Some Biochemical Essentials of Malignancy*

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SUMMARY

Biologically speaking, one of the simplest and most comprehensive definitions of a neoplasm is that it is a relatively autonomous growth of tissue. Such a definition has certain implications, which are discussed here. When one attempts to define the biochemical characteristics of all neoplasms, exceptions arise. However, if one specific neoplastic cell type is carefully studied—e.g., the hepatocellular carcinoma-liver system—certain biochemical characteristics unique to the tumor become apparent. Notable among these is the defective control of enzyme synthesis which is manifest in all systems studied to date. The correlation of the biologic essentials with the apparent biochemical essentials of the malignant hepatic cell is presented as a model system with which to study other neoplastic vs. normal cell types.

The biologic aspects of malignant disease have been as thoroughly described as any in pathology. Volumes have been written on the subject of its etiology, natural history, and therapy. In this century, with the advances in our knowledge of the chemistry of living cells, man has attempted to extend his knowledge of the biologic essentials of neoplasia to the chemical processes responsible for the described phenomena. Unfortunately, to date these attempts have not met with a great deal of success. The reasons for this failure are not altogether clear, but they are probably not primarily because of an insufficiency of facts as evidenced by the world literature. Rather, it would seem that a lack of correlation and comprehension of the facts at hand is a major obstacle to progress. Perhaps one glaring cause for our continued ignorance in the face of millions of hours of work, millions of words in print, and millions of dollars in expenditures is the relative lack of correlation between the descriptive biology and the descriptive and deductive biochemistry of neoplasia. The late Dr. Greenstein recognized the need for such correlations when he wrote “...The chemical description of a tumor has little meaning unless it is read against the background of pathology and is rigidly controlled by microscopic criteria” (11).

It behooves us thus to look closely at the critical biologic characteristics of the malignant process and to interpret the biochemical results of our experiments in light of the pathologic process under investigation. Biologically speaking in the simplest of terms, a neoplasm is a relatively autonomous growth of tissue (8). Such a definition implies several things. First the term relative autonomy requires that neoplasms do not exhibit all the biologic controls which regulate the metabolism and growth of normal tissue but does not state the degree of loss of biologic control. Secondly, growth is a general term stating nothing about rate or degree or whether the growth signifies hypertrophy or hyperplasia. Today we recognize the fact that neoplasms may exist for a lifetime in the host without ever undergoing demonstrable cell division (9), a sobering fact when one considers that anyone may now possess the neoplastic state within his or her body. Third, the word tissue requires that the neoplastic state is only defined at our present state of knowledge in a multicellular organism. Bacteria, amebae, and other unicellular organisms, by definition as well as by their nature, are free of this curse of evolution known to us as cancer. Thus, whereas life is essential to all malignancy, malignancy is not characteristic of all life, suggesting that knowledge of the basic processes of life itself is not necessarily the key to an understanding of malignancy.

It is the hope of us all that the biologic definition of neoplasia requires a biochemical one, which deviates enough from that of a normal cell as to make the selective toxicity of neoplasia an abso-
lute reality. The elucidation of these biochemical essentials of malignancy is the difficult problem that confronts us.

For the biologist the multicellular organism is a gold mine of descriptive facts and interpretations, for the biochemist it is a nightmare of complexity. Each cell type in the organism has its own characteristic morphology as viewed under the microscope. Responsible for this specific morphology are equally unique, fantastically complex arrays of lipides, proteins, and other macro and micro molecules of known and unknown structure, not the least of which are the numerous enzyme proteins whose catalytic activity and spatial arrangement result in the cell's function. Thus, the human organism possesses more than 70 morphologically and, in most cases studied, biochemically distinct cell types. The detailed biochemistry of these cell types is far from understood, but the fact remains that unique morphology denotes unique biochemistry in the majority of cases. The pathologist utilizes the morphologists' histologic classification in diagnosing and typing each malignancy. As a result, we know of more than 70 morphologically distinct neoplasms, implying that each one has its own unique enzyme pattern. Apparently, the nightmare has multiplied 70-fold. Is neoplasia, then, 70 different diseases in the human? It is possible, but the biologic manifestations of malignancy are ubiquitous in the disease, no matter what its classification. The unifying concepts of biochemistry which relate in chemical terms seemingly unrelated biological facts would also speak for certain common chemical characteristics of all malignant cells. But what are these?

THE WARBURG THEORY

Perhaps the best known attempt at a biochemical definition of malignancy was that of Warburg and his associates (36) who, after surveying the glycolytic and respiratory capacity of hundreds of normal and neoplastic tissues, proposed the hypothesis that the cancer cell results from some damage to the respiratory mechanism of the normal cell. Table 1 shows some of the older data compiled from this work. It is evident that there were overlapping values among the three classes of cells. In fact, some embryonic and even adult (the renal medulla) tissues exhibit as high or higher rates of glycolysis than most neoplasms. Thus, as might be expected from the wide variation in the glycolysis of normal cells, a wide variation in the fermentation of neoplastic cells is evident. However, until recently, despite the several weaknesses of this generalization as cited by Weinhouse (38) and others, no definitive exception to the theory was found. As long as no exception was available, the theory certainly could be cited as a biochemical essential of malignancy. But events would not have it so.

| TABLE 1 |
| RESPIRATION AND GLYCOLYSIS OF NORMAL AND MALIGNANT TISSUES |
| Condensed from Burk (5) |

<table>
<thead>
<tr>
<th>Process</th>
<th>Normal non-growing</th>
<th>Malignant</th>
<th>Normal growing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(14)*</td>
<td>(15)*</td>
<td>(7)*</td>
</tr>
<tr>
<td>Respiration</td>
<td>9.3 (8—11)</td>
<td>11.8 (5.3—19.8)</td>
<td>9.7 (1—14)</td>
</tr>
<tr>
<td>Aerobic glycolysis</td>
<td>2.1 (0—10)</td>
<td>14.0 (4.7—24.6)</td>
<td>7.0 (0—15)</td>
</tr>
<tr>
<td>Anaerobic glycolysis</td>
<td>7.2 (2—10)</td>
<td>25.6 (14.0—34.8)</td>
<td>20 (13—28)</td>
</tr>
</tbody>
</table>

* Numbers of tissues used to obtain the averages recorded.

The Greenstein Hypothesis

During and for some time after the elucidation of Warburg's work, the late Dr. Greenstein undertook to survey other aspects of the enzymology of normal and neoplastic cells in general. This work, culminating in the now famous text, "The Biochemistry of Cancer" (11), has been hailed as monumental in its field. From the results obtained, a small portion of which are presented in Chart 1, it was, in general, evident that the neoplasms studied had a strikingly uniform enzymatic pattern as compared with normal tissues each of which, as we have iterated, possesses an enzymology which is as unique and characteristic of each tissue as is its morphology. Thus it would appear, in line with Warburg's theory, that not only are neoplasms characterized by an impaired respiration, but also their general enzymatic patterns are in many cases very similar and actually appear to be "converging" on some general metabolic pattern, characteristic of many neoplasms.

Dr. Greenstein himself (12), however, was quick to point to exceptions of this rule, unlike Warburg's concept, which actually had no exception at that time. A few exceptions can be seen on
the chart—e.g., osteogenic sarcoma and phosphatases; mammary carcinoma and arginase. In fact, an interesting degree of divergence is seen (Table 2) when a single mouse tissue is compared with transplanted neoplasms derived from it. Thus, again it might appear that we are approaching a range of values in neoplasms just as we see them in normal cells.

**THE DELETION HYPOTHESIS**

Unlike the Warburg and Greenstein hypotheses which were based on results obtained with a host of tissues, normal and neoplastic, the deletion hypothesis was advanced by the Millers in 1947 (18) to explain azo dye carcinogenesis in one tissue, liver. These authors found that livers of rats fed aminoazo dyes produced a covalent bonding of the carcinogenic dye to the proteins of the liver but that neoplasms produced by dye feeding bound no dye to their proteins. In addition, the soluble proteins binding the major part of the dye were almost absent from the tumors. This work was extended to include skin carcinogenesis by polycyclic hydrocarbons (13, 17), and more recently other hepatic carcinogens have been found bound to protein (39) as well as to nucleic acid (16).

The approach here was unique in that a hypothesis based on a single set of experiments in a single tissue, liver, was proposed and then extended to other tissues. In part as a result of this theory, as well as Greenstein's and other studies, biochemists became more aware of the 70-fold
nightmare of cell populations in the mammal and in the middle of the last decade turned their attention to delineating the biochemical essentials of a single malignancy where compared with the tissue from which it arose. Because of the extensive work on hepatocarcinogenesis making hepatic tumors readily available, as well as the relative cellular homogeneity of the organ, the liver appeared to be ideally suited to the elucidation of the biochemical essentials of the malignant liver cell.

During the 10 years after the deletion hypothesis was presented, it gained support in biochemical as well as biological terms (32). In particular, Potter and his associates proposed that the deleted proteins may be enzymes associated with catabolism, thus directing metabolites into synthetic pathways and promoting cell hypertrophy and cell division. By 1958 it had become evident, partly from Greenstein’s work (11) and more recently from the work of Weber et al. (37), Novikoff (20), Auerbach and Waisman (5), and others using the transplantable Novikoff hepatoma, that many catabolic enzymes characteristic of liver were deleted in this tumor as well as some primary hepatomas. Thus, the enzymatic concept of the deletion hypothesis appeared well substantiated in liver.

DIVERGENCE IN HEPATOMAS

As Potter pointed out (32), the results with the Novikoff hepatoma were meaningful if this tumor were hepatocellular—i.e., derived from a liver cell. Also if a hepatic tumor were found that possessed a different enzymatic profile from the Novikoff, then the results to date had to be re-evaluated. Actually Greenstein’s work foretold that the latter must occur (Table 2). Work in 1959 (24) with another tumor, the Dunning hepatoma, confirmed this (Table 3). This tumor possessed several enzymes absent from the Novikoff hepatoma and in turn, did not possess deoxycytidylate (dCMP) deaminase, an enzyme present at high levels in the Novikoff hepatoma but virtually absent from normal liver (29). In view of these findings it became of extreme importance to study a number of hepatomas, much as Greenstein did, to see whether in fact a spectrum of enzyme content would be found and, most important, what enzyme deletions were really necessary for carcinogenesis.

Through the cooperation of Drs. Van R. Potter, Tetsuo Ono, Harold Morris, and many others, a survey of ten primary and transplanted rat hepatomas was made. Chart 2 portrays the results obtained by assaying dCMP deaminase in these tumors, liver, and thymus. With the exception of the Dunning and the 5123 hepatomas, readily measurable amounts of this enzyme were found in all these tumors. The two exceptions, like liver, had virtually no deaminase activity. When another enzyme, glucose-6-phosphatase, was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3). When the degradation of thymine to CO₂ was measured in the same tissues, only liver, the 5123, and the ethionine tumors had measurable levels (Chart 3).
biochemically separable hepatomas was found. As might be expected, certain morphologic characteristics were associated with those tumors having qualitatively unique patterns as based on the results of these three enzymes. The morphologic and biochemical characteristics are correlated in Table 4. The Novikoff and Morris tumors are at the opposite ends of the spectrum, and the Dunning is in between, failing to possess any measurable activity of these three enzymes. The ethionine tumor appears to be a hybrid both morphologically and biochemically, since it has characteristics of all the other neoplasms in the table. Continued transplantation of this tumor led to a Novikoff type both biologically and biochemically. In addition, explanation to tissue culture of the ethionine tumor gave at least three different cell types resulting in morphologically, and in some respects biochemically, different neoplasms when reinoculated into suitable hosts. The other tumors breed relatively true in tissue culture giving the original tumor upon reinoculation into the host.

Such studies emphasize another biological pitfall when dealing with neoplasms, that of cellular heterogeneity of an apparently homogeneous neoplasm. The stability of the tumor to transplantation, as well as its homogeneity, are prerequisite to satisfactory biochemical study.

When the enzyme studies mentioned above were extended (Table 3) it was found that the 5123 hepatoma resembled liver in almost every respect enzymatically. Thus the enzyme deletions found previously with other hepatomas were apparently not essential for biologic malignancy. In addition, other characteristics of the Morris 5123 soon became apparent. It possessed essentially no aerobic glycolysis (1) and did have the dye-binding proteins and could bind hepatic carcinogens to soluble proteins. Its ultrastructure and morphology were similar to those of liver (21), and after 25 transplant generations it still possessed the rat diploid chromosome complement (26). Thus, in the case of one neoplasm, the Morris 5123 hepatoma, many of the previously held biochemical essentials of malignancy were not applicable, and yet this was a biologically malignant neoplasm.

1 P. A. Morse, H. E. Swin, and H. C. Pitot, unpublished observations.
SOME BIOCHEMICAL ESSENTIALS OF MALIGNANCY IN HEPATOCELLULAR CARCINOMA

Taken literally, these findings were somewhat discouraging, but actually they were to be expected if a correlation of biology and biochemistry exists. Furthermore, these facts were remarkably encouraging, since now in at least one case the biochemist could logically hope to define the minimum essential biochemical changes required for the conversion of a normal cell type to its malignant counterpart without being led astray by factors which were probably secondary to the neoplastic transformation itself, such as aerobic glycolysis, rapid growth, aneuploidy, etc. The biological-biochemical correlation established also suggested the avenue of future research, since the one unique characteristic of neoplasia at the biochemical level involves derangement of control mechanisms. To loosely paraphrase a great statesman, never before had such an approach been suggested by so many (6, 32) and yet utilized by so few. Recently, as Dr. Pardee will point out, our knowledge of intracellular control mechanisms, especially those associated with the regulation of enzyme synthesis and activity, has expanded to the degree that a systematic understanding of some of the mechanisms is possible (7). It is these mechanisms in the tumor, as compared with its cell of origin, that are of interest now.

Regulation of enzyme activity by products or distal products of the reaction has been termed end-product or feedback inhibition. In three instances where this type of regulatory mechanism has been investigated in the 5123 or similar types of hepatoma—i.e., aspartic transcarbamylase inhibition by cytidine nucleotides (4), thymidine kinase by thymidine triphosphate (14), and pyrroline carboxylate reductase by proline2—no differences in inhibitory properties of the tumor and liver enzymes were noted. However, when the regulation of the synthesis of specific enzymes was investigated, marked discrepancies in these tumors as compared with liver were noted (Table 5). In fact, with no rat hepatoma studied thus far, including transplanted and primary, grade I to grade IV, and fast-growing or slow-growing, has the specific induction of tryptophan pyrrolase by its substrate, tryptophan, been within normal limits for adult rat liver. Two hepatomas studied, the Reuber H-35 and the Morris 7793, did show significant enzyme induction by tryptophan in tumors growing in intact hosts. However, when the hosts were adrenalectomized, no induction occurred in the tumors growing in these operated hosts, whereas enzyme induction in host liver still took place (27). Such an intricate host-tumor relation was shown to an even greater degree (Table 5) by tyrosine transaminase in a number of highly differentiated hepatocellular carcinomas (27, 28).

In these neoplasms the level of the enzyme was very high—actually at the level to which cortisone induced the enzyme in liver. Thus, cortisone administration to the intact tumor-bearing host had little effect on the tumor enzyme but caused a large increase in the host-liver enzyme. Adrenalectomy of the tumor-bearing host equalized the tyrosine transaminase activity of liver and tumor. Further examples of the breakdown in hepatomas of normal, controlled enzyme synthesis is seen when dietary induction of enzymes is studied. It is possible, by suitable dietary manipulations, to alter many enzymes in liver (15). Notable among these are glucose-6-phosphate dehydrogenase and threonine dehydrase (34). In recent investigations in this laboratory (22) force-feeding of

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TABLE 4

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>MORPHOLOGY</th>
<th>BIOCHEMIST*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell type</td>
<td>Growth pattern</td>
</tr>
<tr>
<td>Novikoff</td>
<td>Small</td>
<td>Undifferentiated sheets</td>
</tr>
<tr>
<td>Ethionine</td>
<td>Small+large</td>
<td>Glands and sheets</td>
</tr>
<tr>
<td>Dunning</td>
<td>Large</td>
<td>Plates</td>
</tr>
<tr>
<td>Morris 5123</td>
<td>Large</td>
<td>Plates</td>
</tr>
</tbody>
</table>

* See legend, Table 5.

dCMPase = Deoxyribonucleoside diphosphatase.
G6Pase = Glucose-6-phosphatase.
T → CO₂ = Thymine reductase.

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1 C. Persino and H. C. Pitot, unpublished observations.
casein hydrolysate has resulted in marked short-term (24-hr.) induction (100-fold) of threonine dehydrase. This induction is completely inhibited by the administration of puromycin. Administration of actinomycin D at the initiation of enzyme induction completely prevents the increase. However, when actinomycin is given 12 hours after the feeding is begun no inhibition results, suggesting

that, once an RNA template is formed, it is stable for some time. Potter and Ono (34) have also shown that puromycin or x-radiation markedly inhibits the increase in glucose-6-phosphate de-

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inducer</th>
<th>Associated process</th>
<th>Host liver</th>
<th>Hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan pyrrolase</td>
<td>None</td>
<td>Protein synthesis (10)</td>
<td>2-4</td>
<td>0.1-0.4</td>
</tr>
<tr>
<td></td>
<td>Tryptophan</td>
<td>Protein and RNA (10) synthesis</td>
<td>10-30</td>
<td>0.5-0.6</td>
</tr>
<tr>
<td></td>
<td>Cortisone</td>
<td></td>
<td>4-12</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td>Tyrosine α-ketoglutarate</td>
<td>None</td>
<td>Protein and RNA (10) synthesis</td>
<td>30-90</td>
<td>150-450</td>
</tr>
<tr>
<td>transaminase</td>
<td>Cortisone</td>
<td></td>
<td>150-850</td>
<td>500-250</td>
</tr>
<tr>
<td></td>
<td>Adrenalectomy</td>
<td></td>
<td>20-70</td>
<td>80-180</td>
</tr>
</tbody>
</table>

* Units are in μmoles product/gm tissue/hr. The values given for tryptophan pyrrolase are a composite of about 80 different animals bearing primary and transplanted hepatomas (23-25, 28) with the modifications noted in the text. The values for tyrosine transaminase are taken from two references (27, 28), with the modifications noted in the text.
hydrogenase resulting from refeeding after a 3-day fast. Thus, dietary-induced changes of these enzymes appears to be a reflection of net enzyme synthesis which, in some cases, may require a prior DNA-dependent synthesis of RNA (see Table 5).

The recent work of Bottomley et al. (3) has shown that changes in the protein and carbohydrate content of the diet cause reciprocal changes in the levels of these two enzymes so that, if the activities of the two under each condition are plotted against each other, a set of points along the ordinate and abscissa is obtained as seen in Chart 5. Thus, each hepatic enzyme may vary 100-fold in either direction, but only reciprocally in relation to the other in this tissue. However, when the same enzymes are studied in a variety of hepatomas (Chart 6) under both static and changing dietary conditions, a similar plot results, but only when all the tumors are included—i.e., any single tumor, with few exceptions, cannot alter either enzyme in response to dietary stimuli. Taken together, the spectrum of values for all hepatomas studied falls roughly within the range for liver. It is as though each malignant liver cell were equivalent to a normal hepatocyte whose control mechanisms for these enzymes were frozen in an instant of time.

Thus, in essentially every instance investigated thus far of a specific mechanism controlling the synthesis of a specific enzyme in liver, the same system in the hepatocellular carcinoma shows definite abnormalities by comparison. These abnormalities vary quantitatively in each tumor as to the degree of the effect; however, qualitatively, the derangements are ubiquitous in all hepatic neoplasms studied. Such an extensive defect apparently involving most if not all of the mechanisms regulating protein synthesis has not yet been found in the wide spectrum of microbial mutations and thus may be a change unique in multicellular organisms. Such a hypothesis is compatible with the biological essentials of malignancy. In fact, are derangements in regulatory systems some of the biochemical essentials in determining the critical difference between a normal and neoplastic liver cell? We feel they are and that the problem now facing us is the definition of these defects at the molecular level in order that the biochemical essentials of malignancy may lead to a molecular definition of malignancy.

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