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COMMENTARY

CLONAL ADAPTATION DURING CARCINOGENESIS

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Living forms have evolved in a largely unfriendly or even hostile environment in which adaptation to different forms of potential harm has been essential for survival and reproduction. In fact, an important attribute of any living organism is the ability to respond in different ways to a wide variety of environmental agents including many xenobiotics.

Diffuse or zonal adaptation

One basic form of adaptation to xenobiotics is induction of enzyme patterns that are related to their metabolism and detoxification [1–4] including the repair of alterations in DNA with some alkylating agents [5, 6]. Another form of adaptive response to some forms of injury is the synthesis and secretion of "acute reactive proteins" by the liver [7]. A third general adaptive response to heat, toxic chemicals and a wide spectrum of other environmental perturbations is the synthesis of "heat shock proteins" [8–10]. It is likely that other patterns of adaptive metabolic cellular response may be uncovered as further probes into adaptation mechanisms are made.

These changes in enzymes and other proteins are often accompanied by increases in organelles such as endoplasmic reticulum ("microsomes") (most often smooth; SER), peroxisomes and mitochondria (cell hypertrophy). The response of the SER is seen with many drugs and other forms of xenobiotics. The peroxisome response is seen with a more limited spectrum of drugs and other chemicals (e.g. hypolipidemic agents, some pesticides and plasticizers) and the mitochondrial response with an even more limited number of agents including the antihistaminic methapyrilene [11] and alcohol (e.g. Ref. 12). In addition, the liver may show cell proliferation (hyperplasia) as a response to some xenobiotics.

In the liver, and probably also in other organs and tissues, these transitory [13] adaptive responses are in general diffuse, zonal or regional, involving a considerable proportion of the original target cells. These responses usually are coincident with the duration of exposure to the xenobiotic and most often are readily reversible after termination of the exposure. Where studied, these responses are mainly associated with reversible changes in the patterns of gene expression [1–4], although controls at other sites in the transcription–translation sequence are possible [14].

The diffuse or zonal responses to many physiological or environmental perturbations have been discussed periodically as "physiological adaptations" in the context of differentiation and of neoplasia [15–18].

Clonal adaptation

In a systematic study of the sequence of steps during the carcinogenic process in the liver [19, 20], it became evident that another form of adaptation exists, one that originates in rare single hepatocytes scattered throughout the liver and is constitutive, rather than readily reversible and diffuse [21, 22]. This response pattern has been seen with many chemical carcinogens. It involves the induction of a new resistance phenotype in a rare hepatocyte and the expansion of these rare hepatocytes to form focal proliferations ("hepatocyte nodules"). With a vigorous expansion, these new nodule-arranged hepatocytes can replace at least two thirds of the original hepatocyte population, thus constituting a major new cell population [23]. A role for these resistant hepatocytes in the adaptive response of the whole organism as well as the liver to a wide variety of xenobiotics and environmental perturbations is indicated. Since these nodules of resistant hepatocytes probably arise from single hepatocytes, we have designated this form of adaptation as "clonal".

The evidence available indicates that these hepatocyte nodules represent a new form of differentiation that appears as an adaptive response to selected "genotoxic" xenobiotics, such as chemical carcinogens, and that has survival value for the organism in a hostile environment.

The recognition of this new adaptive phenomenon has important potential implications in tumor promotion, in the analysis of resistance of many primary cancers to chemotherapy, and in the analysis of how some living forms have adapted, presumably through evolution, for survival in a hostile environment as well as for the development of a conceptual framework for cancer development.

Clonal adaptation during liver carcinogenesis

Cancer development in many sites in humans and

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in animals is usually preceded by the appearance of focal proliferative lesions (so-called benign neoplasms) such as papillomas, polyps or nodules [21, 24]. In the liver, as well as in most other tissues and organs in both animals and humans, one observes a very long delay between the initial exposure to an appropriate chemical carcinogen, radiation or a DNA virus and the first evidence of cancer [21, 24]. During this long period of development, a constant finding is the appearance of focal proliferations or nodules, composed of hepatocytes with occasional bile ducts. In several studies, unequivocal liver cell cancer, with metastatis, has been found to arise within such hepatocyte nodules (see Refs 19 and 20 for references).

Historically, these hepatocyte nodules (also designated "hyperplastic nodules," "neoplastic nodules," "regenerative nodules," and "adenomas") have been considered as preneoplastic or early neoplastic lesions or as regenerative or compensatory in nature. However, their biological potential as well as their origin and properties have been most difficult to study. Among the major impediments to their study has been the asynchronous nature of their appearance in most experimental models and, of course, in humans. The failure of the nodules to appear together as a cohort but rather their appearance at different times throughout the carcinogenic process has made it virtually impossible to follow their slow evolution to cancer, i.e. "what precedes or follows what," in most instances in animals and in humans. The availability of a model, the "resistant hepatocyte model" [23, 25] that generates focal proliferations of altered hepatocytes, induced during initiation, in a rapid and remarkably synchronous manner has allowed for a new perspective on liver carcinogenesis in particular and chemical carcinogenesis in general.

Background

During the first few years of our study of mechanisms of chemical carcinogenesis in the liver, we gradually became impressed by five considerations, each of which suggested that some new approach was needed: (a) cancer development is an inordinately long process, requiring one-third to one-half the life span of the organism (humans and animals); (b) autonomous or semi-autonomous growth of initiated cells is a property acquired late in the carcinogenic process; (c) virtually every chemical carcinogen is an inhibitor of cell proliferation; (d) the response of tissues or organs during carcinogenesis, most relevant as potential precursors for cancer, is focal, not general; and (e) carcinogens (chemicals, viruses, non-ionizing and ionizing radiations) are widespread in nature. Humans and animals have been exposed continuously for millions of years to carcinogens, both present in the external environment including the food and generated endogenously. How has nature handled carcinogens and carcinogenesis? What systems have evolved that could allow for the relatively long life span of so many species? Could any insight into these questions generate clues to mechanisms and interpretation of the carcinogenic processes?

(a) A strange and very puzzling characteristic of almost all carcinogenic systems in many different tissues with different agents (chemicals, DNA and some RNA viruses, radiations, diet) is the inordinately long time period between the initial exposure to a carcinogenic agent or stimulus and the first appearance of malignant neoplasia. This long time span is seen with continuous or repeated exposures to carcinogens as well as with systems using initiator–promoter regimens.

(b)(i) Initiation with one of many different carcinogens is at no time followed by spontaneous proliferation of any cells in the liver (and in other organs as well). Only with doses of carcinogens and periods of exposure much greater than are required for initiation are focal proliferations seen. This failure to proliferate and grow is seen for many months after initiation, even though the initiated hepatocytes can be shown to persist [26].

(ii) Hepatocyte nodules, generated under several different promoting circumstances by proliferation of hepatocytes altered during initiation, fail to show any degree of *autonomous* growth of their hepatocytes *in vitro* [27–30] and *in vivo* [31–34]. The latter includes autochthonous transfers to kidney, cleared fat pad, subcutaneous tissue and subcapsular region of the liver and syngeneic transfer to newborns, with or without cortisone treatment. The only known exception to date is the syngeneic spleen [35–37]. In this site, hepatocytes from normal liver as well as hepatocytes from early or late persistent nodules grow slowly. The hepatocytes from control livers or from early nodules grow diffusely with progressive replacement of the splenic pulp with functional liver [35–37]. In contrast, the hepatocytes from late persistent nodules grow in a nodular pattern and generate metastasizing hepatocellular carcinomas after about 18 months [38]. Thus, even in this special site, the spleen, the early nodules behave in a manner similar to hepatocytes from mature adult liver.

If the initiated altered cells induced by exposure to a carcinogen do not grow spontaneously, how might a differential stimulation of their growth be created? The growth of the rare altered cell leading to focal proliferations is a key phenomenon in promotion in virtually all experimental carcinogenesis and in many human systems of cancer development.

(c) It is interesting that many carcinogens are quite potent inhibitors of cell proliferation ("mitoinhibition") and/or DNA synthesis. Based upon his own research on the inhibition of cell growth of normal and cancer cells with some pure polycyclic aromatic hydrocarbons that were being identified and synthesized at the Chester Beatty Research Institute, Haddow [39] suggested in 1938 that inhibition of cell proliferation could be an early effect of carcinogens and that, in such an environment, resistant cells may arise and are encouraged to proliferate. Although this suggestion was directed toward tissues or organs other than the liver, subsequence work with many different carcinogens indicated that a similar phenomenon might occur in the liver [40–42]. Inhibition of hepatocyte proliferation and/or DNA synthesis has been shown to occur with carbon tetrachloride, ethionine, 2-acetylaminofluorene (2-AAF), 4-dimethylaminoazobenzene (DAB) and derivatives, thioacetamide, aflatoxin B_1, dimethyl-

nitrosamine (DMNA), diethylnitrosamine (DENA), pyrrolizidine alkaloids, 7,12-dimethylbenz[*a*]-anthracene, urethane and *N*-methyl-*N*-nitrosourea (see Ref. 43 for references). The clearest manifestation of this phenomenon is the inhibition of hepatocyte proliferation following partial hepatectomy in animals exposed for short periods (few days to 2–3 weeks) to the carcinogen. An equally impressive characteristic is the rapid recovery of the original hepatocytes when the exposure to the carcinogen (DAB and derivatives, 2-AAF, DMNA or DENA) is terminated. Recently, it has been reported that two non-carcinogenic promoting agents for hepatocarcinogenesis, phenobarbital [44, 45] and orotic acid [46], also show considerable inhibition of hepatocyte proliferation, not unlike that seen with the carcinogens.

(d) In the majority of instances of cancer development in humans or in animals in which a precursor cell population or lesion has been identified or proposed, the "preneoplastic" and "precancerous" changes are always focal, involving only a very small number of altered cells [21, 24]. This is most clearly seen in the respiratory, gastrointestinal and urinary tracts, in the skin and in almost every organ. Under these conditions, it is to be anticipated that a selection pressure needs to be established if the altered hepatocytes are to proliferate selectively, relative to the surrounding cells. The creation of a differential phenotype would seem to be critical for any expansion of a minority cell population such as that of rare initiated hepatocytes during promotion [47].

(e) The majority of cancers are seen in humans and in animals relatively late in the life history of any organism. Most often, the cancers appear late in the reproductive phase or following the end of the reproductive period of the life history. This is interesting and puzzling, since it can be clearly shown in experimental animals and in many humans that exposure to a carcinogen began or occurred many years previously, often during the early portion of the reproductive period. Although a dominant role for immunologic surveillance was suggested by Burnet [48], the evidence for this during chemical carcinogenesis is indeed unimpressive if not non-existent [49–51]. Alternative hypothesis for the very prolonged nature of the carcinogenic process seem necessary if we are to understand its essence. As is strongly suggested by the responses of the liver, the inordinately long period for cancer development, coupled with its adaptive nature, points to quite a different interpretation of how the organism responds positively to some carcinogenic stimuli.

The resistant hepatocyte as an early response to carcinogens

On the basis of the five considerations above, we postulated that *one type* of initiated cell in the liver, perhaps even a very common type, induced by carcinogens is a resistant hepatocyte that can grow in an environment that inhibits the majority of uninitiated cells from growing and thus can rapidly become hepatocyte nodules by clonal expansion of each rare resistant hepatocyte [25, 33]. One or very few of these nodules would be precursors for the ultimate development of liver cell cancer. This could explain the regular occurrence of focal proliferations in the liver during the long exposure to strong mitoihibitory carcinogens used in the earlier studies on chemical carcinogenesis.

After obtaining considerable evidence that hepatocyte nodules induced by two quite different carcinogens, 2-AAF and ethionine, were resistant to some carcinogens and other hepatotoxins [52], we devised an initial test for the hypothesis [25].

There is now a large body of evidence indicating that the genesis of resistant hepatocytes is commonly seen with many different carcinogens. With well over seventy-five different chemical carcinogens of quite different chemical structures and properties as well as in hepatocarcinogenesis seen in rats fed a choline devoid diet, a resistant hepatocyte is a common accompaniment of initiation [25, 53–61].

The resistance phenotype is manifested at three levels of organization: (a) physiological (behaviour): resistance to inhibition of cell proliferation ("mitoinhibition"); (b) cellular: resistance to visible cytologic responses to some cytotoxic environments; and (c) biochemical–molecular: the presence of a biochemical pattern that diminishes or counteracts possible toxic effects of xenobiotics.

(a) At the physiological level, the key manifestation of the resistance phenotype in the rare hepatocyte is the ability to proliferate vigorously in response to a proliferative stimulus in an environment, such as that created by the presence of one of many carcinogens, in which the vast majority of hepatocytes, the non-resistant ones, are inhibited. As already indicated, this striking common property is induced by many different carcinogens during initiation of hepatocarcinogenesis, is a common basis for the promoting ability of many hepatocarcinogens and may also be important with two non-carcinogenic promoting agents, the drug phenobarbital and the normal metabolite and obligatory precursor of pyrimidine nucleosides, orotic acid.

(b) The hepatocyte nodules show resistance to metabolic and cytologic or cytotoxic effects of the agents used in their generation (Table 1) and cross-resistance to other xenobiotics and to a choline deficient diet (Table 2).

(c) Hepatocyte nodules induced with quite different regimens show an unusually common biochemical pattern (Table 3). This pattern shows consistent decreases in several Phase I components including total cytochromes P-450 and several mixed-function oxygenases, consistent increases in many Phase II conjugating systems, characteristic alterations in glucose metabolism and a reproducible altered pattern of iron and heme metabolism (see Refs 70–72 for references). This biochemical pattern offers a reasonable basis for the observed decrease in metabolic activation of some xenobiotics and the more efficient detoxification of active derivatives by conjugation as well as for the broad physiological resistance to the cytotoxic and mitoinhibitory effects of many xenobiotics. Because of this, the pattern seen in the hepatocyte nodules is designated as a "resistance phenotype" [75].

It is important to emphasize that many different enzymes and components contribute to this phenotype. For example, the decrease in total cytochromes

Table 1. Resistance of hepatocytes in nodules to the toxic effects of the promoting environments used to generate the nodules

Method of generating the hepatocyte nodules		Properties of hepatocyte nodules
Initiator	Promoting regimen	
Wide variety of initiators	RH model (2-AAF + PH/CCl_4)	Nodules are resistant to the mitoinhibitory effects of 2-AAf.
DENA	CD	Nodules are resistant to fat (triglyceride) accumulation induced by a CD diet [62, 63].
DENA	OA	Nodules are resistant to the OA-induced accumulation of uridine nucleotides [64].
DENA	Lasiocarpine	Nodules are resistant to the megalocytic effects of lasiocarpine [65].
DENA	Polybrominated biphenyls	Nodules are resistant to fat accumulation with polybrominated biphenyls [66].

Abbreviations: DENA, diethylnitrosamine; PH, partial hepatectomy: CCl_4, carbon tetrachloride; AAF, 2-acetylaminofluorene; CD, choline-deficient diet; and OA, orotic acid.

P-450 is so large (60–80%) that several different ones must be involved [76]. This multiplicity of active components in the resistant hepatocytes could readily allow for considerable variation in the levels of one or more components in different clones derived from these hepatocytes [77, 78] without compromising their overall resistant behaviour in an appropriate selecting environment.

It is interesting and noteworthy that the resistance phenotype in the hepatocyte nodules has several resemblances to the phenotype of some human cancer cells that are resistant to some chemotherapeutic agents [79–82].

Nodules of resistant hepatocytes as a type of adaptation with survival value

What is the evidence that the nodules of resistant hepatocytes which we designate as "hepatocyte nodules" are better considered as a form of physiological adaptive response [21, 22] (Fig. 1) rather than as collections of *abnormal* cells, namely benign neoplasms, that are part of a pathologic sequence specifically related to the development of cancer? According to the latter view, the nodules of hepatocytes derived by clonal expansion of potential initiated hepatocytes are *abnormal* from the outset and represent foreign cell populations derived on the basis of abnormal genes and gene products.

The most telling evidence that points overwhelmingly to the physiological adaptive nature of the hepatocyte nodules exists at three levels of organization: (a) the cell and tissue architecture and organization; (b) the whole organism; and (c) the biochemical.

(a) The hepatocytes in the nodules are organized differently than are the hepatocytes in the mature liver, including those in the surrounding liver. In contrast to the normal and surrounding liver, in which hepatocytes are arranged predominantly as single cell plates, those in the nodules are in the form of double cell plates and acini [83, 84]. However, this difference in organization and architecture is not an expression of abnormality, since the hepatocytes in the nodules undergo *spontaneously* a radical restructuring and remodelling [85, 86]. Over 95% of the nodules rapidly undergo this major restructuring at about 6 weeks after initiation such that these nodules virtually disappear and become integrated into the structure of the surrounding liver. The hepatocytes now have changed their architecture and arrangement and cannot be distinguished from the surrounding liver [85]. This restructuring involves the cell to cell organization and the blood supply as well as the biochemical pattern.

Thus, clearly, most of the nodules must have the pre-existing genetic information to allow them to undergo automatically or spontaneously this very complicated restructuring and remodelling. In every respect, this is truly a differentiation, analogous to other examples of differentiation seen in normal development.

This organizational pattern of hepatocytes as well as remodelling is seen with several quite different models of experimental hepatocarcinogenesis, including the earlier ones with long-term exposure to hepatocarcinogens such as the azo dyes. It is

Table 2. Cross-resistance of hepatocytes in foci/nodules to the toxic effects of liver tumor promotors and other xenobiotics

Systems used to generate hepatocyte foci/nodules	Resistance of hepatocyte foci/nodules
DENA + CD diet	Nodules are resistant to the mitoinhibitory effects of 2-AAF.
DENA + RH model (2-AAF + pH/CCl_4)	Nodules are resistant to fat (triglyceride) accumulation induced by CD diet and to uptake of OA [67].
DENA + various promoters	γ-Glutamyltransferase-positive foci are resistant to the mitoinhibitory effects of 2-AAF [68].
2-AAF; ethione	Nodules are resistant to the toxic effects of dimethylnitrosamine and CCl_4 [52].
2-AAF	Nodules are resistant to the toxic effects of phalloidin [69].

Abbreviations: DENA, diethylnitrosamine; CD, choline-deficient diet; 2-AAF, 2-acetylaminofluorine; RH, resistant hepatocyte; PH, partial hepatectomy; and OA, orotic acid.

Table 3. A common biochemical pattern associated with the resistance phenotype in hepatocyte nodules in rats

(A) Decrease in	xenobiotic metabolizing and activating components*
	Cytochromes P-450
	Mixed-function oxygenases
	Sulfotransferase
(B) Increase in	detoxification components*
	Glutathione
	Glutathione-*S*-transferases including glutathione-*S*-transferase 7-7 (P)
	UDP-glucuronyl transferase I
	γ-Glutamyltransferase
	Epoxide hydrase (hydrolase)
	DT-diaphorase (quinone reductase)
	P-Glycoprotein (*mdr*)
(C) Alterations in	glucose metabolism [73, 74]
	Altered glycolytic enzymes
	Alterations in pentose shunt including increase in glucose-6-phosphate dehydrogenase
(D) Altered iron metabolism*	
Decrease in	iron uptake and/or concentration
	Total iron
	Total heme
	Heme enzymes—Cytochromes P-450, cytochrome b_5, catalase, tryptophan 2,3-dioxygenase
	Heme binding protein (cytosolic)
Increase in	heme oxygenase
	Transferrin receptors (60X)

* See Refs 70–72 for references.

only more evident in the resistant hepatocyte model because of the presence of a high degree of synchrony of nodule development.

(b) A recent study has shown clearly that rats with hepatocyte nodules induced using the resistant hepatocyte model are unusually resistant to a lethal dose of a potent hepatotoxic agent, carbon tetrachloride (Fig. 2) [87]. As shown in Fig. 2, rats with nodules show complete resistance to a dose of CCl_4 that is lethal for 100% of normal rats and for almost 70% of rats treated with agents used in either initiation or promotion but without the three treatments needed to generate hepatocyte nodules.

These important findings indicate that treatment of rats so as to generate hepatocyte nodules in their livers has an obvious survival value for the *whole organism* when exposed to at least one potent hepatotoxin. This demonstration of survival value indicates that the genetic information necessary to develop hepatocyte nodules and to have them remodel by differentiation could have been favoured during the long period of evolution over the millennia.

Another aspect, possibly related to survival, is the resistance of the hepatocyte nodules to the hepatotoxic effects of some hepatotoxic xenobiotics. Not only are the hepatocytes in hepatocyte nodules resistant *in vitro* [27, 88–90] to many toxic xenobiotics but they also show unusual resistance to hepatotoxic effects *in vivo* [52, 65, 68, 69]. Doses of CCl_4 or DMNA that induce severe hepatotoxity *in vivo* in control animals do not induce comparable effects on

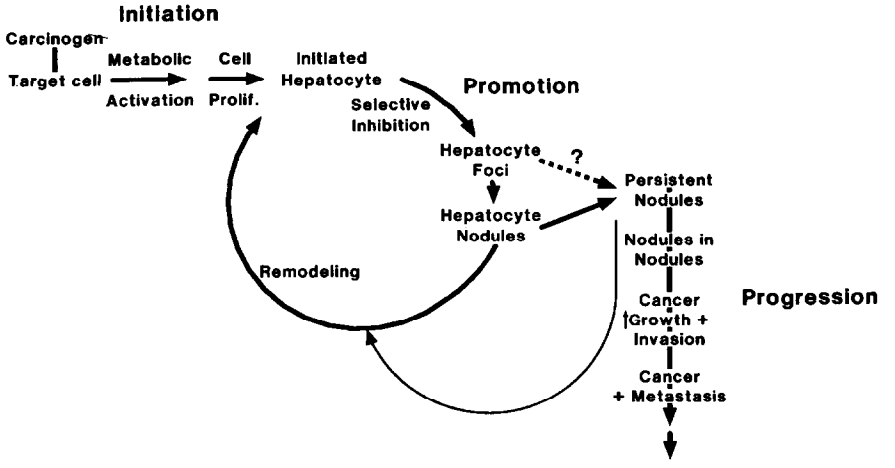

Fig. 1. Schematic representation of clonal adaptation as a central component in the step-by-step development of liver cancer in the rat initiated with genotoxic carcinogenesis. Modified from Ref. 19.

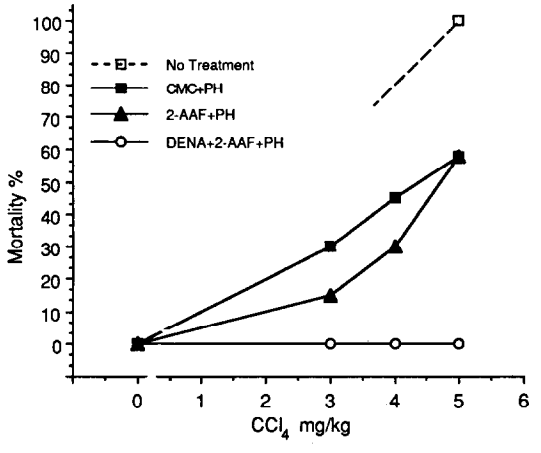

Fig. 2. Mortality (within 14 days) versus dose of CCl_4 in rats with hepatocyte nodules (DENA + 2-AAF + PH) in comparison to control animals with no treatment or treatment not generating nodules (CMC + PH, 2-AAF + PH). Note the striking difference in mortality. The hepatocyte nodules were generated by initiation with diethylnitrosamine, followed by selection by treatment with 2-AAF plus PH. Abbreviations: 2-AAF, 2-acetylaminofluorene; CMC, carboxylmethylcellulose; DENA, diethylnitrosamine; and PH, partial hepatectomy. Modified from Ref. 87.

the hepatocyte nodules. Thus, the replacement of a significant percentage of normal hepatocytes with nodule hepatocytes, as occurs during the process of hepatocarcinogenesis, imparts to the liver a resistance to at least some toxic agents.

(c) At the biochemical–molecular level, the biochemical pattern, or at least a major part of it (A and B in Table 3), can readily account for the resistance of the nodules to cytotoxic effects of xenobiotics, as indicated above, as well as the more efficient excretion of at least one carcinogen, 2-AAF [91, 92]. A marked decrease in activation associated with the large decrement in Phase I components, coupled with the more efficient conjugation and excretion of active moieties generated, associated with the several conjugating systems in Phase II, match quite well.

The virtual certainty that this pattern is physiologically programmed is indicated by its manifold-nature with at least 25–30 different enzymes and other components and by the observations that some agents such as lead nitrate [93], an interferon [94], butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [95, 96] induce a biochemical pattern in normal livers very similar to that seen in the hepatocyte nodules but in a reversible or transient manner.

This special biochemical pattern is not seen in fetal, neonatal or regenerating liver but seems to be uniquely associated with the hepatocyte nodule induced by one of many different carcinogens. These results suggest that hepatocytes in nodules represent a new state of differentiation, better adapted to survive in a hostile environment. The resistance, coupled with the capacity of the majority of nodules to undergo spontaneous differentiation to normal-appearing mature liver [85, 86], indicates that the clonal expansion seen in the early phase of carcinogenesis is genetically programmed and is part of a normal physiological pattern of adaptation to some types of xenobiotics [21, 22, 97]. It should be mentioned, parenthetically, that the minor population of hepatocyte nodules, those that persist, show the same biochemical pattern as do the majority of nodules that remodel. Also, most of the persistent nodules show the same pattern of remodelling by differentiation as do the majority but much more slowly. Thus, there is no evidence that the nodules that remodel and those that persist represent two entirely different responses to carcinogens.

Whether the hepatocytes remain resistant after they remodel to mature liver is an important question that has no answer as yet. Since the bulk of the hepatocytes surrounding the few persistent nodules are derived in large part from differentiated nodules,

the retention of some resistance could have a continuing protective effect against some xenobiotics. The model is now sufficiently advanced to allow critical tests of this possibility.

Is clonal adaptation also probable in other carcinogenic systems?

Superficially, there are several similarities in the overal patterns among many experimental and human systems for cancer development. The very long so-called "incubation period" during which the cellular evolution of malignancy from non-malignant precursor lesions is occurring, the apparent reversibility of putative preneoplastic or precancerous lesions, the common biological behaviour and appearance of early focal proliferations such as polyps, papillomas and nodules with many different carcinogens and promoting environments all point to at least some basic similarity between carcinogenesis in several organs and tissues and carcinogenesis in the liver with chemicals [20, 21, 24]. It would seem prudent, therefore, to explore some of these systems from the point of view of clonal adaptation. In my opinion, it is quite likely that this type of adaptation, as well as perhaps others, may well be important in cancer development in several systems.

The most telling is in the case of malignant melanoma in humans. Clark and his associates [98, 99] have shown that the genesis and behaviour of nevi and their role in the development of melanoma have many similarities to the pattern seen in the rat liver. The nevus, like the hepatocyte nodule, shows at least two options, differentiation to nerve endings as a major pathway and persistence with further cellular evolution to cancer as a minor one. Whether the resemblances between rat liver and human melanocytes extend to biochemical–molecular mechanisms remains a fascinating problem for study.

Relevance of clonal adaptation to carcinogenesis and cancer

Tumor promotion, despite its frequent association with an element of mystique, is basically clonal expansion of initiated cells. In the liver, this leads to the genesis of hepatocyte nodules. The concept of clonal adaptation places tumor promotion in the liver in quite a different perspective. This part of the carcinogenic process is only in part and perhaps only peripherally related to the ultimate development of cancer but becomes an important way in which the living organisms responds in an adaptive fashion to commonly occurring xenobiotics in the environment. On this basis, scientifically, the neoplastic derivative, the cancer, is perhaps only incidental while the adaptive component is the most important from the perspective of biology. In this regard, the "turning on" of a new phenotype in a rare cell during initiation becomes the key to what is seen in promotion. Conceivably, the same type of induction is occurring in other tissues and organs during initiation.

How a genotoxic carcinogen induces a new common constellation of biochemical components that go to make up the resistance phenotype is not understood. Although the induction of mutations in a rare cell by genotoxic carcinogens or metabolites is very well established, whether this is the molecular mechanism for the induction of the resistant hepatocyte as a common product is not evident at this time. Altered regulatory genes, rare gene rearrangements, gene translocations or gene amplifications are some possibilities.

It would appear that the study of the control of transcription of several of the discrete components of the resistance phenotypes, such as glutathione-*S*-transferase 7-7 (P), γ-glutamyltranspeptidase, DT-diaphorase and discrete relevant cytochromes P-450, to name but a few, could in turn suggest possible underlying mechanisms for clonal adaptation.

An interesting aspect of clonal adaptation of great practical importance relates to resistance of many cancers to chemotherapy [100]. It is often implied that resistance to chemotherapy may be a property acquired during the schedule of treatment as a mutation or some other genomic alteration including gene amplification. The experience in the liver and the existence of clonal adaptation suggest the possibility that resistance may be a primary property of the cancer in some instances. Given the derivation of cancer by cellular evolution from a nodule with a resistance phenotype that was used mainly for clonal expansion, it is certainly conceivable that many cancers in some tissues may begin with a resistance phenotype. The presence in cancer precursor cell populations of P-glycoprotein, as the product of *mdr* gene and other components of resistance to xenobiotics including chemotherapeutic agents [75, 79–82], would suggest that some cancers may begin as resistant and that a positive response to chemotherapy may require a further more or less permanent change in the phenotype.

In view of the widespread occurrence of many potential carcinogens in the environment, it remains important to understand how evolution has enabled organisms to adjust or adapt to this ever-present hazard. The long life span of many species in the face of this hazard attests to the efficiency with which evolution has succeeded.

In my opinion, the long precancerous period, lasting as long as one-third to one-half or more of the life span, has largely relegated neoplasia to the post-reproductive period. The acquisition of a mechanism to adapt, such as by clonal adaptation, would seem to be an important way in which this has been accomplished. If this viewpoint is valid, one would have to consider neoplasia as mainly a manifestation of the imperfection of the adaptive process and cancer as a deviant of adaptation [97]. Such a viewpoint may open several new avenues for the development of novel ways to interrupt the carcinogenesis process and thereby prevent cancer.

Finally, the presence of a major adaptive component during carcinogenesis would offer interesting alternatives to the rigid current paradigm of carcinogenesis, the progressive acquisition of abnormal genes and gene products without any regard to the adaptive ability and talent of biological systems. This formulation denies the exquisite ability of almost all organisms, down to the single cell bacterium, to adapt and adjust to environmental perturbations. A formulation more consistent with modern biology, such as here proposed, seems not only attractive

theoretically but offers many possible practical approaches to the ultimate prevention of cancer.

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