of these fluctuations, it has several times been noted that, when the respiration is undoubtedly declining generally, as proved by a record lasting several days, there may yet occur in the course of it a level sequence of no less than four identical readings—covering 12 hr.—before the falling drift comes into evidence again. Had the record been stopped just at the end of this 12 hr. it might have been concluded, confidently, that the fall had passed into a definite level phase.

The range of the fluctuations indicated by the distance apart of the two 'contour lines' is about the same in the different experiments when respiration is running an approximately level course, and amounts to 0.7 mg. CO₂, but occasionally the readings seem to swing with greater amplitude. Apple VIII provides a unique case; this was the one apple that developed a patch of fungus mycelium, involving a big rise in the respiration. Here the fluctuations became very great and the successive readings were most irregular, which we attribute to the irregular growth and activity of the fungus on the apple tissue.

The 'air-line'. When some partial pressure of oxygen, other than that of air, is given it will be seen in the various records that the CO₂ production may be either much increased or much depressed, causing a deflexion of the double contour line which indicates the drift of respiration. At such times it is important to know the ratio of this increase or decrease to the magnitude of respiration that would have occurred had the apple been kept in air all the time. For this purpose it is necessary to join together the air records before and after by an interpolation. This is represented in the records as a single median line, and not by contour lines. Often these

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interpolations have to be long, and much study of the records has been required to carry them out with confidence.

Joining up the actual air records by interpolations, and sometimes adding extrapolations, we can get a continuous line, which we shall call the 'air-line', running right through the experiment and suitable for comparisons and controls.

Respiration in air: the special problems. We may conclude this introduction by indicating the chief features and problems presented by the respiration of our twenty-one apples, when we came to review the records obtained. The earliest apples, V and VI, were investigated when they had been in store for only 30 days; the latest, XXIV and XXV, when they had been stored for 260 days.

(a) The primary variable was the absolute magnitude of the respiration for different apples. Some air-lines started as high as 20 mg. CO₂ per 300 g.hr. apple, while others were as low as 12 mg. CO₂. To some extent the drift of these initial magnitudes was temporal, but clearly some other quite different factor was involved as well. This complication is to be examined in the first section.

(b) Apart from differences of pitch the air-line records were not all of the same type. Some declined fairly fast, some kept level for a time and then fell, while others rose day after day. The outstanding complication was that these different forms did not present themselves as one uniform drift of type, as the individuals were examined month after month. The resolution of these complications in the form of the drift of the air values is the subject of the second and third sections.

(c) A minor feature that attracted attention was the form of the air-line initially, immediately after heating up from 2.5 to 22° C. The rising respiration did not simply mount up to the air-line value but in many cases clearly overshoot that value and then fell back to it. This special disturbance of initial rates is examined in §§ 4 and 5 as the 'change of temperature effect'.

1. THE INTENSITY OF THE RESPIRATION OF APPLES DURING
 THE SENESCENT PHASE

The problem that we have to take up in this section is that of the great variation of intensity of respiration shown by apples removed at intervals from the cool store over a period of 8 months. We have twenty-one cases to consider, and the ideal values to work on would be the initial air-line value for each case at 22° C. Brought, as they are, from 2.5° C. the respiration rises rapidly at first but presently settles down to proceed along the air-line. Extrapolation of this air-line back to zero hour would give, for each apple, what we may call its ideal initial respiration value. There are certain complexities about the early course of observed respiration, which are to be explored in § 4, and these affect the estimation of such initial values; but fortunately the divergences of the apples one from another are so great that for the present section it is a matter of indifference how the initials are arrived at, so long as the same method is followed for every apple. We will therefore adopt the ideal initial values just mentioned, which will be found set out in full in column 7 of the table in Appendix I. These twenty-one initials are represented in fig. 1, plotted against a time axis which gives the date when each apple

was removed from store and the number of days that it had existed in store since picking. Clearly the early apples give respiration values that are lowest of all, after which come slightly higher values. Subsequently there is great divergence of values, the high values mounting up to over 20 mg. in March and falling off somewhat towards June. But all through this drift there are occasional occurrences of medium and quite low values. In the figure the highest values have been connected up with one line and the lowest with another, while a median line has been carried throughout the assembly. On this unanalysed presentation of the data one might conclude that there was a marked tendency for the mean value to rise till about March and then to decline somewhat to June. But more marked than the

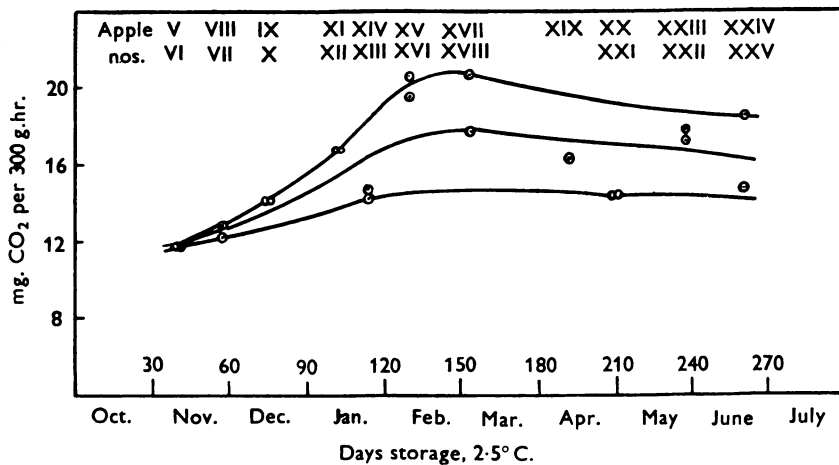


Fig. 1. The initial respiration values at 22° C. arranged in chronological series. The time axis gives dates of removal from cool storage at 2.5°. Twenty-one apples were examined at eleven dates. The exact dates and respiration values will be found in columns 2 and 7 of the table in Appendix I. When two apples at one date gave identical values, this is indicated by the point having a dumbbell surround instead of a circle. The serial numbers of the apples are given by the roman numerals along the top of the figure; the top numeral refers to the apple having the higher respiration value. Lines are drawn connecting up the drift of the highest values, and the drift of the lowest values. A mean line is added equidistant from the two extreme lines.

drift of the mean would be the enormous increase of the scatter about the mean with progressing ripeness. Another curious feature would be the number of examples that lie on the extreme lines and the few that are found on the median line, so that the whole assembly does not at all resemble one showing a normal scatter of chance divergences about a slowly drifting mean. Could apples really diverge so much from one another by unanalysable chance variations, then nothing would be gained by working with single individual apples.

Our first progress in the analysis of this complexity came from comparing with it the grades of ripeness indicated by the colour of the individual apples at the dates when their initial respirations were determined. As the months from November to June passed there were of course changes in the appearance of the apples in store. At first, from October to March, all were full green, but then the slow progression towards ripeness caused visible change of surface colour, through

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yellow-green, to full yellow and then on to partial or complete brownness. While the whole population of the store was drifting through this series of changes it was clear that all individuals were not moving at anything like the same rate. In April apples representing all stages from green to brown were present. As time went on, more and more apples had to be set aside as brown in parts. Most of the apples came to ripeness (yellow colour) in April to May, but some were still yellow-green at the end of June, by which time no pure green apples were left.

The correlation of colour with initial respiratory magnitude for the last seven cases first supplied a clue to the system underlying the irrational distribution of values. The earlier cases had led one to associate very low initial respiration with unripeness, and high initial respiration with ripening, but when apples XXIV and XXV were selected as the two most extreme apples for unripeness and ripeness, respectively, to be found in the store at that date, XXV being 'golden yellow' and XXIV only just beyond full green, namely, 'yellow-green', then it was found that XXV had a low initial respiration value, 14.7, while XXIV was high, 18.5. This distinction was supported by the preceding apples, for XIX, XX and XXI, with low respiration, had been recorded as yellow, but XXII and XXIII, with higher values, as 'green-yellow'. Thus low initial respiration is associated with unripeness in November to December and with very full ripeness in May to June. We must conclude then that respiration first rises and then falls, and can be clearly associated with the ripening drift of colour in storage at 2.5° C.

The relation between colour and intensity of respiration (as measured here initially at 22° C.) that came out of a close study of their parallel drifts may be formulated somewhat as follows. Every apple picked unripe drifts during ripening through a special senescent phase of metabolism, the essential nature of which will be discussed in § 3. The passage into this phase from the previous phase of metabolic maturity is marked by a rise in respiration rate, which rise starts slowly, progresses faster, and then slackens off to a maximum value; during the early part of this rise the apple colour is full green, losing its intensity towards the maximum of respiration. After the maximum, the respiration begins to fall, though at first slowly, and during this stage the colour of the apple may be described as typically yellow-green. This stage is succeeded by a quicker fall of respiration and the apple is now full yellow colour. This fall of respiration continues, provided no fungus attack develops, on into the stage when the apple becomes brown. In fig. 2 we present these relations schematically as a time drift of the two characters. We do not predicate a very close correlation of colour and respiration, since the former is determined only by surface cells while the latter is an expression of the whole mass of the apple, but the figure gives the relation which seems to be typical.

Observation of a population of apples in storage teaches us at once that individual apples run through this typical drift at different rates, since some may have drifted right through to brownness when others have only reached the green-yellow stage. The implication of this is that the respiration curves of such contrasted individual apples, plotted on the same time axis, will cross one another, for the quick-ripening apple will show an early maximum and an early fall, while the slow-

rising respiration of the slow-ripening apple will cut across the fall of the other on its progress to its own later maximum. We are inclined to think that the later the maximum is attained the lower is its pitch for a given apple, as compared with the earlier maximal values of the quickly ripening apple individuals. The evidence points to all the apples having much the same low respiration values in the 'mature' stage before the senescent rise sets in.

In conformity with these propositions we have fitted to our observed assembly of initial values of respiration sets of curves representing the complete respiration sequence for the whole of the senescent drift. This is carried out in fig. 3. Four of the initial points are marked by squares, and these will be dealt with later. At present we are concerned to schematize the seventeen cases represented by circles.

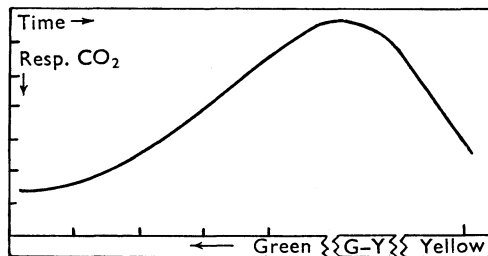


Fig. 2. Schematic form of drift, with time, of intensity of respiration of a ripening apple passing through the senescent phase. The form of curve given here is applicable to the respiration measured initially at 22° C. when the senescence is progressing in cool storage at 2.5° C. In this generalized curve no definite values are given to either ordinate or abscissa axis, but the colour sequence which is associated with the respiration drift is indicated. Specific cases are to be dealt with in fig. 3.

Through these are drawn two sets of illustrative curves, and these curves imply that for any apple in the schema, the curve which passes through its observed initial value at a certain date indicates what its initial respiration would have been, had it been withdrawn from store at any other date, either earlier or later, than it was actually taken out. One striking feature of this figure is the strong suggestion of heterogeneity in the population shown by the fact that the sets of curves fall into two remote groups. Variation could easily be made in the course of the construction curves, but the absence of individuals of intermediate rates of ripening, both in the rising and the falling stages, could hardly be eliminated by an alternative formulation. We propose to distinguish the twelve apples that ripen quickly as representatives of class A, while the five that ripen more slowly may form class B. Below the respiration drifts are set out the colour sequence expected for class A and for class B according to the principles already enunciated. These sequences are based on the recorded colours as given in column 3 of the table in Appendix I.

According to this schema the scatter round the mean is very small for class B, but only a few of this class chanced to be drawn from the population, presumably because they were in small minority. Even in class A the scatter is not great, as the drift lines are here presented. The fact that so often two apples drawn from store at one date give nearly identical respiration values in class A, points also to

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this being a real group of small scatter, rather than part of one wide common group with the remote cases of class B.*

There are still four other apples that have not been brought to account in our treatment of class A and class B in fig. 3. These are VII, VIII, XXII and XXIV,

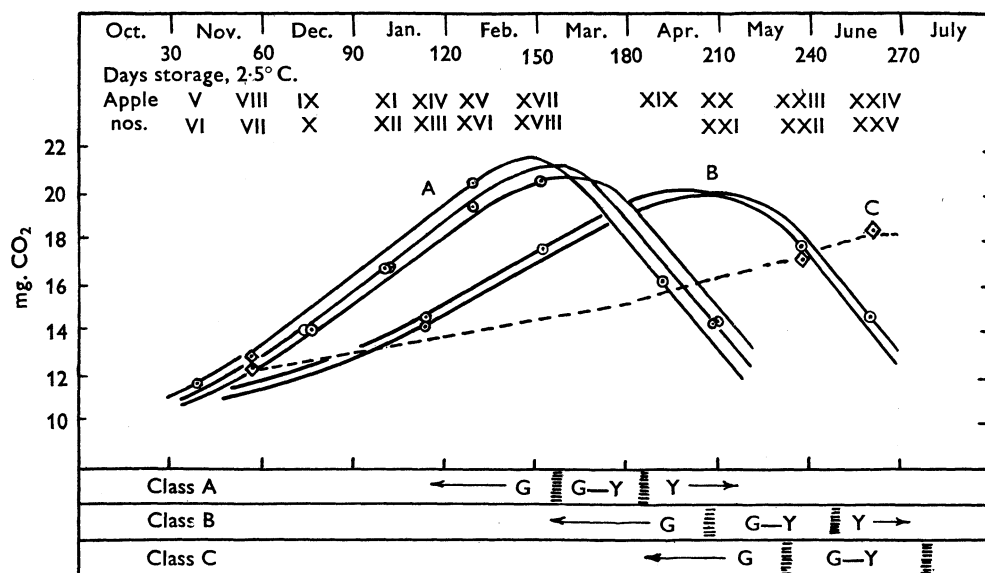


Fig. 3. The observed initial respiration values of the twenty-one apples given in fig. 1 are here grouped into three separate classes A-B-C which ripen and pass through their respiration drift at three separate rates. Through each initial point has been drawn a curve of the type of fig. 2. Twelve apples are allotted to class A, and three typical curves serve to indicate their senescent drift. Five apples are allotted to the later ripening class B, and two curves serve to connect them together. The four apples of class C are connected by a single drift line, which had not run beyond the rising phase when the investigation was ended. The identification numbers that were given to the individual apples are entered in the upper part of the figure over the individual respiration values. Where the two apples at one date have different respiration values the top number is that of the apple with the higher respiration value. The observed colour sequence noted for the apples of each class is set out below to show that the drift from green through green-yellow to yellow takes place at a different rate for each class, and that the relation of these rates for the three classes is the same as that shown by the respiration rates.

represented by squares instead of circles. Apple XXIV presented us with a very high respiration very late in the chronology, and also showed a yellow-green colour. These are features of an apple near its maximal senescent respiration, which suggests that XXIV must represent a class that ripens still much later than

* In addition to a scatter of rates of ripening within class A there must also be some variation between individuals with regard to the respiration value per unit of fresh weight. If this had a considerable range, the vertical divergence of lines within the nest of curves might be wholly or partly an expression of such a scatter. Introduction of this consideration might remove the intersection of curves inside each class at the peak of the schema and substitute a set of three parallel lines, but this would not affect any arguments based on the schema. During storage, month by month, the apples are losing water, so that from this cause alone the respiration values per unit fresh weight must rise. The observed water loss is, however, not enough to make an appreciable contribution towards explanation of the observed large rise.

class B. This apple provides the first individual for our new class C, and to this are assigned also a pair of early apples—VII and VIII. On any evidence that this chart can provide this last attribution is, of course, absolutely arbitrary, for these two apples are perfectly situated for class A apples. But on evidence provided in § 2 on air-line drifts, and confirmed in the next paper where the behaviour in nitrogen is investigated, there is no doubt whatever that VII and VIII must be segregated from their neighbours and classed with XXIV as representatives of a separate class, C. The straight line drawn in fig. 3 from VII to XXIV would serve for the slowly rising limb of the schema of initial respiration values of this class. This line passes through apple XXII, which is also undoubtedly of class C on similar evidence to be set out later.

Such an analytic schema of three sets of lines provides some interesting situations when an apple is found at the intersection of two lines. Thus XIX might be claimed, as far as position on the chart goes, as either rising C or falling A; but the fact that it was full yellow settles it as A. Again, XXII and XXIII are in the chart so balanced between rising C and falling B that we must seek other evidence. A falling B apple, not far below the maximum, should be green-yellow as was XXIII. It would then have been expected that XXII, which we have referred to class C, should have been more green than XXIII. It was not recorded at the time as more green, but only as yellow-green, though a special note was made that XXII was strikingly turgid and fresh in appearance for an apple at that late date, so that on the whole its condition supports its attribution to a rising line.

It may be mentioned that we had no schema of this type before us when the experiments were actually made, but only a growing perplexity about the association of low respiration with both the greenest and the yellowest apples. It was this perplexity that led us to select for the two apples of the late June experiment the greenest apple and the yellowest apple that could be found in the population. This gave a clue which, followed up, has led us ultimately to substitute for the perplexing configuration of fig. 1 the highly rationalized formulation of fig. 3, in which, whether rightly or wrongly, each point finds its place in one of three physiological classes, and also a definite position in the sequence of development of its own class. This formulation on the basis of the evidence so far produced may appear rather unsubstantial, but it will receive further support in later sections on the air-lines as well as from nitrogen effects.

It may have puzzled the reader that, whereas the whole of this section is expounded as a study of senescent drift of a population of apples stored at the temperature of 2.5° C., yet all the respiration values brought to account are for the high temperature of 22° C. The explanation of this indirect approach is that the experimental work was undertaken as a study of the effect of oxygen concentration upon apples at 22° C., and not till after it was finished was it discovered that the data supplied material for the exposition of the various analytic treatments set out in the sections of this paper. The respiration values in the present section are, however, all initial values, at 22° C., and so are determined by the physiological state of the apple at 2° C. when removed from store multiplied by the factor which gives the proper ratio for increase of the respiration rate between