1956 multiple authors

On Respiratory Impairment in Cancer Cells

Some years ago Otto Warburg (*1*) enunciated a theory of cancer which, briefly summarized, proposed that cancer originates when a nonneoplastic cell adopts an anaerobic metabolism as a means of survival after injury to its respiratory system. According to Warburg, the tumor is initiated by a damaged respiration, which persists as a characteristic feature of the neoplastic condition. In a recent paper, entitled "On the origin of cancer cells," originally published in German (*2*) and then translated into English (*3*), Warburg reiterates this hypothesis and claims further support for it on the basis of experiments with ascites tumor cells. I recognize the great debt that biochemists owe this illustrious investigator and regret the necessity of taking issue with his basic biochemical premise, namely, that cancer cells have an impaired respiration.

In a comprehensive review of this subject in 1939, Burk (*4*) first pointed out the essentially fallacious reasoning behind this hypothesis. More recently, Schmidt (*5*) and I (*6*) reviewed this topic independently in the light of modern findings and concluded similarly that there is no sound experimental basis for the belief that oxidative metabolism in tumors is impaired. It is recognized by all, including Warburg, that despite their high glycolysis, oxygen consumption is not quantitatively diminished; by and large, a representative group of tumors absorb oxygen about as rapidly as a comparable group of nonneoplastic tissues (see, for example, Burk's extensive tables, *4*). An early statement by Warburg, illustrative of his views concerning the relationship between the high aerobic and anaerobic glycolysis of tumor cells and their oxygen consumption, is the following (*1*, pp. 139–141).

"We determined the Meyerhof quotient for carcinoma tissue, lactic acid bacteria, embryonic tissue and a number of other glycolyzing tissues, and as a rule obtained the same mean values as Meyerhof. As a rule 1 mol. of breathed oxygen, just as in muscle, causes the disappearance of 1–2 mol. lactic acid. This result . . . proves that the influence of the respiration on the cleavage metabolism in the carcinoma-cell is normal. . . . Although in the tumor every oxygen molecule breathed is just as effective as in muscle—the Meyerhof quotient is equal in the two cases—yet the respiration does not cause the glycolysis to disappear. The respiration of the carcinoma tissue is too small in comparison with its glycolytic power."

Thus, according to Warburg, the Meyerhof quotient (a quantitative expression of the Pasteur effect) is normal in carcinoma, and oxygen consumption is also not quantitatively diminished; but respiration is disturbed, because glycolysis persists in oxygen. As I pointed out earlier (*6*, p. 276), I believe it would be more accurate to state that anaerobic glycolysis is so high in tumors that a normal respiration and a normal Pasteur effect are incapable of eliminating it.

Although Warburg still states categori-

cally that ". . . the respiration of all cancer cells is damaged. . . ." (*3*, p. 309), he has given no clearer justification now for this view than he did 26 years ago. In Table 1 (*3*), he shows that whereas liver, kidney, and embryo have Q_{O_2}'s of -15, the ascites tumor has a Q_{O_2} of -7. On these grounds he concludes that the tumor cannot utilize sufficient oxygen for its needs and thus requires fermentative energy. On the assumption that each mole of lactic acid formed from glucose yields 1 mole of ATP, and each mole of oxygen consumed gives rise to 7 moles of ATP, he calculates that the tumor obtains more than half of its potential phosphate bond energy by glycolysis, whereas the three noncancer tissues obtain theirs mainly by respiration. Accepting these results at their face value, it is still necessary to ask why some normal tissues manage to survive without glycolysis with Q_{O_2}'s of -3 to -6; also, why some tumors glycolyze highly with Q_{O_2}'s as high as -10 to -20 (*4*, pp. 438–441)? It is also pertinent to ask why certain nonneoplastic tissues, with moderate to high oxygen uptakes—for example, brain, retina, kidney medulla, and intestinal mucosa—glycolyze as highly as many tumors (*7*)? It is evident that all tumors produce large amounts of lactic acid, but so do many noncancer tissues; and just as noncancer tissues display a wide diversity in oxygen uptake, so do tumors.

Although it is unintentional, I am sure, Table 1 (*3*) gives misleading impressions that the respiratory and glycolytic activities are constant and characteristic for a single tissue, and that all tissues produce essentially the same amounts of phosphate bond energy. Actually, tissue even from a single organ will vary considerably in Q_{O_2} from one animal to another. In our experience, rat liver slices display Q_{O_2}'s ranging from -6 to -12 (usually about -7), and rat kidney cortex from -15 to -25. Biochemists are confronted with a wide variety of tissues of the most diverse respiratory behavior. Our present state of knowledge does not allow any categorical statements about what represents a proper respiratory activity for maintenance of either a normal or a cancer cell; nor can we state what is an optimal enzyme activity for a particular cell function.

Perhaps the most damaging evidence against the Warburg hypothesis has been obtained in isotope tracer studies (*6*, pp. 303 ff). The results of such studies leave no doubt of the ability of miscellaneous tumors to convert glucose (and fatty acids) to carbon dioxide at rates similar in magnitude to that of nonneoplastic tissues (*5*, *6*). It is difficult to imagine a type of respiratory disturbance not involving either a diminution in oxygen consumption or a loss in the ability to convert glucose and fatty acids to CO_2.

Warburg suggests in the present paper that perhaps the respiratory impairment may involve an inability to couple oxidation with phosphorylation. Here again, the available evidence does not support such a concept. Effects of inhibitors such as fluoride ion or dinitrophenol are similar in neoplastic and nonneoplastic tissues (*8*) and data of other investigators (*6*, p. 321) have given no indication that oxidative phosphorylation occurs in tumor mitochondria in a manner different from that in their noncancerous counterparts.

Another pertinent illustraton of the inadequacy of the Warburg concept is that it fails to consider that a large part of the respiration of cancer cells may be due to fatty acid oxidation. Although it seems fairly certain now that animal tissues generally, including the neoplastic, use fatty acids as a metabolic fuel, nowhere in Warburg's writings is there any consideration of the possible role played by fatty acids in the respiration of cells.

Another weakness of the Warburg hypothesis is that it does not fit in with what we have learned in recent years of chemical mechanisms of glycolysis and respiration. Again, Warburg states, "We need to know no more of respiration and fermentation here than that they are energy-producing reactions. . . ." (*3*, p. 309). This attitude may have been justified 25 years ago when little was known of their chemical nature. At present we recognize that they are not "independent metabolic processes" but are intimately related. To assume that respiration and glycolysis are separately activated, alternate means of cellular energy production, it is indeed necessary to ignore all that has been learned of their chemical mechanisms.

According to our present conceptions, the major pathway of oxidation of glucose to carbon dioxide in most animal cells, whether normal or neoplastic, involves its conversion to pyruvic acid by way of the Embden-Meyerhof process, oxidative decarboxylation of pyruvic acid to acetyl coenzyme A, and condensation of the latter with oxaloacetic acid to enter the citric acid cycle. Down to the pyruvic acid stage, respiration and fermentation follow a common pathway. The extent to which pyruvic acid, a common intermediary in both respiration and glycolysis, competes for electrons held by the pyridine nucleotides with those factors that transport electrons to oxygen—namely, the flavoproteins and cytochromes—should be a crucial factor in determining the degree of aerobic glycolysis.

If there is a disturbance in respiration that leads to an accumulation of lactic acid, it can occur only at or beyond the pyruvic acid stage and must be due either to some aberration in carbon transport through the citric acid cycle or to some "bottleneck" in electron transport. Many enzymatic and isotope tracer studies have fully established that the citric acid cycle operates in tumors (*6*, pp. 311 ff). Although cytochromes are reportedly low in tumors (*9*, pp. 404 ff), as are also some of the B vitamins involved in electron transport (*9*, p. 408), the generally unimpaired oxygen consumption already referred to clearly indicates that electrons reach oxygen about as readily in tumors as in other tissues. Thus, the available evidence indicates to me that high glycolysis occurs, *despite* quantitatively and qualitatively normal occurrence of carbon and electron transport. This can mean only that glucose catabolism is so rapid in tumors that the normal channels for disposal of pyruvic acid are overloaded. Many possibilities exist for explaining this high glucose catabolism, which do not involve disturbances in respiration. My colleagues and I are now attempting to unravel the multiplicity of factors concerned with lactic acid production in the intact cell.

I do not wish to minimize the significance of the high aerobic and anaerobic glycolysis of tumor tissue. It is conceivable that glycolytic activity, though not resulting from a faulty respiration, may play a special role in the neoplastic process. Data are available from the field of lipid metabolism, for example, which suggest that some phase of glucose catabolism in liver is coupled with fatty acid synthesis (*10*). The close association of lactic acid production with the neoplastic process, and with growth in general, makes this phenomenon a worthy subject of study.

Warburg states (*3*, p. 309), "We now understand the chemical mechanism of respiration and fermentation almost completely. . . ." We have, indeed, learned a great deal of what can happen in cells, and much of this can be credited to Warburg, who has played a large part in expanding our biochemical frontiers. The fact that progress has been so great in the past must make us aware, however, that future progress will also be great, and that our present knowledge is still primitive. Certainly we have much to learn before we can feel we understand the mechanisms that underlie the utilization of metabolic fuels for functional activities of cells.

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In 1950 George Klein of the Karolinska Institute, Stockholm, was kind enough to send to Dahlem a strain of mouse ascites cancer that consists of nearly 100 percent cancer cells, that can be transplanted with 100 percent takes, and that is resistant to the shaking necessary in manometry.

Only since that time have we been able to determine quantitatively the metabolism of pure cancer cells. All our previous experiments (*1*) had been carried out with solid tumors—that is, with various mixtures of cancer cells and normal cells. The more cancer cells a tumor contained, the higher was the fermentation and the lower was the respiration. But no absolute values could be obtained.

Thus, the year 1950 brought about the transition from the study of the metabolism of the mixed cells to the study of the metabolism of pure cancer cells, a very great progress in cancer research (*2*). Weinhouse (*3*) is not appreciative of this progress, or of many important discoveries made since 1925—for example, the nonfermentation of the rapidly regenerating liver; the structural difference between energy production by respiration and by fermentation; the genetic autonomy of the respiring grana and the role of grana in the pathology of neoplasms; and carcinogenesis by respiratory poisons. Most of the comment by Weinhouse might have been written before 1925.

As expected, the fermentation of the pure cancer cells was found to be much higher than any previously observed fermentation of cancer cell mixtures. Indeed, mouse ascites cancer cells produced anaerobically per hour nearly 30 percent of their dry weight of lactic acid. In comparison with this enormous fermentation, respiration of the pure cancer cells was, as expected, very low.

Even more important were the results obtained in 1956 with Earle's *in vitro* cultures of pure mouse cancer cells. Such cells were available as two strains of high and low "malignancy," both derived from one and the same single cell. When, in the laboratory of Dean Burk, the respiration and the fermentation of these two strains of different malignancy but the same genetic origin were measured, the result was as follows: the higher the malignancy, the greater the fermentation and the smaller the respiration (*2*, pp. 313–314). The absolute fermentation values for the high-malignancy cells were as high as the values for the mouse ascites cancer cells.

These experiments with pure cancer cells—the ascites cells and Earle's cells of different malignancy—were decisive and conclusive. They correspond, in the problem of relativity, to the observed red displacement in the gravitational field.

Among normal cells, the nearest equivalent to pure cancer cells is the chorion in the first days of embryonic development. The chorion grows rapidly. It is histologically almost pure. It is so thin that it is not necessary to slice it for measurements of metabolism. It is so hardy that it can be shaken many hours in manometric vessels without disintegration. Anaerobically it produces 15 percent of its dry weight of lactic acid per hour, but aerobically it produces no lactic acid at all. Its respiration, in contrast to that of cancer cells, is high—indeed, nearly three times higher than the respiration of highly malignant, pure cancer cells.

Unlike the chorion, the whole embryo itself is unsuitable for such *in vitro* experiments, because it is disintegrated by the motion of the vessels if it is immersed in salt solutions or even in homologous serum. Yet the important question of lactic acid production by the living embryo can easily be decided by lactic acid determinations in the affluent and effluent blood vessels of an embryo *in situ* in a pregnant animal. If an embryo produced as much lactic acid as cancer cells, a very great increase in the effluent vessels would be found. But no increase has been found.

By these experiments—with chorion *in vitro*, and with the total embryo *in vivo* —it has been shown that intact embryonic cells, in contrast to cancer cells, produce no lactic acid aerobically.

Table 1. Average metabolic values obtained in serum with pure cancer cells of mice and with pure embryonic cells of mice. Q_{O_2} means cubic millimeters of oxygen consumed, and $Q_M^{O_2}$ and $Q_M^{N_2}$ mean cubic millimeters of lactic acid produced aerobically and anaerobically, respectively, per milligram (dry weight), per hour.

Cells	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$
Ascites cancer cells	−7	30	70
Earle's cancer cells (high malignancy)	−7	30	70
Earle's cancer cells (low malignancy)	−13	10	25
Chorion of young embryos	−17	0	35

Table 1 summarizes the average metabolic values obtained in serum with pure cancer cells of mice and with pure embryonic cells of mice. They constitute the main basic facts. $Q_M^{N_2} = 70$ means that the high-malignancy cancer cells produce anaerobically per hour an amount of lactic acid equal to 29 percent of their dry weight. The respiration, if normal, should be $Q_{O_2} = -35$, whereas $Q_{O_2} = -7$ was found. This means that the respiration of the high-malignancy cancer cells is only one-fifth of that of normal growing cells with $Q_M^{N_2} = 70$, and only two-fifths of that of growing chorion of young embryo with a $Q_M^{N_2} = 35$.

Obviously, nothing could be less enlightened than the opinion of Weinhouse that the respiration of cancer cells is as high, or even higher, than the respiration of normal growing cells. "High" and "low" respiration have greatest significance, of course, when compared with fermentation, as has been defined and emphasized many times since 1923, not only by the early use of such specific quotients as $Q_M^{O_2}/Q_{O_2}$, and $Q_M^{N_2}/Q_{O_2}$, but in my words of 1924 cited by Weinhouse, "The respiration of the carcinoma tissue is too small in comparison with its glycolytic power." Without such specification, "high" and "low" can become meaningless; thus, the absolute value of respiration of normal connective tissue is very low, and yet it is no cancer, because the fermentation too is very low.

Many years before the respiratory enzymes and the fermentation enzymes of oxidation-reduction were discovered at Dahlem, we reached the conclusion that the biochemical mechanism of fermentation and respiration in cancer cells is qualitatively the same as in normal cells, the differences being only quantitative, the one process being increased and the other decreased. Although later investigators have confirmed this general conclusion, Weinhouse takes exception to the phraseology "damaged respiration" of cancer cells. But because the facts are so clear and well defined, our abridged expression should not be objectionable. We have here a perfect example of a dispute about words.

It is something deeper when Weinhouse dislikes the statement that the shifting of the energy production from the aerobic to the anaerobic state is the cause of cancer. He feels that this is far too simple: How can cancer, as mysterious as life itself, be explained by such a simple physicochemical principle?

Yet this feeling is not justified. The problem of cancer is not to explain life, but to discover the differences between cancer cells and normal growing cells. Fortunately this can be done without

knowing what life really is. Imagine two engines, the one being driven by complete and the other by incomplete combustion of coal. A man who knows nothing at all about engines, their structure, and their purpose, may discover the difference. He may, for example, smell it.

OTTO WARBURG

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A mass of experimental data published during the past 33 years has established the phenomenon of a metabolism characteristic of living cancer cells, including high anaerobic and aerobic glycolysis (formation of lactic acid from glucose) and an impaired respiration (*1, 2*). The impaired respiration may involve any combination—usually at least three—of the following experimental quantities readily measurable under appropriate conditions: (i) a high ratio of glycolysis to respiration; (ii) a low absolute value for oxygen consumption (Q_{O_2}); (iii) an inefficient or uncoupled respiration; or (iv) a low paraphenylenediamine (succinate) oxidative response (*3*). This list could be expanded.

Respiratory impairment in living cancer cells, first discovered by Otto Warburg in 1923, is an experimental fact, and not, as described by Weinhouse (*4, 5*) a hypothesis based on "essentially fallacious reasoning." Statements of Weinhouse indicating that the author of "Burk's extensive tables" has at any time ever denied the factual existence of an impaired respiration in cancer cells are categorically untrue.

The overwhelming evidence for the occurrence of various forms of respiratory impairment in neoplastic cells, available in some 1000 experimental papers, but denied by Weinhouse, obviously cannot be recapitulated in this note. It is, however, possible to outline here decisive errors in the most tangible support offered by Weinhouse for his central, underlying view that "by and large, a representative group of tumors absorb oxygen about as rapidly as a comparable group of nonneoplastic tissues . . . . oxygen consumption is not quantitatively diminished" (*6*). Curiously, this long-discarded but now exhumed view, which specifically denies a low Q_{O_2} in most cancer cells (impairment ii), is alleged to derive its main support from "Burk's extensive tables" (*7*). Weinhouse (*4*, p. 271) has selectively condensed parts of these extensive tables, on some incompletely defined basis, and arrived at the following average tissue slice Q_{O_2} values for three groups of tissues, each of which has a wide spread of individual values: 15 types of malignant tumors, −11.8; seven growing tissues (three types), −9.7; 14 nongrowing tissue types, −9.3. In short, by these statistics, the tumors would *appear* to have an average ("by and large") respiration that is not lower, but even higher, than that of either the growing or nongrowing tissues selected as "comparable."

But such average Q_{O_2} values are meaningless as they stand, both statistically and otherwise. They have not been adjusted for important species differences among the three groups, for medium effects, for cellular impurity in the tumors (normal tissues, ordinarily being 100 percent nonneoplastic, need no such adjustment), for differences of technique among the different investigators cited, and so forth. A remarkable error is Weinhouse's inclusion, in the already small group of growing tissues, of certain tissues that had first been severely predamaged by treatment with cyanide and anaerobiosis (!), thus lowering notably the calculated average Q_{O_2} for this group.

Weinhouse's statistical approach to the problem of possible Q_{O_2} impairment in cancer cells requires the use of the foregoing adjustments just as surely as cancer mortality and morbidity statistics require adjustment, in the absence of complete and perfect data. The types of adjustments listed all tend, without exception, to raise the Q_{O_2} values of normal tissues relative to tumor tissues, with the end result that, as one approaches truly physiological comparisons, one obtains average Q_{O_2} values for normal growing and nongrowing tissues that are "by and large" two to three times higher than those for tumors, with reduction in spread of individual values.

The first two types of adjustment listed are probably the most important quantitatively and will be briefly discussed by way of illustration. It is well established from the data extensively summarized and formalized, for example, by Krebs (*8*) and Adolph (*9*), that tissue respiratory rate, basal heat production, ventilation rate, and a host of other vital properties are marked, linear log-log functions of species body weight. The group of normal tissues selected by Weinhouse were from the rat, rabbit, dog, and man, but none from mice; the tumor tissue types selected were preponderantly from mice, the normal tissues of which have average rates of respiration and basal heat production far above those of the larger species, the basal heat productions being of the rough relative order 160, 100, 60, 35, and 25 in mice, rat, rabbit, dog, and man, respectively. Any reasonable (but imperative!) adjustment of average respiratory rate for species difference alone would place the tumor group well below that of the normal group, with an adjusted average Q_{O_2} value of −6 to −7 (instead of −11.8, (*10*). This value agrees with the grand average value that one obtains directly from all 30 widely varying types of malignant, nonmouse tumors (rat, chicken, man) listed in "Burk's extensive tables" VII and VIII (*7*), many of which were excluded from, or improperly weighted in, the selected and condensed summary prepared by Weinhouse without due regard to the effect of species. This single error in Weinhouse's statistics, by itself, reverses the order of average Q_{O_2} values among his three groups, so that nongrowing > growing > tumor.

The second factor, of equal quantitative importance in tending to widen further the relative difference between normal and tumor tissues, is that in virtually all of the experiments selected by Weinhouse from Burk's tables, simple saline media were employed, instead of sera or similar body fluids that are much more physiological and therefore much more pertinent for the solution of the problem at hand. Extensive modern studies show, even better than earlier studies, that the use of sera instead of saline media increases the average Q_{O_2} values of normal tissues much more than that of tumors—by a relative factor up to 2 and more. This differential response is due partly, though not wholly, to the greater response of normal tissue to respiratory substrates occurring naturally in sera but not added to the early saline media employed in the experiments selected by Weinhouse. This differential response finds equivalent and additional expression in the relatively small succinate oxidative response shown by tumor tissues [impairment type iv; compare Kidd *et al.* (*3*) and Weinhouse (*4*, p. 302)].

It is obvious that in the hands of Weinhouse the statistical approach has led to gross error in final conclusion. This erroneous conclusion has been woven into his papers (*4, 5*) extensively and affects still other conclusions based on it, leading to general confusion not confined to his own papers (*11*).

The error concerning species adjustment that Weinhouse has introduced into his consideration of "Burk's extensive tables" enters with equal force into various of his conclusions concerning his own experimental data (*4, 5*) obtained with isotopically marked substrates, where again results with miscellaneous rat and mouse materials are often mixed indiscriminately; space, however, does not permit tabular detailing of his propagation of errors in this direction. Suffice it to say, his own data reported on Q_{O_2} (sketchy, but republished verbatim many times) show lower average species unadjusted Q_{O_2} values for the tumors (−5.3 for 19 determinations) than for the

normal tissues (−9.5 for 19 determinations) in saline-substrate (nonserum) media. Unfortunately, the mouse data contain only one normal tissue (liver) and several tumor types, whereas the rat data contain only one tumor type (hepatoma) and several normal tissues. If more normal mouse-tissue types and more rat-tumor types had been available for proper species adjustment, the adjusted values (for either species) would have been even wider apart than −5.3 and −9.5, since the Q_{O_2} of hepatoma is relatively high among tumors and the Q_{O_2} of normal liver tissue is relatively low among highly functional normal tissues.

When, in more extensive experimentation, C^{14}-marked substrates were tested (glucose, lactate, acetate, butyrate, octanoate, palmitate, and 2,4-acetoacetate), there was in general a relatively much greater aerobic production of $C^{14}O_2$ by the normal tissue groups than by the tumor groups employed. On an absolute basis, $Q_{C^{14}O_2}$ in the tumors rarely exceeded 2 ("O.C." = 89), and were ordinarily well below 1; but normal tissues ranged up to $Q_{C^{14}O_2} = 4$ and more, notably with lactate, an important constituent of sera. These isotope tracer studies of Weinhouse show that fatty acids and a wide variety of exogenous substrates may be oxidized to carbon dioxide by a miscellany of rat and mouse tumors, but only at quite small initial rates that were on the average lower than the average of initial rates shown by the highly functional normal rat and mouse tissues tested. Such studies largely confirm, albeit with superior quantitative finesse, widely accepted conclusions reached decades ago by investigators who had employed time-honored kinetic, substrate-addition, and respiratory quotient methods.

G. N. Lewis (*12*) has said, "It is a common fault of mankind to refuse to recognize the existence of a phenomenon unless some mechanism has been devised." Much of the general confusion (*11, 13*) evident in the discussion advanced by Weinhouse also derives from his failure to distinguish between various over-all phenomena of respiratory impairment, on the one hand, and detailed mechanisms thereof, on the other. In his own experimental work he has looked for respiratory impairment among details of biochemical respiratory mechanisms, following well-worn channels of possible aberrancy—for example, enzyme content (*14*), citric acid cycle, exogenous substrate oxidation, localized "bottlenecks" in electron transport, pyruvate shunt, hexosemonophosphate shunt, and fluoride and dinitrophenol inhibitions. The results obtained by him, even with isotopic tracers, have in no way contraindicated the over-all phenomena of respiratory impairment, and have indeed provided some valuable supporting evidence. In any event, such conventional and assuredly worthwhile studies are, regardless of outcome, no more essential to experimental recognition of the over-all phenomena of respiratory impairment in living cancer cells than is knowledge of the ultimate physical mechanism of gravitation essential to experimental recognition of the square law of gravitation. In the words of Isaac Newton (*15*), "the main Business of Natural Philosophy is to argue from Phaenomena. . . . we must learn from the Phaenomena of Nature what are the Laws and Properties of the Attraction before we enquire the Cause by which the Attraction is perform'd."

It is a tribute to the genius of Otto Warburg that he discovered the impaired Q_{O_2} of cancers more than 30 years ago with the now obsolete methods and relatively poor (mixed) cancer materials then available, and under the handicap of the statistical approach. It is a further tribute that in recent years Warburg has been the first to appreciate and capitalize on the importance of the use of pure cancer cells (for example, ascites and tissue culture cells) for final decision on the relative status of Q_{O_2} in cancer cells. It should never be forgotten, however, that in the characterization of cancer metabolism, once developed following carcinogenesis, he has always (1924–1956) regarded the invariably high ratio of fermentation to respiration (impairment type i) as of much greater significance than the usually low Q_{O_2} (impairment type ii).

On the basis of impairment type i, and as a confirmatory qualitative demonstration thereof, the following simple manometric test has been devised (*16*) that permits investigators to distinguish cancer cells from virtually all normal body cells, growing or nongrowing. The cells under test are placed in a manometric vessel of any convenient size. The vessel is filled to one-third to one-half of its volume with physiological serum or other equivalent body fluid (5 percent CO_2 in O_2 or air as appropriate, slight alkalinity, adequate glucose and bicarbonate). The volume of cells is of the order of 1/300 the volume of serum. Cancer cells cause the manometer to register a steady and notable increase in pressure with time (minutes to hours), whereas normal (uninjured) body cells cause a negative (or ~ zero) change in pressure with time. The few—if any—exceptions extant are only apparent, or are readily ruled out on other bases; thus, use of nonphysiological media (for example, unfortified saline) may vitiate the test for normal cells, and omission of the carbohydrate glucose certainly will for cancer cells.

This test can be made quantitative, but in the simple form just outlined it possesses the advantage of being qualitative. It offers a distinction between neoplastic and normal cells that is as qualitative as "plus versus minus" or "up versus down," and that is observable in terms of mere pressure change—that is, directional movement of the manometric fluid. The quantitative metabolic basis for the observed qualitative manometry is completely understood. Thus, the sign and magnitude of the pressure change can be expressed as an exact mathematical function of the ratio of the absolute magnitude of aerobic glycolysis to the absolute magnitude of respiration for any given conditions of experimental arrangement (*17*).

It is hoped that this simple test may prove useful or definitive in studies of many tissues or cells of questionable malignancy—for example, in tissue cultures of originally normal cells that are undergoing or have undergone carcinogenesis, as well as numerous *in vivo* instances. In any event, the proposed test is a *post facto* epitomization of three decades of experimental protocols in the literature that are in harmony with the concepts recapitulated in this communication on respiratory impairment in "THE metabolism of THE cancer cell."

DEAN BURK
ARTHUR L. SCHADE

References and Notes

1. O. Warburg, *Biochem. Z.* 142, 317 (1923); *Metabolism of Tumors,* translated by F. Dickens (Constable, London, 1930); *Science* 123, 309 (1956); *Science,* this issue.
2. Various words expressing "impaired" respiration have been employed by various writers, all, however, to much the same gross end result, semantics aside: damaged, destroyed, defective, diminished, disturbed, eliminated, harmed, inadequate, inhibited, injured, insufficient, limited, restrained, uncoupled, weakened, *eingeschränkt, entkoppelt, gehemmt, geschädigt, insuffizient, unzureichend, vergiftet, vermindert, zerstört, zugrunde gegangen,* and *zurückgegangen.* In any event, the irreversible respiratory limitation referred to in cancer cells is always partial, never total; otherwise, their life and continued growth would totally cease, so far as is known.
3. J. G. Kidd, R. J. Winzler, D. Burk, *Cancer Research* 4, 457 (1944).
4. S. Weinhouse, *Advances in Cancer Research* 3, 269 (1955); *Science,* this issue.
5. ———, *Antimetabolites and Cancer* (AAAS, Washington, D.C., 1955), p. 1; *Cancer Research* 11, 585, 845 (1951).
6. Weinhouse's frequently reiterated statement that this view held by him has now come to be held *also by Warburg* is categorically incorrect, as simple inspection of the two recent articles by the latter in *Science* (*1*) will suffice to show, in addition to many others that date back to 1924. Questioned directly regarding the accuracy of this footnote, Warburg has provided written, emphatic confirmation (July 1956).
7. D. Burk, *Cold Spring Harbor Symposia on Quant. Biol.* 7, 437, 441 (1939).
8. H. A. Krebs, *Metabolism and Function* (Elsevier, Amsterdam, 1950), p. 249.
9. E. F. Adolph, *Science* 109, 579 (1949).
10. It is important to note that the Q_{O_2} values for the mouse tumors of Crabtree and Murphy and Hawkins (1925–29), selected by Weinhouse from Burk's tables and averaging −13.9 for ten types, are, in fact, *exceptionally high* when compared with most values for mouse tumors obtained during the last 25 years by others using improved manometric methods. In the work of Dickens and Simer in 1930–31 (also cited in Burk's tables) the Q_{O_2} values

for the five mouse tumor types reported on averaged only −7.1! Three of these types (sarcoma 37S, tar carcinoma 2146, and spindle-cell tar tumor 173) had an average Q_{O_2} of −5.6, compared with −16.6 for the *same* tumor strains as measured by Crabtree and used by Weinhouse. Thus, even the *unadjusted* average tumor Q_{O_2} value employed by Weinhouse (−11.8) is, as the result of unwarranted bias in selection and incomplete utilization of available data, much too high.

11. Weinhouse's confusion is infectious, although mainly among investigators who do not themselves perform laboratory experiments on cancer metabolism. One may read, for example, "We hear about THE metabolism of THE cancer cell. Unfortunately, no such phenomenon has been established. . . . As Dr. Weinhouse said at the opening of the symposium, the critical difference between metabolism in malignant tissues and in normal tissues does not appear to reside in the major ways in which they handle carbohydrate metabolism." [*Antimetabolites and Cancer* (AAAS, Washington, D.C., 1955, pp. 305, 308)]. Such statements could scarcely be more incorrect or uninformed. They set the clock back and encourage the empirical approach to the problem of cancer by a sheer and vicarious denial of available fundamental information.
12. G. N. Lewis, *The Anatomy of Science* (Yale Univ. Press, New Haven, Conn., 1926), p. 171.
13. In paragraphs 4, 5, and 6 of his note in this issue of *Science*, Weinhouse asks or raises several questions that have been asked, discussed, and answered many times in the literature of cancer.
14. The mechanism of the cancer respiratory impairment may indeed often involve lowered content of a particular respiratory enzyme, but this is not a necessary general requirement, since internal cellular arrangement and chemical or structural restraint of other correlated enzymes are, as in so many living phenomena, often of more decisive importance. Thus, certain ascites cancer cells have been found [B. Chance and L. N. Castor, *Science* 116, 200 (1952)] to have unusually high contents of cytochrome *c*; but even in such ascites cells, the paraphenylenediamine and succinate oxidative responses are characteristically low or zero (*1*, p. 314); this is clearly indicative of respiratory restraint in spite of abnormally high absoute content of cytochrome *c* (compare *4*, pp. 295–6). Oxidation-reduction potential restraints may well be involved here, as well as low contents of cytochrome *b* or DPNH demonstrated.
15. I. Newton, *Opticks* (W. and J. Innys, London, ed. 2, 1718), pp. 344, 351.
16. Presented orally at the 1956 meeting of American Association for Cancer Research. [*Proc.* 2, 98 (Natl. Inst. of Health Information Release, 13 Apr.)].
17. A description of these quantitative potentialities and other qualitative aspects is in preparation.

2 July 1956

On the Biosynthesis of the Porphyrinlike Moiety of Vitamin B_{12}

The investigations performed during the past decade have elucidated many of the intimate biosynthetic steps by which the cell elaborates the porphyrin molecule. It has been found that "active" succinate (*1*) and glycine (*2*) are the sole precursors of the porphyrin compounds in all biological systems studied. The glycine and succinate condense to form α-amino-β-ketoadipic acid. This β-keto acid on decarboxylation yields δ-aminolevulinic acid (*3*). Condensation of 2 mole of the aminoketone results in the formation of the precursor monopyrrole, porphobilinogen (*4*). Four mole of this pyrrole then condenses to form a porphyrin, and modification of the side chains in the β-positions gives rise to a particular porphyrin.

The chemical work of the Merck group (*5*) and of the English workers (*6*) and the x-ray studies of Hodgkin *et al.* (*7*) have culminated recently in the proposal of a very probable structure of vitamin B_{12} which contains a porphyrinlike structure (*6, 7*). Although this latter component of the vitamin differs somewhat in structure from that of porphyrins (that is, the vitamin molecule contains one pyrrolidine and three pyrroline rings, a methyl group on two of the bridge-carbon atoms, four extra methyl groups in the β-positions of the rings, and an α-methyl group instead of a bridge-carbon atom), there are sufficient similarities to lead to the suspicion that the basic mechanism of synthesis of this part of the vitamin is similar to that known for porphyrins. It would seem possible that the porphyrinlike moiety of the vitamin is synthesized by the mechanism known for pyrrole and porphyrin synthesis and that the modified structure is subsequently methylated in the afore-mentioned positions to form the final product. This conclusion, of methylation subsequent to ring formation from δ-aminolevulinic acid (*6*), is supported by structural considerations. If a methylated derivative of δ-aminolevulinic acid were the precursor, one would expect that the extra methyl groups would be on only those β-positions that bear acetic acid side chains. However, this is the case with only rings A and B; in ring D the methyl group is attached to the carbon atom that bears the propionic acid group.

In order to check this hypothesis, we have carried out a microbiological synthesis of vitamin B_{12} in the presence of 125 mg of δ-aminolevulinic acid-1,4-C^{14} having a molar activity (*1*) of 8.3×10^5 count/min for each active carbon. The culture was agitated in a medium containing the following nutrients, in addition to the δ-aminolevulinic acid: sucrose, 8.75 g; L-glutamic acid, 2.5 g; $(NH_4)_2HPO_4$, 0.5 g; Na_2SO_4, 0.5 g; KCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.125 g; $MnSO_4 \cdot 4H_2O$, 0.05 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; $ZnSO_4 \cdot 7H_2O$, 0.005 g; and $Co(NO_3)_2 \cdot 6H_2O$, 0.01 g. Under these fermentation conditions, the culture produced 0.163 mg of vitamin B_{12}. After the addition of 10.1 mg of nonradioactive B_{12}, 6.294 mg of B_{12} was isolated. The molar activity of the undiluted B_{12} was 30×10^5 count/min. Therefore, in the unlikely possibility that endogenous synthesis of aminoketone be disregarded, at least four carbon atoms of the vitamin must have contained C^{14}.

On the reasonable assumption, based on previous studies on porphyrin formation, that 2 mole of aminoketone is utilized for each ring, one can postulate that 15 labeled carbon atoms (16 minus the carboxyl lost from ring C) of the porphyrinlike structure of the vitamin were derived from our labeled substrate. On this basis the molar activity of each of these 15 carbon atoms would be 2×10^5 count/min. This represents a mere fourfold dilution of the radioactive carbon atoms of the labeled substrate in the course of the synthesis of the vitamin. It may therefore justifiably be concluded that the porphyrinlike structure of vitamin B_{12} is synthesized from δ-aminolevulinic acid, as are the porphyrins, and that the mechanism of synthesis of the ring system in the vitamin is similar to that of the porphyrins.

We are presently engaged in degrading the labeled vitamin in order to isolate those carbon atoms which we predict should contain all the radioactivity.

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