Respiratory Behavior of Bacteria-Free Crown-Gall Tissues

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(Received for publication October 26, 1944)

INTRODUCTION

The respiratory processes of malignant tissues of animals and human beings have long been considered to differ in important respects from those of non-malignant tissues of the same creatures. This difference expresses itself in several ways, especially in a low R.Q. (0.8 or less) and a decided anaerobic tendency to accumulate acid products, lactic acid in particular. It seems possible, indeed, if we may draw an analogy from plant materials (6, 9), that the characteristic independence from normal morphogenetic restraint shown by tumor tissues may be essentially an expression of independence from the formative influences of oxygen gradients. These evidences of a high degree of anaerobiosis are considered typical of malignant growths, and their possible use in the diagnosis of malignancy has been suggested by some (2, Footnote, p. 242).

Routine observations on in vitro cultures of tissues from cancer-like, bacteria-free, crown-gall tissues of sunflower (12) disclosed 3 traits that suggest the existence of a similar picture in plant tumors. In the first place, old cultures have a distinctly yeasty or alcoholic odor. Since this odor, in yeasts, is an accompaniment of an anaerobic type of respiration, it may be interpreted in plant tumor materials as suggesting the accumulation of incompletely respired materials even when oxygen for complete respiration is available to the tissues. Since these cultures are bacteriologically sterile this accumulation must result from respiratory processes in the tumor tissues themselves. In the second place, such cultures tend to acidify the subjacent nutrient (unpublished data), indicating again the accumulation of incompletely respired, in this case acid, products. There seems to be no general agreement as regards the behavior of normal plant tissues in this respect. Robbins and Maneval (5) found that excised corn root tips tended to raise the pH of the nutrient, while White (7) reported a lowering of pH by wheat root tips. In the third place, such cultures, when immersed in a liquid nutrient, do not undergo the formative changes evinced by similarly treated cultures of Nicotiana-hybrid tissues, which changes have been attributed to effects of an altered oxygen gradient (9). These observations, together with the obvious possibility of an analogy between these plant tumor cells and those of animal tumors, led to a more careful examination of the respiratory processes involved. The work reported here is the result of preliminary studies on this problem.

MATERIALS AND METHODS

Interest centered particularly around the behavior of tissues of the bacteria-free crown-gall tumors of sunflower, which have been shown to possess in high degree the properties of malignancy; transplantability, and the quantitatively and qualitatively unrestrained growth commonly associated with animal and human cancers (12). The respiratory processes of 3 types of bacteria-free tumor tissues of sunflower were studied: (a) secondary tumors arising as a direct result of the inoculation of young sunflower plants with crown-gall bacteria (1); (b) tissue cultures isolated from secondary tumors and maintained in vitro through repeated (40 or more) passages (12); and (c) tumors arising as a result of implantation of fragments of tissue cultures under the bark of otherwise healthy plants (12). The respiration of these 3 types of bacteria-free crown-gall tumors were compared with a second type of bacteria-free plant tumor, of abiotic origin; that arising in consequence of hereditary defect in tissues of plants resulting from the cross Nicotiana langsdorffii × N. glauca (4) represented by: (a) tissue cultures obtained from the procambial region of such plants and maintained in vitro for many (100 or more) passages (8); and (b) tumors resulting from implantation of such tissue cultures under the bark of otherwise healthy plants of Nicotiana glauca (11). All these were further compared with tissues of bacteria-containing crown-gall tumors of sunflower, with healthy tissues of sunflower represented by internodal segments taken at a distance of 3 to 4 cm.
from transplant galls, and with stem growing points and young inflorescences from healthy plants. Records were thus obtained from 3 types of healthy tissue, from bacteria-containing tissue, and from 5 types of bacteria-free tumor tissue, including material of 2 unrelated host plants.

Respiration of these tissues was studied in a standard Barcroft-Warburg apparatus. The vessels used were of about 7.0 ml. capacity and of the standard type, with center well for caustic paper and a single side arm for acid. For most experiments 0.5 ml. of White's nutrient (10) containing 2 per cent sucrose was used to bathe the tissues; this was just enough to keep the tissues thoroughly moist, but not enough to cover them. A like amount of 20 per cent KOH was employed in the center well with a filter-paper roll. Tissues to give wet weights of between 30 and 300 mgm. were torn into fragments small enough to enter the flasks comfortably, tearing rather than cutting being considered preferable as introducing a lesser degree of trauma. All tissues were dried after respiratory test to constant weight at 120° C. and all calculations were reduced to cu. mm. of gas absorbed or released per mgm. dry weight of tissue. The manometers were shaken at an arbitrarily chosen speed of 120 r.p.m. with a 2.5 cm. stroke, though in view of the small volume of fluid used shaking is, of course, probably of little significance. The bath was kept at 25° ± 0.1° C. Most series of experiments were run in sevens; 6 flasks containing tissue, the seventh serving as thermobarometer.

Theoretically, the use of dry weights in dealing with plant tissues, with their relatively high and often widely differing content of inert storage carbohydrate, introduces an unknown and possibly important error into the calculations (see also discussion by Burk, 3, p. 207-208). Protein nitrogen should give a much better basis for comparison of the respiratory activity of plant tissues. Actually, however, the water content of the tissues studied here was relatively high and fairly constant. The highest individual dry weight recorded was 19 per cent, in a flower bud, which class had an average dry weight of 11.6 per cent and a range from 9.7 to 19.0. Bacterial tumors, and bacteria-free tumors arising from grafting of tissue cultures, had essentially identical percentage dry weights—11.6 (8.8 to 15.0) per cent, and 11.8 (8.4 to 17.1) per cent respectively. Healthy stem growing points were slightly lower—10.5 (8.0 to 15.0) per cent, and healthy internodal tissue was still lower—8.1 (3.6 to 12.9) per cent. Tissues cultures from Nicotiana had slightly higher dry weights than these last—8.7 (5.4 to 15.8) per cent, while Helianthus tumor tissue cultures were again lower—7.3 (3.3 to 12.9) per cent. Yet the widest range of averages, from 11.8 per cent dry weight for Helianthus graft tumors to 7.3 per cent for Helianthus tumor tissue cultures, represents a difference of only 40 per cent, based on the higher figure. Some of this dry weight is certainly inert in all tissues studied, so that all Qo½'s must be considered minimal values. And much of the variation between Qo½ values for different samples may be due to differences in content of respiring protoplasm. The over-all picture, however, seems unlikely to be materially altered by this factor. As long as we realize the existence of this uncertainty and weigh our interpretations accordingly, dry weight can be employed as a useful though not fully satisfactory basis of reckoning.

Since many readers of this journal may be unfamiliar with the metabolic characteristics of plant materials, and since a discussion of respiration in plants involves certain factors not present in animal tissues, it may be desirable to review these matters briefly.

Respiration, as defined in plant physiology, is any vital process that frees energy for the use of the organism. This is to be clearly distinguished from the mechanical exchange of gases, which in higher animals is the most obvious accompaniment of this process. The two are sometimes distinguished as egressis and breathing. In higher organisms, both plant and animal, the commonest type of respiration is the oxidative catabolism of carbohydrate, but it should be kept in mind that there are processes like lactic fermentation that release energy by means of a drop in energy level of the internal system (C₆H₁₂O₆ → 2C₂H₅OH·COOH + 18 Cal.). These are also respirations under the definition given above, although no "oxidation" in the usual sense takes place.

When the oxygen involved in carbohydrate catabolism is in the form of the free gas, the process is known as aerobic respiration. When free oxygen is not involved, the oxygen coming from internal sources, the process is called anaerobic respiration. Although anaerobic respiration commonly occurs where free oxygen is not available, it should be remembered that it may also occur in the presence of free oxygen, the crucial question being not what gases are available, but rather what gases are utilized. Yeasts, for example,
regularly respire anaerobically even in high oxygen atmospheres. Palladin, indeed, believed that all CO₂ produced in oxygen respiration is of anaerobic origin, the oxidative process going on at the expense of the pigment and not of the carbohydrate.

In aerobic respiration there is a patent absorption of oxygen, which can be measured manometrically, and is expressed as $Q_{O_2}$.² This is further elaborated as $Q_{O_2}^{AR}$ or $Q_{O_2}^{DA}$ to distinguish the two common types of gaseous media in which it is determined.

Complete oxidative catabolism of carbohydrate gives rise to CO₂ and H₂O, so that a second significant respiratory criterion is the $Q_{CO_2}$. This can be measured continuously by electrometric methods, or intermittently by absorption in caustic in the Pettenkoffer apparatus. In the Barcroft-Warburg apparatus it cannot be measured continuously in air or oxygen, but only as a final over-all reading by absorption in caustic, as in the Dixon-Keilin method. It can be measured continuously in nitrogen, since here the total gas pressure change is due to evolution of CO₂. We therefore can record the $Q_{CO_2}^{N_2}$ as a direct measure of anaerobic respiration.

When oxidative catabolism goes to completion the total reaction may be expressed by the equation

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 674 \text{ Cal.}$$

In this case the amounts of CO₂ and O₂ are equal and the process may be characterized by the quotient $CO_2/O_2 = 1$. This is called the R.Q. When the Barcroft-Warburg apparatus is employed in its simplest form, without caustic, the readings obviously represent the algebraic sum of the exchange of these two gases,

$$Q_{CO_2} \cdot O_2 = Q_{CO_2} = R.Q.$$  

If the R.Q. is 1 (complete and unmodified respiration) there will be no change in volume of the gaseous system, and the readings will be consistently zero.³ Any increase in volume of the system (positive readings) would represent

an excess of CO₂ over O₂, hence an anaerobic respiration; any decrease in volume (negative readings) would represent an excess of O₂ over CO₂, as occurs in the oxidation of fat. Since complete anaerbiosis would give $R.Q. = \frac{nCO_2}{nO_2} = nCO_2$, and since $Q_{CO_2}$ can be measured directly in nitrogen, the degree with which the directly read apparent R.Q. departs from the zero line in a positive direction (Fig. 2, left hand column) and approaches the $Q_{CO_2}^{N_2}$ (Fig. 2, second column) is a measure of the relative completeness of anaerobiosis in the presence of oxygen; that is, of the degree to which anaerobiosis is obligate.

Anaerobic respiration in animal tissues commonly takes the form of incomplete degradation of carbohydrate to lactic acid, according to the formula

$$C_6H_{12}O_6 \rightarrow 2C_3H_6OH + CO_2 + 18 \text{ Cal.}$$

Here there is no gas exchange, but only a change in energy level within the system. Such a reaction is commonly called "glycolysis." In animal tissue studies glycolysis has come to be used as synonymous with lactic acid fermentation; it should be remembered, however, that this is only a special case. Any degradation of carbohydrate involving hydrolysis, carboxylation, or hydroxylation, whether it be to lactic acid, butyric acid, or alcohol, is essentially a glycolysis in the same sense. Since anaerobic respiration studies with animal tissues are generally carried out in media containing bicarbonate, the lactic acid can be calculated from the CO₂ displaced from the bicarbonate, and is generally expressed as $Q_{G}^{AR}$, $Q_{G}^{O_2}$ or $Q_{G}^{DA}$; the G representing glycolysis. Measurement of anaerobic respiration in plants encounters two differences; first, that lactic acid is not the typical end product in plant tissues, and second, that plant tissue nutrients generally do not contain carbonate or bicarbonate. The characteristic anaerobic degradation (fermentation) in plants is to alcohol, according to the formula

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 28 \text{ Cal.}$$

Here no oxygen is absorbed, but CO₂ is released and can be determined directly by absorption in caustic. One can thus calculate a figure for $Q_{G}^{AR}$ (or $Q_{G}^{O_2}$) by subtracting the observed $Q_{O_2}$ from the observed $Q_{CO_2}$. This difference is of course synonymous with the $Q_{CO_2}$—it is, in fact, a real $Q_{CO_2}$ even in

³ Although commonly so stated this is not strictly true, since the solubilities of O₂ and CO₂ differ. This difference is, in fact, the basis for the so-called "Direct Method of Warburg." The difference in solubility makes the slope of the curve dependent not only on the volumes of gas absorbed or evolved but also on the total flask volume, and the volume of fluid present as well. The formula is

$$\frac{\Delta V}{\Delta t} = \frac{x_{O_2}(K_{CO_2} - R.Q \cdot K_{O_2})}{K_{O_2} \cdot K_{CO_2}}$$

If the volume of fluid used is large in proportion to the amount of gases present the error may be important. In these experiments, however, with a fluid volume of 0.5 ml. in a 7 ml. flask the error is small. A typical example (flask No. 1) gives

$$\frac{\Delta V}{\Delta t} = \frac{x_{O_2}(1.15 - (1 \times 1.09)) \cdot x_{O_2}(0.06)}{1.09 \times 1.15} = \frac{0.05x_{O_2}}{1.25}$$

in which case the error is 5 per cent of the volume of oxygen absorbed.

⁴ The dictionary (Webster's, Stedman's) defines glycolysis as "hydrolytic decomposition of sugar." Strictly speaking, lactic or alcoholic fermentations, which involve formation of carboxyl or hydroxyl groups without addition of water (hydrolysis), are not glycolyses according to this definition. The use of the term to apply to lactic fermentation has, however, become so universal in animal respiration studies that it must be accepted. There seems, nevertheless, to be no justification for making it synonymous with lactic fermentation alone, as is so often done by animal physiologists.
the case of alcoholic fermentation, in contradistinction to the apparent \( Q_{co_{2}} \) (really \( Q_{\text{lactate}} \)) observed in animal tissues. Only when its value is greater than the \( Q_{o_{2}} \), however, does it signify a glycolysis.

Some authors designate these various quotients as positive or negative. These signs are merely the expression of change in volume of the closed Barcroft-Warburg system. The \( Q_{o_{2}} \) is thus characteristically negative, the \( Q_{co_{2}} \) positive. One might, however, reason with equal logic that the \( Q_{o_{2}} \) represents a gain to the tissue, and should therefore be designated with a positive sign! Moreover, the use of signs leads to the anomalous result that the respiratory quotient \( \left( \frac{Q_{co_{2}}}{Q_{o_{2}}} \right) \) will generally be negative. To think of respiration as a negative process is certainly not helpful, and the author prefers to discard the use of algebraic signs in this discussion. This should introduce no misunderstanding so long as the essential nature of the processes is understood.

One other factor needs to be considered in dealing with plant materials, which does not enter into the picture at all in studying animal tissues. Green plant tissues, in the presence of light, carry on the process of photosynthesis, which is the exact reverse of catabolic respiration,

\[
\text{Photosynthesis} \quad 6\text{CO}_2 + 6\text{H}_2\text{O} + 674 \text{ Cal.} \rightarrow \text{C}_{6}\text{H}_{12}\text{O}_{6} + 6\text{O}_2
\]

This requires very little light, but does require the presence of chlorophyll. In the closed Warburg system this process results in no change in volume, but does alter the proportion of gases available for other reactions. Since this process may go on simultaneously with that of respiration, and since its intensity will vary with the intensity of light impinging on the tissue and with the amount of chlorophyll present (high in internodal tissues, inflorescences, and growing points; moderate in primary, secondary, and graft tumors depending on age; and nil in tumor tissue cultures), it is obvious that the interpretation of results obtained in the light might be completely impossible. Light must be entirely excluded from the respiration flasks during all measurements, and when green tissues are used sufficient time should be allowed in the dark to permit completion of all residual photosynthetic processes before readings are begun. Light was excluded by means of a matt-black hood placed over the water bath and over the manometer heads, with baffles so arranged as to permit shaking and reading the manometers without admitting any traces of reflected light to the flasks.

One deviation was made from the standard procedure, in order that R.Q.’s and \( Q_{o_{2}} \)’s might be obtained on each sample. Instead of running parallel tests with and without KOH and in air and \( N_2 \) atmospheres on separate samples, which introduces unpredictable errors due to variations in behavior of different samples, each sample was in most experiments followed for from 1 to 3 hours under each condition without removal from the vessel. Thus, in the most complete type of test, the vessel was first shaken for an hour with tissue and nutrient in air, without KOH, giving a measure of the apparent aerobic R.Q. directly. It was then flushed out for 10 minutes with a stream of \( N_2 \), the stopcock closed, equilibrated for 10 minutes, and then shaken for a second hour in an \( N_2 \) atmosphere, without KOH, giving a measure of the anaerobic respiration. The flask was then removed and charged with KOH, freshly flushed with \( N_2 \) and shaken for a third hour, giving a check on the anaerobic respiration and on the functioning of the apparatus, the theoretical reading being zero. The stopcock was then opened, the flask flushed thoroughly with air, the stopcock closed, and the manometer shaken for a fourth hour with KOH, giving a direct measure of oxygen absorption. From these figures \( Q_{co_{2}}^{\text{air}}, Q_{co_{2}}^{\text{air}}, Q_{o_{2}}^{\text{air}}, \), \( Q_{co_{2}}^{\text{air}}, \) and R.Q. are all can be calculated on the same sample of tissue. This procedure assumes, of course, that previous treatment with one atmosphere does not result in a residual change in behavior in another atmosphere. The experiments show, in fact, that an hour’s shaking in \( N_2 \), either with or without KOH, does not alter the subsequent shape of the \( Q_{o_{2}} \) curve. The approximately 30 minute period of adjustment involved between procedures while fluids were changed, gases flushed out, final readings recorded, manometers equilibrated, etc., does seem to have been sufficient to eliminate any residual effects, at least within the range of precision of the methods used.

This procedure also introduces certain complications into the calculations. The \( V_F \) of tests 1 and 2 differs by 0.5 ml. from that of tests 3 and 4. The solubility correction for tests 1 and 2 involves the summation of \( a_{o_2} + a_{CO_2} \) while that for tests 3 and 4 is dependent on \( a_{o_2} \) only. The appropriate constants calculated to correct for these differences were introduced into the records for each procedure.

Animal respiration studies, especially those carried out in bicarbonate, encounter one problem, the presence of "bound" (dissolved, occluded, or adsorbed) \( CO_2 \) in or on tissues or nutrients, which has to be corrected

\[ \text{Anaerobic respiration was measured manometrically only, and was not checked by chemical determination of the metabolic product. Lactic acid determination, the usual criterion in studies of animal tumors, would obviously be meaningless in studies of plant tumors. Determinations of alcohol might or might not prove significant.} \]
for by measurement after freeing with HCl. Experiments with plant tissues, in which bound CO$_2$ was determined by adding HCl to the tissue-nutrient charge of aliquot vessels at the beginning and at the end of a group of tests, showed that under the experimental conditions used (unbuffered solution containing no carbonate or bicarbonate and very little phosphate) neither nutrient nor tissue contained significant quantities of CO$_2$. This factor is so small as to be within the experimental error of the method; it can therefore be ignored in all calculations.

Tissue cultures of Helianthus tumor tissue, and secondary tumor tissues, were sliced on a microtome to 0.1 mm. thickness. Their respiration was then compared with that of similar cultures torn to a thickness of 1 to 2 mm. The results showed a consistently wider variation in QO$_2$ values and a lower absolute level of oxygen consumption in sliced than in unsliced samples. The result was interpreted as indicating that tissues containing as great a proportion of large cells as do these, are significantly injured by slicing, reducing their oxygen intake, while oxygen diffusion is adequate for the desired interchange without dividing beyond the 1 to 2 mm. limits set by the size of the vessel openings. Tissues were therefore not sliced in the definitive experiments.

This same conclusion was borne out by a second type of test. Tank oxygen was flowed through the vessels containing tissue culture material for 10 minutes so as to introduce a high-oxygen atmosphere. Readings on such vessels were then compared with those on similar vessels containing air (Fig. 1). Cultures in a high-oxygen atmosphere showed a considerable initial increase in total gas volume, resulting in an apparent anaerobiosis. The rate of increase was, however, not constant, decreasing rapidly and approaching a zero

Respiration studies of animal tissues are usually made on slices of 0.1 mm. or less in thickness and in high-oxygen atmospheres, so as to obtain a maximum oxygen gradient and assure an adequate supply of oxygen to all parts of the tissue. The first procedure involves considerable trauma, which would be even more pronounced with plant tissues (10, pp. 9-11). The second introduces an environment obviously differing radically from that in which metabolism goes on in vivo. Both are hence theoretically objectionable, and are used only because of the necessities imposed by the slow diffusion rate of O$_2$ in animal tissues. Preliminary tests were run to determine if these procedures are likewise necessary for plant tumor materials.

Fig. 1.—Effect of high oxygen (O$_2$) and low oxygen (Air) atmospheres on the R.Q. and QO$_2$ of pieces of living tissue cultures of Helianthus. The equilibrium slopes for both values are essentially identical in the 2 atmospheres, indicating that maximum rates are reached without resorting to increased oxygen levels. This is in distinct contrast to the behavior of most animal tissues.
value. When a steady rate was reached (after about 2 hours) this rate did not differ significantly from that obtained in air. The rapid initial increase in volume of gas would seem, therefore, to have been referable not to increased CO₂ output, but to release of excess O₂ adsorbed on the tissue or dissolved in the nutrient. There is no indication that increase in oxygen tension actually increases the rate of respiration. This result was again interpreted to indicate that oxygen diffusion through unsliced tissues is adequate to support the maximum respiration rate characteristic of these tissues. In definitive experiments cultures were therefore neither sliced nor supplied with a high-oxygen atmosphere.

One feature of these tissues did become evident during the experimentation, which requires attention since it introduces a possible error in the calculation on the basis of the classic Barcroft-Warburg formulae. These formulae are all based on the assumption that the nitrogen of the air, or the pure nitrogen used in measurement of anaerobic respiration, is completely inert and can be ignored. In running tests to check the reliability of the apparatus it became evident that this assumption is not always a valid one. When certain tissues, especially young sunflower inflorescences, are shaken in an atmosphere of pure nitrogen there is a rapid decrease in pressure within the closed system, indicating that nitrogen is absorbed by these tissues. This is not because of the use of impure gas containing traces of oxygen, for the rate of decrease was not reduced when the nitrogen used was first bubbled through concentrated alkaline pyrogallol solution until it no longer darkened pyrogallol in a trap. Approximately the same rate of absorption was also maintained when nitrogen was replaced by helium. This phenomenon thus cannot involve any significant chemical reaction. It seems to be purely mechanical. It is possible that nitrogen (or helium) dissolves in the fatty materials in which young inflorescences abound. The rate of absorption at first appears linear, but after 2 to 3 hours' continuous shaking it begins to fall off rapidly, and usually ceases to be significant by the end of 4 or 5 hours. One cannot, however, run each test in a closed atmosphere for 4 or 5 hours before beginning to take readings, especially in the presence of a nutrient capable of rapid fermentation if contaminated. We have no data on the exact solubilities of N₂ or He in this nutrient and in the various tissues under investigation. The best that can be done is to run at least a brief empirical test to determine the $Q_{O_2}^N$ for each sample and then correct the readings of $Q_{O_2}^{N-He}$ by this amount. Fortunately this

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We are also faced with the question as to how much absorption goes on in air. If we assume that the nutrient and tissues absorption is not important with most tissues and is negligible with the tissue cultures, which are perhaps the most important materials under consideration.

EXPERIMENTAL RESULTS

Healthy tissues.—Bacterial tumors, secondary tumors (bacteria-free), and graft (tertiary, metastatic) tumors (also bacteria-free) all are located on the older parts of the sunflower stem, adjacent to healthy internodal tissues. Tissues of the internode including epidermis, cortex, vascular material, and a large proportion of pith are thus geographically nearer to the tumor tissues than are any of the more rapidly growing regions such as stem tips. This does not, of course, imply any physiological or anatomical resemblance. For comparative tests segments of internodes from 2 month old sunflower plants were excised, usually about 30 to 40 cm. from the growing point, and split lengthwise into 6 approximately equal sectors. These were cut to such a length as to give wet weights of about 300 mgm. and introduced into the respiration vessels, where possible without further cutting. Four runs, totaling 19 samples, were made. Tests on vegetative stem growing points and on young inflorescences have also been included, since they represent rapidly growing tissues, which may be presumed to bear a closer physiological resemblance to tumor tissues than do the slow-growing internodal tissues. The results are illustrated in Fig. 2, top row, and Table I.

The respiratory levels, with the exception of the 2 winter runs (Dec. 11, 1942 and Feb. 5, 1943), were sufficiently high so that the results should possess a fair degree of precision ($Q_{O_2}=3.2$ to 5.5).

The R.Q.'s for internodal tissue (1.1) and young inflorescences (1.1) were not in any way unusual. For stem growing points they were low (0.9), suggesting an anaerobic (incomplete) type of respiration, but the deficiency was not pronounced. The level of anaerobic respiration was low in internodal tissue ($Q_{O_2}=1.3$) but more than twice as high in meristematic tissue (3.2 for stem growing points and 3.7 for young inflorescences). Evidently healthy meristematic tissues are capable of a much higher anaerobic respiration than are internodal tissues, although their aerobic respiration levels were about the same.

 Diseased tissues; bacterial galls.—Although the tissues are saturated with nitrogen at the partial pressure of atmospheric air, a reasonable assumption, then we need make no correction on the $Q_{O_2}^{N-He}$ and R.Q.$^{N-He}$, but only on the $Q_{O_2}^{N}$. If, on the other hand, we assume that the cuticular layers form a barrier to atmospheric nitrogen so that the internal tissues are at an unsaturated level with respect to atmospheric nitrogen, then a correction would have to be introduced. This last possibility seems extremely unlikely and has been ignored in the following treatment.
Respiratory quotient

\[ RQ = \frac{Q_{CO_2} + Q_{O_2}}{Q_{CO_2}} \]

Anaerobic respiration

\[ \frac{Q_{N_2}}{Q_{CO_2}} \]

Nitrogen error

\[ \frac{Q_{N_2}}{Q_{N_2}} \]

Oxygen consumption

\[ \frac{Q_{Air}}{Q_{O_2}} \]

Healthy tissues

(\textit{Helianthus})

- Internode
- Vegetative meristem
- Inflorescence

(\textit{BACTERIA FREE})

Primary tumors

(bacterial)

\textit{Helianthus}

Secondary and tertiary (graft) tumors

- Secondary tumors (\textit{Helianthus})
- Graft tumors (\textit{Helianthus})
- Graft tumors (\textit{Nicotiana})

(\textit{BACTERIA FREE})

Tissue cultures

- \textit{Helianthus}
- \textit{Nicotiana}

(\textit{BACTERIA FREE})

Fig. 2.—Summarized respiratory results for 9 types of tissues. Detailed explanation in text.
sues in which we are primarily interested, those of secondary and tertiary (metastatic) galls and tissue cultures derived therefrom, are all bacteria-free, the existence of such tumor tissues is referable in some as yet obscure fashion to the interaction of healthy host tissues with actively metabolizing colonies of Phytomonas tumefaciens. The behavior of these bacteria-free tumor tissues is therefore to be compared not only with healthy host tissues but also with the bacteria-infested tissues of primary crown-galls. A

active bacterial colonies does not appear to alter the respiratory picture qualitatively to any significant degree, so far as can be shown by the methods employed here. The only change is a quantitative one, a reduced respiratory level.

**Bacteria-free tumor tissues.**—Three types of bacteria-free tumor tissues of sunflower were studied: (a) secondary galls, (b) tissue cultures, and (c) graft tumors (tertiary, metastatic). Results with these and with comparable tissues from hereditary tumors of

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<th>Percentage</th>
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<td>Intermediate</td>
<td>8.1</td>
<td>6.0 4.9 4.8</td>
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<tr>
<td>Feb. 5, 1943</td>
<td>Secondary</td>
<td>10.5 (5.6-12.9)</td>
<td>4.0 3.5 3.2</td>
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<tr>
<td>Sept. 30, 1943</td>
<td>Tertiary</td>
<td>12.0 (6.5-15.0)</td>
<td>6.0 5.6 5.2</td>
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<td>Oct. 6, 1943</td>
<td>Primary</td>
<td>14.0 (12.5-15.0)</td>
<td>4.0 3.5 3.2</td>
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<tr>
<td>Nov. 18, 1943</td>
<td>Graft tumors</td>
<td>15.0 (13.5-16.0)</td>
<td>6.0 5.6 5.2</td>
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<tr>
<td>Averages</td>
<td></td>
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<td>10.0 4.8 4.8</td>
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* See Footnote 2.

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<td>15.0 (10-15)</td>
<td>4.0 3.5 3.2</td>
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* Nicotiana are presented in Fig. 2, third and fourth rows, and Table III.

Here the respiratory level was again in all cases low, the value of 3.6 for Nicotiana graft tumors being the highest. The respiratory quotients were normal, with the exception of that for graft tumors of sunflower (1.3), which may be due to an excess of necrotic tissue. Anaerobic respiration was fairly low except in the March 5 and 11 runs on sunflower graft tumors, and even here it did not equal the values for healthy growing tissues of sunflower. Nowhere does the picture appear to be clearly out of the ordinary except, again, as regards level of activity.
DISCUSSION

The results of all these tests are summarized in Table IV. The respiratory level was high in healthy meristems (growing regions); intermediate in tissues containing bacteria and in the hereditary tumors of Nicotiana, both in vitro and in vivo; and low in bacteria-free crown-gall tissues in whatever form. If a result, however, of the excess of woody and necrotic tissues in these tumors. Anaerobic respiration was high in healthy meristems, but not decidedly so in any type of diseased tissue. This characteristic appears to be bound up not with the tumorous state, but with actively growing meristem. Absorption of nitrogen is an important factor requiring correction in healthy

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<td>2.6</td>
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<td>Dec. 2, 1943</td>
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<td>Tissue cultures, Nicotiana</td>
<td>6.1</td>
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Table IV: Summary of Respiratory Behavior of Tissues of Tumor-Bearing Plants of Helianthus and Nicotiana

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Q_{\text{air}}^{O_2}</th>
<th>Q_{\text{air}}^{\text{CO}_2}</th>
<th>Q_{N_2}^{\text{CO}_2}</th>
<th>Q_{N_2}^{O_2}</th>
<th>R.Q.</th>
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<tr>
<td>Healthy internodal tissue</td>
<td>3.7</td>
<td>3.9</td>
<td>1.3</td>
<td>0.0</td>
<td>1.1</td>
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<tr>
<td>&quot;    stem-tips</td>
<td>4.5</td>
<td>3.8</td>
<td>3.2</td>
<td>1.0</td>
<td>0.9</td>
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<tr>
<td>&quot;    inflorescence tissue</td>
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<td>5.3</td>
<td>3.7</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Primary (bacterial) gall</td>
<td>3.7</td>
<td>3.4</td>
<td>2.8</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Secondary (bacteria-free) gall</td>
<td>1.8</td>
<td>1.9</td>
<td>1.7</td>
<td>0.4</td>
<td>1.0</td>
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<tr>
<td>Tertiary (graft, bacteria-free) gall, sunflower</td>
<td>1.7</td>
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<tr>
<td>&quot;    &quot; Nicotiana 3.6</td>
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<td>3.9</td>
<td>*</td>
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</tr>
<tr>
<td>Tissue culture (bacteria-free), sunflower</td>
<td>3.0</td>
<td>3.2</td>
<td>1.9</td>
<td>0.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Not determined.

this low respiratory level is real and not referable to the amount of woody or necrotic (non-respiring) material present in the samples—and tests based on protein nitrogen instead of dry weight determinations should answer this question, then it is important, and agrees with the Burk-Warburg idea that respiratory derangement or deficiency is characteristic of tumors. The respiratory quotients were normal (0.9 to 1.1) in all tissues with the exception of the graft tumors of sunflower, where the R.Q. was 1.3. This also may be ininflorescence tissue and in primary crown-galls (both rapidly growing), but not in other tissues. Although such qualitative deviations from normal as do occur may be indicative of real and significant abnormalities in respiratory behavior, it seems more probable that they are to be attributed to differences in density of tissue, rate of gas diffusion, and similar factors, and to the inaccuracies inherent in the experimental method when applied to materials having a low respiratory level, rather than to any qualities of tumor...
tissues as opposed to healthy tissues. The tumefacient change that these tissues have undergone does not express itself in any qualitative change in the respiratory picture that is clearly distinguishable by this method of study. Other methods may of course reveal such changes but, so far as one can distinguish by this particular use of the Barcroft-Warburg method, the respiratory process has not been altered in kind. This conclusion agrees with that arrived at by Burk and his associates (3) in their studies of chicken tumors.

The work presented here is admittedly incomplete, since the writer has had no previous experience or training in the investigation of problems of this nature. Nevertheless, it is hoped that it may serve to stimulate others of greater competence to see what more can be extracted from the study of respiratory processes in plant tumors.

**SUMMARY**

Studies by the Barcroft-Warburg method on the respiration of healthy vegetative growing points, inflorescences, and internodes of *Helianthus annuus*; of pathological tissues of the same plant represented by crown-gall tumors containing active colonies of *Phytophthora tumefaciens*, secondary tumors free of bacteria, tertiary (metastatic, graft) tumors arising as a result of implantation of bacteria-free tumor tissue cultures under the bark of healthy plants, and tissue cultures derived from bacteria-free secondary tumors; of genetically tumefacient tissues of the hybrid, *Nicotiana langsdorffii* × *N. glauca*, tissue cultures derived from the meristem of this hybrid, and tumors arising as a result of implantation of such tissue cultures under the bark of *Nicotiana glauca* have led to the conclusion that these various pathological states do not result in any apparent significant qualitative change in the respiratory picture, but do result in a definite lowering of the respiratory level. If this lowering is real and not merely an artefact due to the greater amount of nonliving tissue present in pathological growths, it may be considered similar in kind to long recognized characteristics of animal neoplasia.

**REFERENCES**

Respiratory Behavior of Bacteria-Free Crown-Gall Tissues

Philip R. White

Cancer Res 1945;5:302-311.