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A BIOCHEMICAL BASIS FOR CARCINOGENESIS[1]

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Abstract

Recent results on enzyme induction in rat liver and kidney tumors make it apparent that former hypotheses on carcinogenesis are inadequate. Instead, a new hypothesis is proposed. It takes note of certain remarkable similarities in enzyme regulation between the perinatal and the malignant states. It considers oncogeny as resulting from reactivation or derepression of certain specific genes normally suppressed in cells beyond the perinatal state. Hence, the reversion hypothesis. Results from independent immunologic studies also support this concept. Although the detailed mechanism awaits further elaboration, this hypothesis does predict the direction in which changes in gene regulation will take place during carcinogenesis.

During oncogenesis, definitive alterations in the regulation of certain genes, and hence enzymes, must have taken place. Hence, the biochemical investigation of carcinogenesis has a two-fold purpose: to discover the biochemical changes essential for the induction and to elucidate the mechanism by which these changes take place. Although the logical sequence would appear first to identify the essential changes and then to probe the underlying mechanism, our experience suggests otherwise. To date no specific biochemical alteration has been recognized as a universal property of cancer. Moreover, the question whether there is a common biochemical denominator in all forms of cancer remains unanswerable. Then what justification do we have in proposing to study the mechanism of carcinogenesis when the very nature of the biochemical changes obligatory to it has not been defined?

Our experience with Morris hepatomas has shown that no two lines of these tumors are alike biochemically (Wu, 1967). We infer from this diversity

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that during carcinogenesis many biochemical changes irrelevant to malignancy occur at random along with those essential to cancer induction. We assume that some of these irrelevant changes take place by the same mechanism that brings about the obligatory changes. Therefore, by understanding how the concomitant non-essential changes take place, we hope we will have understood how the essential changes are brought about.

From experiments on enzyme induction and immunological studies in tumors, certain implications in the mechanism of oncogenesis have emerged. In this paper, I shall present a hypothesis describing such a mechanism. Before doing this, however, I should like to review the earlier hypotheses in this area and to show how each of them has proved inadequate in the light of subsequent findings.

A Brief Historical Survey(2)

The Warburg hypothesis of respiratory defect

The successful use of the tissue slice technique made it possible for Warburg to examine numerous cancer tissues with respect to their glycolytic capacity under aerobic and anaerobic conditions. Out of this study came the bold generalization by Warburg (1930) that all cancer tissues had high glycolysis when compared with normal tissues. Warburg's original observations were confirmed in many laboratories (see Burk, 1939). Table 1 shows some representative results.

Table 1. *Tumor Glycolysis Data of Warburg and Others*[1]

Glycolysis	Normal	Malignant
Aerobic	2.1 (0-10)	14.0 (4.7-24.6)
Anaerobic	7.2 (2-19)	25.6 (14.0-34.8)

[1] Data condensed from Burk (1939) and given as microliters of gas corresponding to lactic acid produced per mg dry weight of tissue per hour.

These results convinced Warburg that high glycolysis was a universal characteristic of cancer. On the basis of these findings, Warburg (1930) proposed that there was a respiratory defect in cancer, and because of this defect cancer cells must look for another way to provide energy for metabolism and growth. Hence, the observed high glycolysis in these tissues. For more

(2) In discussing the hypotheses of carcinogenesis, some authors include the Greenstein concept of convergence (Greenstein, 1956). This concept is primarily descriptive of the enzyme activity in cancer and proposes no mechanism for carcinogenesis. Hence, this concept is deleted from the present discussion

than two decades, the Warburg hypothesis occupied the center stage and formed the main guiding principle in cancer research.

We should remember that Warburg and his contemporaries carried out their experiments at a time when undifferentiated, advanced cancer tissues were apparently the only specimens available for investigative work. Warburg's interpretation of high glycolysis in cancer as resulting from respiratory defect was subsequently challenged by Weinhouse (1955). However, the frontal attack on the Warburg hypothesis came in 1961 when Aisenberg and Morris (1961, 1963) showed that well differentiated hepatomas in their early stages following induction did not exhibit high glycolysis. Table 2 shows that the values for glycolysis and respiration in the hepatomas are within the range of normal liver. These findings clearly repudiate the generalization that high glycolysis is a universal alteration in cancer, and the rejection of the Warburg hypothesis as a *sine qua non* to explain carcinogenesis becomes inevitable.

Table 2. *Glycolysis and Respiration of Slices of Normal Liver and Hepatomas*[1]

Tissue	Q^{N2}_{Lactic}	Q^{O2}_{Lactic}	Q_{O_2}
Normal liver	0.7–1.0	0.6	4.9–5.2
Hepatoma 5123	1.0	0.5	5.0
Hepatoma 7800	1.0	1.0	4.6
Hepatoma H-35	1.0	0.9	5.0

[1] Data taken from Aisenberg and Morris (1961, 1963) and expressed as microliters of O_2 consumed or acid formed per mg dry weight of tissue per hour.

The deletion hypothesis

The deletion concept was originally put forth by Miller and Miller (1947) to explain azo-dye carcinogenesis as resulting from an "alteration or loss of proteins essential for the control of growth but not for life." The experimental basis for the interpretation lies in their observation that certain carcinogenic dye-binding proteins present in rat liver were deleted from primary hepatomas. This concept was later extended by Potter (1958) to include deletion of catabolic enzymes in cancer cells. Subsequently, the availability of hepatomas with substantial amounts of the catabolic enzymes forced Potter to abandon the catabolic deletion concept and to replace it with the feedback deletion concept (Potter, 1964). This latter concept is based on a series of experiments on enzyme induction carried out with transplantable, well differentiated rat hepatomas possessing "marker" enzymes of rat liver. In general, there was a lack of response or a diminished response by the

Table 3. *Lowered Response of Enzymes in a Rat Hepatoma to Metabolic Alterations*[1]

Treatment	Host liver	Hepatoma 5123
(a) *Tryptophan pyrrolase*[2]		
None	0.26-0.53	0.13-0.20
Cortisone	0.6 -1.3	0.07-0.13
Tryptophan	2.4 -6.3	0.07-0.13
(b) *Threonine dehydratase*[3]		
None	<10	440
91% protein diet (7 days)	158	560
2% protein diet (4 days)	<10	440

[1] Data read off from the charts (Pitot and Morris, 1961; Pitot *et al.*, 1961).
[2] Expressed as micromoles per 200 mg tissue per hour.
[3] Expressed as micromoles per gram protein per 30 minutes.

Table 4. *Increased Response of Enzymes in Rat Hepatomas to Hormonal and Nutritional Stimuli*[1]

Rats and treatment	Enzyme activity	
	Host liver	Hepatoma
(a) *Glutamine synthetase*[2]		
Hepatoma 7787-bearing		
Control	183	66
Cortisol-injected	205	136
Hepatoma 7800-bearing		
Control	243	69.6
Adrenalectomized	254	15.2
Cortisol-injected	246	796
Thyroxine-injected	290	624
Hepatoma 9618A-bearing		
Control	173	911
Glycine-fed	180	461
Hepatoma 8999-bearing		
Control	183	695
Glucagon-injected	52.0	28.5
(b) *Argininosuccinate synthetase*[2]		
Hepatoma 7800-bearing		
Control	83.4	101
Thyroxine-injected	79.1	312
(c) *Arginase*[3]		
Hepatoma 7800-bearing		
Control	115	18.6
Cortisol-injected	157	90.2

[1] Data taken from Wu and Morris (1970), Wu and Bauer (1971), and Wu *et al.* (1971)
[2] Expressed as micromoles per gram per hour.
[3] Expressed as millimoles per gram per hour.

enzymes in the hepatomas to the administration of hormones or substrates, and to the feeding of different diets. Table 3 shows the unresponsiveness of tryptophan pyrrolase and threonine dehydratase in a rat hepatoma under the conditions that effected responses in liver. This lack of response in cancer tissues is what Potter (1964) referred to as indicating that "one or more connecting links in feedback loops have been deleted or altered to the point of functional ineffectiveness."

A few years later, Wu and his associates (Wu and Morris, 1970; Wu and Bauer, 1971; Wu *et al.*, 1971) found that certain enzymes in slow-growing, well differentiated hepatomas were *more* responsive to metabolic modulations than were those in normal liver. Table 4 shows the overresponsiveness of glutamine synthetase, argininosuccinate synthetase, and arginase to hormonal and dietary alterations. Evidently, the feedback deletion hypothesis formulated to explain the underresponsiveness of enzymes in cancer is limited in scope and cannot accommodate these newer findings, since in overresponsiveness there appears no "functional ineffectiveness."

These and other similar results led Wu *et al.* (1971) "to conclude that lack of responsiveness of enzymes in tumors is not the rule." Hence, a broader mechanism must be considered to explain not only the lack of responsiveness but also the overresponsiveness of enzymes in cancer. And the reversion hypothesis was born.

The Reversion Hypothesis

That there is a similarity in enzyme activity between fetal liver and hepatomas is an old story. Greenstein (1947) pointed out that some parallelism existed between these two types of tissues. However, during the early decades of biochemical research in cancer, the results were generally descriptive of what cancer was in biochemical terms. Since the enzyme activity of a tissue in the resting state tells little about its potentiality when challenged, the early observations of similarity have shed little light on the mechanism of cancer induction. Now we must examine the phenomenon in the light of current knowledge of molecular biology.

In the course of studying the responses of several enzymes in well differentiated rat hepatomas to metabolic modulations *in vivo*, Wu and his associates (Wu and Bauer, 1971; Wu and Morris, 1970; Wu *et al.*, 1971) observed a striking similarity in the responsiveness of the enzymes between the suckling rat liver and the hepatomas. At the same time, the responsiveness of these enzymes showed a marked contrast between the adult liver and the hepatomas. Tables 5 and 6 present succinctly the results in support of this correlation. Subsequently, the observation has been extended to include

Table 5. *Responsiveness of Enzymes in the Suckling and Adult Rat Liver and in the Hepatomas to Hormonal Administration*[1]

Tissue and age	Enzyme activity	
	Control	Treated
(a) *Arginase*[2] *after cortisol*		
Liver, 4 days	30.0	60.0
Liver, 50 days	57.0	68.0
Hepatoma 8999	0.65	6.2
Hepatoma 7800	18.6	90.2
(b) *Glutamine synthetase*[3] *after cortisol*		
Liver, 15 days	95.6	155
Liver, adult	250	251
Hepatoma 9618A	124	395
Hepatoma 8999	695	1,424
(c) *Glutamine synthetase*[3] *after thyroxine*		
Liver, 15 days	95.6	161
Liver, host	176	171
Hepatoma 9618A	130	984
Hepatoma 7800	54.4	366

[1] Data taken from Greengard *et al.* (1970), Wu (1964), Wu and Morris (1970), Wu *et al.* (1971), and Wu (1973).
[2] Expressed as millimoles per gram tissue per hour.
[3] Expressed as micromoles per gram tissue per hour.

Table 6. *Responsiveness of Adenylate Kinase in the Neonatal and Adult Rat Liver and in the Hepatomas to Dietary Manipulations*[1]

Tissue and age	Enzyme activity		
	Control	Fasted	Refed
Liver, 4 days	84	87	76
Liver, adult	151	287	92
Hepatoma 16	52	65	65
Hepatoma 9618A	109	95	88
Hepatoma 9633	79	82	81

[1] Data taken from Criss *et al.* (1970) and Wu (1973), and expressed as micromoles per gram tissue per minute.

a kidney tumor as shown in Table 7. Hence, the phenomenon appears to have general validity among tumors of different origins. On the basis of these observations, they proposed that the correlation reflects a reacquisition or reactivation during carcinogenesis of certain enzyme regulating systems that are operative during the early periods of ontogeny but are suppressed later

Table 7. *Responsiveness of Ornithine Aminotransferase in the Fetal and Adult Rat Kidney and in a Kidney Tumor to Estradiol*[1]

Tissue and age	Enzyme activity	
	Control	Treated
Kidney, fetal	15	No precocious increase
Kidney, adult female	377	1,224
Kidney tumor MK3	17.7	23.2

[1] Data taken from Herzfeld and Knox (1968) and Wu (1973), and expressed as micromoles per gram tissue per hour.

in life. In other words, the cancer cell has adopted a mode of regulation prevailing in the cell of a growing organism but inactivated in the cell of an adult. In a sense, the regulatory mechanism in the cancer cell represents a reversion from that in the mature state to that in the early ontogenic state. Although certain aspects of gene regulation in cancer may be identical with those in the perinatal state, as a whole there is no identity between them (Wu, 1973).

Since this concept relates ontogenic biochemical events to oncogeny, I should like to discuss the manifestation of gene regulation as seen in enzyme induction during ontogeny. Greengard (1970) has written *in extenso* a lucid review on the enzyme formation in the developing rat liver and concluded that individual enzymes emerge stepwise and in clusters, and each stage of emergence may be associated with certain hormonal changes. An injection of hormones or their ablations may result in a premature evocation of an enzyme or a delay in its appearance. On the other hand, certain enzymes decrease to vanishing activities with development. Since during ontogeny the structural genes remain unchanged, both the appearance and the disappearance of enzymes with development must be controlled by the regulatory genes probably with the mediation of hormones. Thus, the close resemblance in the responsiveness, or the lack of it, of an enzyme to hormonal and nutritional stimuli between the perinatal liver and the hepatoma suggests a like mechanism in gene regulation.

In addition to our own results, compilation of results from other laboratories also substantiates the reversion concept. Furthermore, studies of isozyme distribution in hepatomas show good correspondence with that in the perinatal liver but not with that in the adult liver. I have documented these findings elsewhere (Wu, 1973); they need not be repeated here. However, I should like to mention briefly findings in immunological studies, which lend further support to the biochemical conclusion.

The association of certain embryonic antigens with hepatomas in man (Abelev, 1968), the rat (Stanislawski-Birencwajg *et al.*, 1967) and the mouse (Khramkova and Guelstein, 1965) has been reported. Apparently, the genes for the production of these cellular constituents are active during an early period in ontogeny but are suppressed later in life. Reactivation of the suppressed genes takes place during the oncogenic transformation. Huebner and his associates (Huebner and Todaro, 1969; Huebner *et al.*, 1970) reported the presence of a group-specific antigen of the C-type RNA tumor virus during the murine embryonic development and speculated of the indigenous, vertically transmitted viral genome as being responsible for cancers in possibly all vertebrates. Hence, the RNA tumor virus genome may play an important role in embryogenesis, but if reactivated later in life, causes carcinogenesis.

Figure 1 is a diagrammatic, oversimplified representation of the reversion hypothesis as it relates ontogeny to oncogeny. Since there has been no direct way to probe the regulation of a small segment of a genome *in situ* in the mammalian system, a study of the induction-repression of its target protein provides the only means to understand gene regulation.

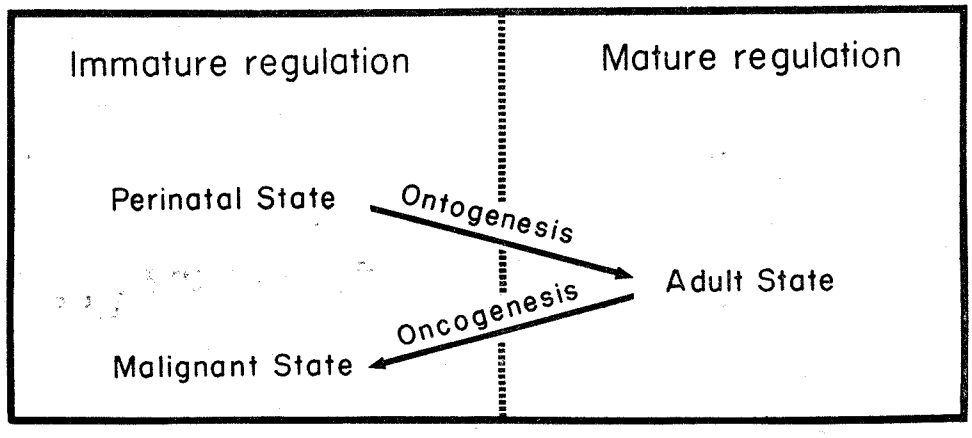

Fig. 1. Gene regulation as seen in enzyme induction in oncogeny as a reversion of that in ontogeny.

Advancement in cancer research depends on advancement in other branches of science, notably molecular biology. We cannot at present discuss the reversion hypothesis in greater detail because we cannot answer the question of exactly what is reversed or how the reversion takes place. The answer will come. I hope before long we shall have it.

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