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On the Origin of Cancer Cells

Otto Warburg

Our principal experimental object for the measurement of the metabolism of cancer cells is today no longer the tumor but the ascites cancer cells (1) living free in the abdominal cavity, which are almost pure cultures of cancer cells with which one can work quantitatively as in chemical analysis. Formerly, it could be said of tumors, with their varying cancer cell content, that they ferment more strongly the more cancer cells they contain, but today we can determine the absolute fermentation values of the cancer cells and find such high values that we come very close to the fermentation values of wildly proliferating *Torula* yeasts.

What was formerly only qualitative has now become quantitative. What was formerly only probable has now become certain. The era in which the fermentation of the cancer cells or its importance could be disputed is over, and no one today can doubt that we understand the origin of cancer cells if we know how their large fermentation originates, or, to express it more fully, if we know how the damaged respiration and the excessive fermentation of the cancer cells originate.

Energy of Respiration and Fermentation

We now understand the chemical mechanism of respiration and fermentation almost completely, but we do not need this knowledge for what follows, since *energy alone* will be the center of our considerations. We need to know no more of respiration and fermentation here than that they are energy-produc-

ing reactions and that they synthesize the energy-rich adenosine triphosphate, through which the energy of respiration and fermentation is then made available for life. Since it is known how much adenosine triphosphate can be synthesized by respiration and how much by fermentation, we can write immediately the potential, biologically utilizable energy production of any cells if we have measured their respiration and fermentation. With the ascites cancer cells of the mouse, for example, we find an average respiration of 7 cubic millimeters of oxygen consumed per milligram, per hour, and fermentation of 60 cubic millimeters of lactic acid produced per milligram, per hour. This, converted to energy equivalents, means that the cancer cells can obtain approximately the same amount of energy from fermentation as from respiration, whereas the normal body cells obtain much more energy from respiration than from fermentation. For example, the liver and kidney of an adult animal obtain about 100 times as much energy from respiration as from fermentation.

I shall not consider aerobic fermentation, which is a result of the interaction of respiration and fermentation, because aerobic fermentation is too labile and too dependent on external conditions. Of importance for the considerations that follow are only the two stable independent metabolic processes, respiration and *anaerobic* fermentation—respiration, which is measured by the oxygen consumption of cells that are saturated with oxygen, and fermentation, which is measured by the formation of lactic acid in the absence of oxygen.

Injuring of Respiration

Since the respiration of all cancer cells is damaged, our first question is, How can the respiration of body cells be injured? Of this damage to respiration, it can be said at the outset that it must be *irreversible*, since the respiration of cancer cells never returns to normal. Second, the injury to respiration must not be so great that the cells are killed, for then no cancer cells could result. If respiration is damaged when it forms too little adenosine triphosphate, it may be either that the oxygen consumption has been decreased or that, with undiminished oxygen consumption, the coupling between respiration and the formation of adenosine triphosphate has been broken, as was first pointed out by Feodor Lynen (2).

One method for the destruction of the respiration of body cells is removal of oxygen. If, for example, embryonal tissue is exposed to an oxygen deficiency for some hours and then is placed in oxygen again, 50 percent or more of the respiration is usually destroyed. The cause of this destruction of respiration is lack of energy. As a matter of fact, the cells need their respiratory energy to preserve their structure, and if respiration is inhibited, both structure and respiration disappear.

Another method for destroying respiration is to use respiratory poisons. From the standpoint of energy, this method comes to the same result as the first method. No matter whether oxygen is withdrawn from the cell or whether the oxygen is prevented from reacting by a poison, the result is the same in both cases—namely, impairment of respiration from lack of energy.

I may mention a few respiratory poisons. A strong, specific respiratory poison is arsenious acid, which, as every clinician knows, may produce cancer. Hydrogen sulfide and many of its deriv-

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atives are also strong, specific respiratory poisons. We know today that certain hydrogen sulfide derivatives, thio-urea and thioacetamide, with which citrus fruit juices have been preserved in recent times, induce cancer of the liver and gall bladder in rats.

Urethane is a nonspecific respiratory poison. It inhibits respiration as a chemically indifferent narcotic, since it displaces metabolites from cell structures. In recent years it has been recognized that subnarcotic doses of urethane cause lung cancer in mice in 100 percent of treatments. Urethane is particularly suitable as a carcinogen, because, in contrast to alcohol, it is not itself burned up on the respiring surfaces and, unlike ether or chloroform, it does not cytolize the cells. Any narcotic that has these properties may cause cancer upon chronic administration in small doses.

The first notable experimental induction of cancer by oxygen deficiency was described by Goldblatt and Cameron (3), who exposed heart fibroblasts in tissue culture to intermittent oxygen deficiency for long periods and finally obtained transplantable cancer cells, whereas in the control cultures that were maintained without oxygen deficiency, no cancer cells resulted. Clinical experiences along these lines are innumerable: the production of cancer by intermittent irritation of the outer skin and of the mucosa of internal organs, by the plugging of excretory ducts of glands, by cirrhoses of tissues, and so forth. In all these cases, the intermittent irritations lead to intermittent circulatory disturbances. Probably chronic intermittent oxygen deficiency plays a greater role in the formation of cancer in the body than does the chronic administration of respiratory poisons.

Any respiratory injury due to lack of energy, however, whether it is produced by oxygen deficiency or by respiratory poisons, must be cumulative, since it is irreversible. Frequent small doses of respiratory poisons are therefore more dangerous than a single large dose, where there is always the chance that the cells will be killed rather than that they will become carcinogenic.

Grana

If an injury of respiration is to produce cancer, this injury must, as already mentioned, be irreversible. We understand by this not only that the inhibition of respiration remains after removal of the respiratory poison but, even more, that the inhibition of respiration also continues through all the following cell divisions, for measurements of metabolism in transplanted tumors have shown that cancer cells cannot regain normal

respiration, even in the course of many decades, once they have lost it.

This originally mysterious phenomenon has been explained by a discovery that comes from the early years of cell physiology (4). When liver cells were cytolized by infusion of water and the cytolizate was centrifuged, it was found that the greater part of the respiration sank to the bottom with the cell grana. It was also shown that the respiration of the centrifuged grana was inhibited by narcotics at concentrations affecting cell structures, from which it was concluded—already in 1914—that the respiring grana are not insoluble cell particles but *autonomous organisms*, a result that has been extended in recent years by the English botanist Darlington (5) and particularly by Mark Woods and H. G. du Buy (6) of the National Cancer Institute in Bethesda, Md. Woods and du Buy have experimentally expanded our concepts concerning the self-perpetuating nature of mitochondrial elements (grana) and have demonstrated the hereditary role of extranuclear aberrant forms of these in the causation of neoplasia. The autonomy of the respiring grana, both biochemically and genetically, can hardly be doubted today.

If the principle *Omne granum e grano* is valid for the respiring grana, we understand why the respiration connected with the grana remains damaged when it has once been damaged; it is for the same reason that properties linked with genes remain damaged when the genes have been damaged.

Furthermore, the connection of respiration with the grana (7) also explains a carcinogenesis that I have not mentioned previously, the carcinogenesis by x-rays. Rajewsky and Pauly have recently shown that the respiration linked with the grana can be destroyed with strong doses of x-rays, while the small part of the respiration that takes place in the fluid protoplasm can be inhibited very little by irradiation. Carcinogenesis by x-rays is obviously nothing else than a destruction of respiration by elimination of the respiring grana.

It should also be mentioned here that grana, as Graffi has shown (8), fluoresce brightly if carcinogenic hydrocarbons are brought into their surroundings, because the grana accumulate the carcinogenic substances. Probably this accumulation is the explanation for the fact that carcinogenic hydrocarbons, although almost insoluble in water, can inhibit respiration and therefore have a carcinogenic effect.

Increase of Fermentation

When the respiration of body cells has been irreversibly damaged, cancer cells

by no means immediately result. For cancer formation there is necessary not only an irreversible damaging of the respiration but also an increase in the fermentation—indeed, such an increase of the fermentation that the failure of respiration is compensated for energetically. But how does this increase of fermentation come about?

The most important fact in this field is that there is no physical or chemical agent with which the fermentation of cells in the body can be increased directly; for increasing fermentation, a long time and many cell divisions are always necessary. The temporal course of this increase of fermentation in carcinogenesis has been measured in many interesting works, among which I should like to make special mention of those of Dean Burk (9).

Burk first cut out part of the liver of healthy rats and investigated the metabolism of the liver cells in the course of the ensuing regeneration, in which, as is well known, the liver grows more rapidly than a rapidly growing tumor. No increase of fermentation was found. Burk then fed rats for 200 days on butter yellow, whereupon liver carcinomas were produced, and he found that the fermentation slowly increased in the course of 200 days toward values characteristic of tumors.

The mysterious latency period of the production of cancer is, therefore, nothing more than the time in which the fermentation increases after a damaging of the respiration. This time differs in various animals; it is especially long in man and here often amounts to several decades, as can be determined in the cases in which the time of the respiratory damage is known—for example, in arsenic cancer and irradiation cancer.

The driving force of the increase of fermentation, however, is the energy deficiency under which the cells operate after destruction of their respiration, which forces the cells to replace the irretrievably lost respiration energy in some way. They are able to do this by a selective process that makes use of the fermentation of the normal body cells. The more weakly fermenting body cells perish, but the more strongly fermenting ones remain alive, and this selective process continues until the respiratory failure is compensated for energetically by the increase in fermentation. Only then has a cancer cell resulted from the normal body cell.

Now we understand why the increase in fermentation takes such a long time and why it is possible only with the help of many cell divisions. We also understand why the latency period is different in rats and in man. Since the average fermentation of normal rat cells is much greater than the average fermentation

of normal human cells, the selective process begins at a higher fermentation level in the rat and, hence, is completed more quickly than it is in man.

It follows from this that there would be no cancers if there were no fermentation of normal body cells, and hence we should like to know, naturally, from where the fermentation of the normal body cells stems and what its significance is in the body. Since, as Burk has shown, the fermentation remains almost zero in the regenerating liver growth, we must conclude that the fermentation of the body cells has nothing to do with normal growth. On the other hand, we have found that the fermentation of the body cells is greatest in the very earliest stages of embryonal development and that it then decreases gradually in the course of embryonal development. Under these conditions, it is obvious—since ontogeny is the repetition of phylogeny—that the fermentation of body cells is the inheritance of undifferentiated ancestors that have lived in the past at the expense of fermentation energy.

Structure and Energy

But why—and this is our last question—are the body cells dedifferentiated when their respiration energy is replaced by fermentation energy? At first, one would think that it is immaterial to the cells whether they obtain their energy from respiration or from fermentation, since the energy of both reactions is transformed into the energy of adenosine triphosphate, and yet adenosine triphosphate = adenosine triphosphate. This equation is certainly correct chemically and energetically, but it is incorrect morphologically, because, although respiration takes place for the most part in the structure of the grana, the fermentation enzymes are found for a greater part in the fluid protoplasm. The adenosine triphosphate synthesized by respiration therefore involves more structure than the adenosine triphosphate synthesized by fermentation. Thus, it is as if one reduced the same amount of silver on a photographic plate by the same amount of light, but in one case with diffused light and in the other with patterned light. In the first case, a diffuse blackening appears on the plate, but in the second case, a picture appears; however, the same thing happens chemically and energetically in both cases. Just as the one type of light energy involves more structure than the other type, the adenosine triphosphate energy involves more structure when it is formed by respiration than it does when it is formed by fermentation.

In any event, it is one of the fundamental facts of present-day biochemis-

try that adenosine triphosphate can be synthesized in homogeneous solutions with crystallized fermentation enzymes, whereas so far no one has succeeded in synthesizing adenosine triphosphate in homogeneous solutions with dissolved respiratory enzymes, and the structure always goes with oxidative phosphorylation.

Moreover, it was known for a long time before the advent of crystallized fermentation enzymes and oxidative phosphorylation that fermentation—the energy-supplying reaction of the lower organisms—is morphologically inferior to respiration. Not even yeast, which is one of the lowest forms of life, can maintain its structure permanently by fermentation alone; it degenerates to bizarre forms. However, as Pasteur showed, it is rejuvenated in a wonderful manner if it comes in contact with oxygen for a short time. "I should not be surprised," Pasteur said in 1876 (10) in the description of these experiments, "if there should arise in the mind of an attentive hearer a presentiment about the causes of those great mysteries of life which we conceal under the words youth and age of cells." Today, after 80 years, the explanation is as follows: the firmer connection of respiration with structure and the looser connection of fermentation with structure.

This, therefore, is the physicochemical explanation of the dedifferentiation of cancer cells. If the structure of yeast cannot be maintained by fermentation alone, one need not wonder that highly differentiated body cells lose their differentiation upon continuous replacement of their respiration with fermentation.

I would like at this point to draw attention to a consequence of practical importance. When one irradiates a tissue that contains cancer cells as well as normal cells, the respiration of the cancer cells, already too small, will decline further. If the respiration falls below a certain minimum that the cells need unconditionally, despite their increased fermentation, they die; whereas the normal cells, where respiration may be harmed by the same amount, will survive because, with a greater initial respiration, they will still possess a higher residual respiration after irradiation. This explains the selective killing action of x-rays on cancer cells. But still further: the descendants of the surviving normal cells may in the course of the latent period compensate the respiration decrease by fermentation increase and, thence, become cancer cells. Thus it happens that radiation which kills cancer cells can also at the same time produce cancer or that urethane, which kills cancer cells, can also at the same time produce cancer. Both events take place from harming respira-

tion: the killing, by harming an *already* harmed respiration; the carcinogenesis by the harming of a *not yet* harmed respiration.

Maintenance Energy

When dedifferentiation of the body cells has occurred and cancer cells have thereby developed, there appears a phenomenon to which our attention has been called by the special living conditions of the ascites cancer cells. In extensively progressed ascites cancer of the mouse, the abdominal cavity contains so many cancer cells that the latter cannot utilize their full capacity to respire and ferment because of the lack of oxygen and sugar. Nevertheless, the cancer cells remain alive in the abdominal cavity, as the result of transplantation proves.

Recently we have confirmed this result by direct experiments in which we placed varying amounts of energy at the disposal of the ascites outside the body, *in vitro*, and then transplanted it. This investigation showed that all cancer cells were killed when no energy at all was supplied for 24 hours at 38°C but that one-fifth of the growth energy was sufficient to preserve the transplantability of the ascites. This result can also be expressed by saying that cancer cells require much less energy to keep them alive than they do for growth. In this they resemble other lower cells, such as yeast cells, which remain alive for a long time in densely packed packets—almost without respiration and fermentation.

In any case, the ability of cancer cells to survive with little energy, if they are not growing, will be of great importance for the behavior of the cancer cells in the body.

Sleeping Cancer Cells

Since the increase in fermentation in the development of cancer cells takes place gradually, there must be a transitional phase between normal body cells and fully formed cancer cells. Thus, for example, when fermentation has become so great that dedifferentiation has commenced, but not so great that the respiratory defect has been fully compensated for energetically by fermentation, we may have cells which indeed look like cancer cells but are still energetically insufficient. Such cells, which are clinically not cancer cells, have lately been found, not only in the prostate, but also in the lungs, kidney, and stomach of elderly persons. Such cells have been referred to as "sleeping cancer cells" (11, 12).

The sleeping cancer cells will possibly play a role in chemotherapy. From energy considerations, I could think that

sleeping cancer cells could be killed more readily than growing cancer cells in the body and that the most suitable test objects for finding effective killing agents would be the sleeping cancer cells of skin—that is, precancerous skin.

Summary

Cancer cells originate from normal body cells in *two* phases. The first phase is the irreversible injuring of respiration. Just as there are many remote causes of plague—heat, insects, rats—but only one common cause, the plague bacillus, there are a great many remote causes of cancer—tar, rays, arsenic, pressure, urethane—but there is only one common cause into which all other causes of cancer merge, the irreversible injuring of respiration.

The irreversible injuring of respiration is followed, as the second phase of cancer formation, by a long struggle for existence by the injured cells to maintain their structure, in which a part of the cells perish from lack of energy, while another part succeed in replacing the irretrievably lost respiration energy by fermentation energy. Because of the morphological inferiority of fermentation energy, the highly differentiated body cells are converted by this into undifferentiated cells that grow wildly—the cancer cells.

To the thousands of quantitative experiments on which these results are based, I should like to add, as a further argument, the fact that there is no alternative today. If the explanation of a vital process is its reduction to physics and chemistry, there is today no other explanation for the origin of cancer cells, either special or general. From this point of view, *mutation* and *carcinogenic agent* are not alternatives, but empty words, unless metabolically specified. Even more harmful in the struggle against cancer can be the continual discovery of miscellaneous cancer agents and cancer viruses, which, by obscuring the underlying phenomena, may hinder necessary preventive measures and thereby become responsible for cancer cases.

Technical Considerations and Comments

Metabolism of the ascites cancer cells. The high fermentation of ascites cancer cells was discovered in Dahlem in 1951 (12) and since then has been confirmed in many works (13, 14). For best measurements, the ascites cells are not transferred to Ringer's solution but are maintained in their natural medium, ascites serum, which is adjusted physiologically at the beginning of the measurement by

addition of glucose and bicarbonate. Because of the very large fermentation, it is necessary to dilute the ascites cells that are removed from the abdominal cavity rather considerably with ascites serum; otherwise the bicarbonate would be used up within a few minutes after addition of the glucose, and hence the fermentation would be brought to a standstill.

Under physiological conditions of pH and temperature, we find the following metabolic quotients in ascites serum (15):

$$Q_{O_2} = -5 \text{ to } -10$$

$$Q_M^{O_2} = 25 \text{ to } 35$$

$$Q_M^{N_2} = 50 \text{ to } 70$$

where Q_{O_2} is the amount of oxygen in cubic millimeters that 1 milligram of tissue (dry weight) consumes per hour at 38°C with oxygen saturation, $Q_M^{O_2}$ is the amount of lactic acid in cubic millimeters that 1 milligram of tissue (dry weight) develops per hour at 38°C with oxygen saturation, and $Q_M^{N_2}$ is the amount of lactic acid in cubic millimeters that 1 milligram of tissue (dry weight) develops per hour at 38°C in the absence of oxygen.

Even higher fermentation quotients have been found in the United States with other strains of mouse ascites cancer cells (13, 14).

All calculations of the energy-production potential of cancer cells should now be based on the quotients of the ascites cancer cells, since these quotients are 2 or 3 times as large anaerobically as the values formerly found for the purest solid tumors. The quotients of the normal body cells, however, remain as they were found in Dahlem in the years from 1924 to 1929 (16–19). It is clear that the difference in metabolism between normal cells and cancer cells is much greater than it formerly appeared to be on the basis of measurements on solid tumors.

Utilizable energy of respiration and fermentation. Since the discovery of the oxidation reaction of fermentation in 1939 (20), we have known the chemical reactions by which adenosine diphosphate is phosphorylated to adenosine triphosphate in fermentation; and since then we have found that 1 mole of fermentation lactic acid produces 1 mole of adenosine triphosphate (ATP).

The chemical reactions by which ATP is synthesized in respiration are still unknown, but it can be assumed, according to the existing measurements (21), that 7 moles of ATP can be formed when 1 mole of oxygen is consumed in respiration.

ATP quotients. If we multiply Q_{O_2} by 7 and $Q_M^{N_2}$ by 1, we obtain the number of cubic millimeters of ATP that 1 milligram of tissue (dry substance) can synthesize per hour (22,400 cubic milli-

millimeters = 1 millimole of ATP). We call these quotients $Q_{ATP}^{O_2}$ and $Q_{ATP}^{N_2}$, according to whether the ATP is formed by respiration or by fermentation, respectively.

Energy production of cancer cells and of normal body cells. In Table 1, the Q values of some normal body cells are contrasted with the Q values of our ascites cancer cells.

The cancer cells have about as much energy available as the normal body cells, but the ratio of the fermentation energy to the respiration energy is much greater in the cancer cells than it is in the normal cells.

Uncoupling of respiration. If a young rat embryo is transferred from the amniotic sac to Ringer's solution, the previously transparent embryo becomes opaque and soon appears coagulated (17). At the same time, the connection between respiration and phosphorylation is broken; that is, although oxygen is still consumed and carbon dioxide is still developed, the energy of this combustion process is lost for life. If the metabolism quotients had previously been

$$Q_{O_2} = -15, Q_M^{O_2} = 0, Q_M^{N_2} = 25, \\ Q_{ATP}^{O_2} = 105, Q_{ATP}^{N_2} = 25$$

in the amniotic fluid, afterward, in Ringer's solution, they are

$$Q_{O_2} = -15, Q_M^{O_2} = 25, Q_M^{N_2} = 25, \\ Q_{ATP}^{O_2} = 0, Q_{ATP}^{N_2} = 25$$

Because of uncoupling of respiration and phosphorylation, the energy production of the embryo has fallen from $Q_{ATP}^{O_2} + Q_{ATP}^{N_2} = 130$, to 25; since the uncoupling is irreversible, the embryo dies in the Ringer's solution.

This example will show that the first phase of carcinogenesis, the irreversible damaging of respiration, need not be an actual decrease in the respiration quotient but merely an uncoupling of respiration, with undiminished over-all oxygen consumption. Ascites cancer cells, which owe their origin primarily to an uncoupling of respiration, could conceivably have the following metabolism quotients, for example:

$$Q_{O_2} = -50, Q_M^{O_2} = 100, Q_M^{N_2} = 100, \\ Q_{ATP}^{O_2} = 0, Q_{ATP}^{N_2} = 100$$

which would mean that, despite great respiration, the usable energy production would be displaced completely toward the side of fermentation. One will now have to search for such cancer cells among the ascites cancer cells. Solid tumors—and especially solid spontaneous tumors—need no longer be subjected to such examinations today, of course, since the solid tumors are usually so impure histologically.

Aerobic fermentation. Aerobic fermentation is a property of all growing cancer cells, but aerobic fermentation

without growth is a property of damaged body cells—for example, embryos that have been transferred from amniotic fluid to Ringer's solution. Since it is always easy to detect aerobic fermentation but generally difficult to detect growth, or lack thereof, of body cells, aerobic fermentation should not be used as a test for cancer cells, as I made clear in 1928 (19).

Nevertheless, misuse is still made of aerobic fermentation. Thus, O'Connor (22) recently repeated our old experiments on the aerobic fermentation of the embryo that has been transferred into Ringer's solution, but he drew the conclusion that the growth of normal body cells is completed at the expense of the aerobic fermentation, even though it has long been established that the embryo does not ferment aerobically when it grows in the amniotic fluid.

Respiratory poisons. The specific respiration-inhibiting effect of arsenious acid and the irreversibility of its inhibitions were discovered in the first quantitative works on cell respiration (23, 24). There is abundant literature on the carcinogenesis by arsenic, particularly on arsenic cancer after treatment of psoriasis and on the cancer of grape owners who spray their vineyards with arsenic. The specific respiration-inhibiting effect of hydrogen sulfide has likewise been described by Negelein (25), and carcinogenesis by derivatives of hydrogen sulfide has been recently described by D. N. Gupta (26).

The irreversible inhibition of cell respiration by urethane was discovered early (27) as well as the fact that the urethane inhibition is more irreversible the higher the temperature. In sea urchin eggs, the effect of urethane was investigated, not only on the metabolism, but also on cell division in studies (28) from which the later urethane treatment of leukemia was developed. The physicochemical mechanism by which urethane and other indifferent narcotics inhibit cell respiration was cleared up in 1921 (29). Only much later did the carcinogenic effect of urethane become known. Actually, multiple lung adenomas can often be produced in 100 percent of the mice treated with small doses of urethane (30).

Oxygen deficiency. Short-period oxygen deficiency irreversibly destroys the respiration of embryos (16) without thereby inhibiting the anaerobic fermenta-

tion of the embryo. If such embryos are transplanted, teratomas are formed (31). It has recently been reported that, in the development of the Alpine salamander, malformations occurred when the respiration was inhibited by hydrocyanic acid in the early stages of embryonal development (32).

Goldblatt and Cameron (3) reported that, in the *in vitro* culturing of fibroblasts, tumor cells appeared when the cultures were exposed to intermittent oxygen deficiency for long periods, whereas, in the control cultures, no tumor cells appeared. In the discussion at the Stuttgart convention, Letré cited against Goldblatt and Cameron the fact that another American tissue culturist, Earle, has occasionally obtained tumor cells from fibroblasts for reasons unknown to him and in an unproduceable manner, but this objection does not seem weighty, and the latter part is untrue (33). In any event, here is an area in which the methods of tissue culture could prove useful for cancer research. But warnings must be given against metabolism measurements in tissue cultures, if and when the tissue cultures are mixtures of growing and dying cells, especially under conditions of malnutrition. An example of the latter type of confusion is involved in the discussion by Albert Fischer (34), especially in the chapter "Energy exchange of tissue cells cultivated *in vitro*."

Rous agent. If the Rous agent is inoculated into the chorion of chick embryos, tumors originate in the course of a few days—as rapidly as in the transplantation of cancer cells. The tumors formed are not chorion tumors but Rous sarcomas. The Rous agent, to which a particle weight of 150 million is ascribed at present, is therefore capable of transmitting the morphological properties of the Rous sarcoma; and whatever we call the Rous agent—"hereditary unit," cell fragment, microcell, or spore—the transmission of the Rous sarcoma by the Rous agent is, in any case, nothing more than a *transplantation* and is to be differentiated strictly from the production of a chicken sarcoma by methylcholanthrene, which is a *neoformation* of a tumor from normal body cells and as such takes a long time.

The metabolism of the chicken sarcomas, whether produced by the Rous agent or by methylcholanthrene, is the

same and does not differ in any way from the metabolism of the tumors of other animals (35). In the first case, however, the fermentation potential has been transplanted with the Rous agent, whereas in the second case the fermentation has been intensified by selection from normal body cells under the action of the methylcholanthrene.

Addendum: *in vitro* Carcinogenesis and Metabolism

Since this paper was prepared, striking confirmation and extension of its main conclusions have been obtained from correlated metabolic and growth studies of two lines of tissue culture cancer cells of widely differing malignancy that were both derived from *one and the same* normal, tissue-culture cell (36). The single cell was isolated some 5 years ago from a 97-day old parent culture of normal subcutaneous adipose tissue of a strain C3H/He mouse by Sanford, Likely, and Earle (33) of the National Cancer Institute. Up to the time that the single-cell isolation was made, no tumors developed when cells of the parent culture were injected into strain C3H/He mice. Injections of *in vitro* cells of the lines 1742 and 2049 (formerly labeled substrains VII and III, respectively) first produced tumors in normal C3H/He mice after the 12th and 19th *in vitro* transplant generations, respectively; after 1½ years, the percentage production of sarcomas was 63 and 0 percent, respectively; and after 3 years, it was 97 and 1 percent, respectively, with correspondingly marked differences in length of induction period. Despite such gross differences in "malignancy" *in vivo*, the rates of growth of the two lines of cells maintained continuously *in vitro* have remained nearly identical and relatively rapid. Nevertheless, the metabolism of the two lines of cancer cells, whose malignancy was developed *in vitro*, has been found by Woods, Hunter, Hobby, and Burk to parallel strikingly the differences in malignancy observed *in vivo*, in a manner in harmony with the predications and predictions of this article.

The metabolic values were measured following direct transfer of the liquid cultures from the growth flasks into manometric vessels, without notable alteration of environmental temperature, pH, or medium composition (horse serum, chick embryo extract, glucose, bicarbonate, balanced saline). The values obtained thus accurately represent the metabolism of growing, adequately nourished, pure lines of healthy cancer cells free of admixture with any other tissue cell type. The anaerobic glycolysis of the high-malignancy line 1742 was $Q_{M}^{N_2} = 60$ to 80, which is virtually max-

Table 1. Contrast of the Q values of some normal body cells with the Q values of ascites cancer cells.

Cells	Q_{O_2}	$Q_{M}^{N_2}$	$Q_{ATP}^{O_2}$	$Q_{ATP}^{N_2}$	$Q_{ATP}^{O_2} + Q_{ATP}^{N_2}$
Liver	-15	1	105	1	106
Kidney	-15	1	105	1	106
Embryo (very young)	-15	25	105	25	130
Cancer	-7	60	49	60	109

imum for any and all cancer cells previously reported, including ascites cells (12-14). The anaerobic glycolysis of the low-malignancy line was, however, only one-third as great, $Q_M^{N_2} = 20$ to 30. The average aerobic glycolysis values for the two lines were in the same order, $Q_M^{O_2} = 30$ and 10, respectively, but of lower magnitude because of the usual, pronounced Pasteur effect, greater in line 1742 than in line 2049 ($Q_M^{N_2} - Q_M^{O_2} =$ about 40 and 15). On the other hand, the rates of oxygen consumption were in the converse order, being smaller in line 1742 ($Q_{O_2} = 5$ to 10) than in line 2049 ($Q_{O_2} = 10$ to 15), corresponding to a greater degree of respiratory defect in line 1742. The respiratory defect in both lines was further delineated by the finding of little or no increase in respiration after the addition of succinate to either line of cells, in contrast to the considerable increases obtained with virtually all normal tissues (9); and the respiratory increase with paraphenylenediamine was likewise relatively low, compared with normal tissue responses.

A further notable difference between the two cell lines was the very much lower inhibition of glycolysis by podophyllin materials (anti-insulin potentiators) observed with line 1742 compared with line 2049 (for example, 10 and 70 percent, respectively, at a suitably low concentration). This result would be expected on the basis of the much greater loss of anti-insulin hormonal restraint of glucose metabolism, at the hexokinase phosphorylating level, as the degree of malignancy is increased, just as was reported for a spectrum of solid tumors (14).

Finally, the high-malignancy line 1742 cells have been found by A. L. Schade to contain 3 times as much aldolase as the low-malignancy line 2049 cells (11, 300 versus 3700 Warburg activity units per milliliter of packed cells extracted), and about 2 times as much α -glycerophosphate dehydrogenase [2600 versus

1400 Schade activity units (13) per milliliter of packed cells extracted]. The potential significance of these indicated enzymic differences in relation to the parallel glycolytic differences, measured with aliquots of the same cell cultures, is evident, and may well be connected with the corresponding hexokinase system differences.

The new metabolic data on the two remarkably contrasting lines of cancer cells, which originated from a single, individual cell and have been maintained exclusively *in vitro* over a period of years, epitomize and prove finally the main conclusions of this article, which are based on decades of research. Such metabolic analyses provide promise of a powerful tool for diagnosis of malignancy in the ever-increasing variety of tissue-culture lines now becoming available in this rapidly expanding biological and medical field, where characterization of malignancy by conventional methods (animal inoculation or otherwise) may be difficult or impracticable. This metabolic tool should be especially important in connection with the use of tissue cultures for the evaluation of chemotherapeutic agents or other control procedures.

References and Notes

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36. This summary of studies of various collaborating groups of investigators was prepared by Dean Burk at Professor Warburg's request.

A university has its responsibility to the student as well as an obligation to add to knowledge. Let us not put our students in the position of observing or being a party to practices contrary to the ideal for a university and for his future career. If the student sees his institution engaged in fundamental research, freely supported and freely conducted, he will learn that independence in investigation is man's right and a university's responsibility to exemplify.—N. PAUL HUDSON, *Ohio State University Graduate School Record*, December 1951.