A DISCUSSION OF RECENT STUDIES ON THE METABOLISM OF NORMAL AND MALIGNANT CELLS.

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The importance of lactic acid as a product in the carbohydrate metabolism of living tissue is well recognized. Salkowski (1) in 1890 stated that muscle produces lactic acid not because it is dying but because it is living and produces it only during life. Fletcher and Hopkins (2) have shown that under anaerobic conditions lactic acid is spontaneously developed in freshly excised resting muscle, but only in very small quantities in the air and not at all in the presence of pure oxygen. Hill and Meyerhof (3) consider that glycolysis is the chemical process involving the whole (or nearly the whole) of the energy liberated in the initial phases of contraction of muscle. Levene and Meyer (4) found that the leucocytes of rats are able to diminish markedly the reducing power of a glucose solution and that kidney tissue of rabbits also has this property but to a lesser extent. They were able to isolate paralactic acid as the zinc salt from the solution, and thus definitely established for the first time the fact that lactic acid is an intermediate product of glycolysis in living tissues.

Warburg (5) and his associates in a series of studies have reported on the metabolism of malignant tissues as compared to that of normal tissues. They found that the respiration of the Flexner-Jobling carcinoma was considerably less than that of normal tissue. Further investigation showed that even this small amount of respiration in tumor tissue suspended in Ringer's solution was inhibited by the addition of glucose. The lactic acid produced by glycolysis was sufficient to stop respiration. The respiration of normal tissue was not influenced by the addition of glucose and only minute amounts of lactic acid were formed.

Warburg found that malignant tumor cells produce three to four times as much lactic acid per molecule of oxygen consumed as do
benign tumors, and that a 3 to 5 day old chick embryo in the absence of oxygen produces lactic acid at almost the same rate as malignant or benign tumor tissues but that in the presence of oxygen normal respiration takes place with the formation of very little lactic acid.

Negelein (6) recently has shown that rat embryos, suspended in inactivated horse serum, produce large amounts of lactic acid in the absence of oxygen and in the earlier stages of development produce some lactic acid in the presence of oxygen. The amnion and chorion of these embryos also produce large amounts of lactic acid in the absence of oxygen. He found that the glycolytic activity of these tissues was at a maximum in the earlier stages of development of the embryo when the growth of the embryo is most rapid.

From these results, Warburg divides tissues into four types on the basis of metabolism: normal resting tissue with a high respiratory rate and slight anaerobic glycolysis; embryonic tissue with a high respiratory rate and high anaerobic but low aerobic glycolysis; malignant tumor tissue with a low respiration and high anaerobic and aerobic glycolysis; benign tumor tissue with the same type of metabolism as malignant tissue but with a lower aerobic glycolysis. He classifies tissues on the basis of their aerobic metabolism, as malignant if the glycolysis-respiration ratio is 3, as benign tissue if the ratio is 1, and as normal growing tissues if the respiration suffices to bring about the disappearance of glycolysis products.

Using Warburg's technique and working with the same types of tissue as used by him we obtained results essentially the same as his and the differences between the groups were clear-cut (7). However, when other tissues were investigated the groups became less definite. For example, rat spleen, embryonic skin, and the wall of a pregnant uterus had the typical embryonic type of metabolism. Rat placenta grouped according to this scheme fell within the malignant group. The transplantable Chicken Tumor 1 could be grouped with the transplantable rat carcinoma, but Chicken Tumor 9 showed considerable variation. The latter tumor, grouped on the aerobic glycolysis respiration ratio, in the majority of instances had about the same range as Chicken Tumor 1; but a rapidly retrogressing tumor composed almost entirely of reactive tissue frequently gave as high a ratio as the progressive tumors made up entirely of intact tumor cells.
The results with the spontaneous tumors of mice were even more varied. Grouped on the basis of their types of metabolism the majority behaved as embryonic tissue, some fell in the group of benign tumors, and a very few could be classed as malignant. Histologically, all of these tumors are typically malignant. Classified on the basis of

![Graph](image-url)

**Fig. 1.** The oxygen consumption in c.mm. per mg. of dried tissue per hour is plotted as ordinates and the aerobic glycolysis in c.mm. per mg. of dried tissue per hour as abscissae. Each point (●) represents an individual observation on normal tissues, each symbol (*) on placenta. Each of the other symbols represents an individual observation on malignant tissues: × mouse tumors, ■ chicken tumors, ◊ Flexner-Jobling tumors. The areas normal, benign, and malignant indicate the areas bounded by the aerobic glycolysis-respiration ratios 0 and 1, 1 and 2, and 2 and over respectively.

For their biological behavior they are in all essentials similar to malignant tumors as they occur in man. The results are shown graphically in Fig. 1.

The aerobic glycolytic activity seems to bear no relationship to
growth rate. A very slowly growing or retrogressing chicken tumor
currently gave as high an aerobic glycolysis-respiration ratio as a
rapidly growing tumor. The rapidly progressing spontaneous mouse
tumors often gave a low ratio while the slower growing ones in some
instances gave a relatively high ratio.

If we examine these results on another basis, i.e. that of energy
production, we find that the total aerobic energy per mg. of dried tissue
falls within the same range for all the tissues.

The amount of energy obtained per mg. of tissue may be calculated
from the following data by Slater (8):

| Heat of combustion of 1 gm. of glycogen monohydrate in dilute solution | 3836 cals. |
| Heat of combustion of 1 gm. of lactic acid in dilute solution | 3601 “ |
| Heat available due to the formation of 1 gm. of lactic acid from 1 gm. of glycogen | 235 “ |
| Heat of neutralization of 1 gm. of lactic acid by alkali protein as determined by Meyerhof | 138 “ |
| Total heat liberated per gm. of lactic acid formed | 373 “ |

Therefore every c.mm. of CO₂ formed by the combustion of glycogen
represents $5.09 \times 10^{-3}$ cals., and every c.mm. of CO₂ obtained by the
splitting of glycogen to lactic acid represents $1.50 \times 10^{-3}$ cals. Consequently the total aerobic energy per mg. of dried tissue per hour is
equal to the c.mm. of CO₂ obtained by respiration multiplied by $5.09 \times 10^{-3}$ cals. plus the c.mm. of CO₂ obtained by glycolysis multiplied
by $1.50 \times 10^{-3}$ cals.

From the results shown in Fig. 2 it is apparent that the energy per
mg. of dried tissue is approximately the same whether this energy is
obtained entirely by the combustion of glucose or by the combustion of glucose and the splitting of glucose to lactic acid. So tissue with a
low respiratory rate and high glycolytic activity obtains practically
the same energy as tissue with high respiration and no glycolysis. Fig. 3 shows the results obtained by using the published data of
Warburg, Posener, and Negelein (9) to calculate the energy per mg. of
dried tissue per hour. There is a close agreement between his results and ours.

That the results obtained per mg. of dried tissue for normal and for
malignant tissues are comparable would seem to be borne out by the
Fig. 2. Each point in the upper chart represents an individual determination of the total aerobic carbon dioxide in c.c.mm. per mg. of dried tissue per hour produced by the various tissues.

Each point in the lower chart represents an individual determination of the total aerobic energy in cals. \( \times 10^{-3} \) per mg. of dried tissue per hour produced by the various tissues.
**Fig. 3.** For explanation see Fig. 2. These determinations were taken from the published data of Warburg.
FIG. 4. Each point represents an individual determination of the anaerobic glycolysis expressed in c.mm. of carbon dioxide per mg. of dried tissue per hour. The crosses represent the average for each class of tissue. The heavy vertical line is drawn to divide normal and malignant groups.

The determinations in the lower chart were taken from the published data of Warburg.
findings of Lewis and Lewis (10) that there is a striking similarity between the growth of normal tissues of embryo chicks in salt solutions, nutrient agar, and plasma, and that pictured by Lambert and Hanes for the growth of rat and mouse sarcoma and carcinoma in plasma.

If we turn now to the anaerobic conditions where glycolysis is favored, we find that all tissues have some glycolytic activity. If the tissues are arranged in groups we find that each group has a different amount of glycolysis as expressed in c.mm. of CO₂ per mg. of dried tissue per hour. Normal tissues produce very little lactic acid, embryonic tissues and placenta slightly more, and malignant tumor tissue still more. If the maximum amount of CO₂ produced by placenta is taken as a limit for normal tissue it is found that malignant tissues as a rule group themselves above this point.

In Fig. 4 are shown the c.mm. of CO₂ produced by various tissues by anaerobic glycolysis. The normal tissues liver, spleen, embryonic rat skin, and placentas, all produce less than 19 c.mm. of CO₂ per mg. of dried tissue per hour. In the Chicken Tumor 9 group, five tumors produce less than 19 c.mm. of CO₂ but four of these were tissues taken from retrogressing tumors, showing practically no intact tumor cells, the mass being composed mostly of reactive tissue. Eight of the spontaneous mouse tumors produced less than 19 c.mm. of CO₂ and seven of these were tumors on which accurate measurements for 4 weeks showed very little growth.

In the lower half of the chart are given the published data of Warburg. The results are not very clear-cut, as five tissues which he classified as benign tumors are above the line while five tumors classified as malignant are below; but, if we used his corrected results, four of these five would be above the line. The 3 to 5 day old chick embryos are on the border line or above, but the growth rate of these could be compared favorably with that of malignant tissue.

DISCUSSION.

It would seem from these results and from the findings of Negelein on the glycolytic activity of young rat embryos and chorion, that glycolytic activity of a tissue is a function of its growth rate. On this basis the malignant tissues in most instances having a more rapid growth rate than normal tissues fall in a group by themselves, and are only approached by the young embryonic tissues.
We have extended the findings of Warburg and differ from him in the interpretation of the results. We believe that the anaerobic glycolytic activity of tissues is a function of their growth rate, and that from this activity a classification of tissues may be made corresponding to their biological groupings much more closely than the classification of tissues from the aerobic glycolysis-respiration ratio as used by Warburg.

BIBLIOGRAPHY.