# BIOCHEMICAL PATHOLOGY

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EMMANUEL FARBER
*Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania*



## INTRODUCTION

This review is concerned with the cellular mechanisms of the toxicity of chemicals or drugs. It is self-evident today that all living organisms of whatever complexity continually respond and adapt to a wide variety of perturbations in their environment. The modern study of the response of an organism to such stresses must include the functional and structural components of cells and tissues as well as the molecular. Although pathology theoretically should include the chemical basis for the response in disease, by tradition it has dealt primarily with the structural and to a lesser degree the physiologic. Because of this, "biochemical pathology" has begun to develop as the study of the development of disease in chemical and molecular terms.

The "toxicity" of any compound in the final analysis is the sum total of its interactions with cell constituents to produce chemical alterations *and* the cell's response to these aberrations. Thus, the field of toxicology overlaps to such a degree with some aspects of pathology that no clear-cut separation between the two is meaningful or useful in terms of modern biology.

The analysis of the mechanism of toxicity of chemicals is an important undertaking from at least two points of view—a *practical* and a *theoretical.* The practical use stems from the need to interrupt or circumvent the toxicological process in the continuing presence of the hazard (e.g. tobacco, etc.). The theoretical is equally important and has broader application in biology—namely the use of "toxic" chemicals as metabolic probes for the analysis of biological systems. This was appreciated almost a century ago by Claude Bernard, when he stated:

> ". . . mais, outre ces deux usages bien connus de tout de monde, il en est un troisième qui intéresse particulièrement le physiologiste. Pour lui, le poison devient un instrument qui dissocie et analyse les phénomènes les plus délcats de la machine vivant, et, en étudiant attentivement le mécanisme de la mort dans les divers empoisonnements, il s'instruit par voie indirecte sur le mécanisme physiologique de la vie." (1)

The extreme complexity of living cells at the chemical level will necessitate the development of a large number of model systems if we are to continue with the fine metabolic dissection required for the analysis of cell

structure and function. In microorganisms, the most elegant and precise tools for metabolic dissection, the single genetic mutants, are becoming available in increasing numbers. Such elegance and precision is not yet available for the vast number of eukaryotic cells, especially in higher organisms. The much more complex genomic organization in such cells, and the present inaccessibility to manipulation of most genetic information, have denied us the single genetic mutant as a tool, except in a rare instance. We are, therefore, forced to use a variety of exogenous environmental variations as the major means for cellular analysis. Dietary manipulations, viruses, radiations, and chemicals or drugs are among the list available. Of these, drugs, as their mechanism of interaction with cells and cell components is elucidated, offer some of the most versatile approaches to the metabolic dissection of the cell.

Thus, there is a good theoretical basis for the opinion that the approach of cell biology through pathology and pharmacology offers unusual opportunities for understanding cell function through cell structure in the living intact organism. The emphasis on continually relating the analysis to the intact organism is a special feature of this approach to biology.

Fundamental to the analysis of the mechanism of action of any toxic agent is the delineation of the "biochemical lesion," a concept formulated and developed especially by Peters (2, 3) in his important studies on arsenicals, carbon-fluorine compounds, and thiamine deficiency. Although this concept stems from the writings of Virchow, who as far back as 1855, stressed the need for a physico-chemical understanding of disease (4), the early attempts in this field were often abortive, because of the primitive state of cell biochemistry. Peters and others have laid a solid modern foundation for "biochemical pathology."

The reviewer has selected arbitrarily examples illustrating two basic approaches in toxicology and biochemical pathology: (*a*) the response of certain cells or organs to interference with selected metabolic processes, i.e. response to known biochemical lesions—ATP deficiency, inhibition of protein synthesis, RNA synthesis, and DNA synthesis; and (*b*) the biochemical mechanisms underlying the induction of discrete and selective biological responses to some toxic agents, i.e. selected experimental disease—carbon tetrachloride and related compounds, trialkyltin compounds, and β-aminopropionitrile and related osteolathyrogens. It is hoped to indicate how the knowledge contributed by each approach may lead to new insight into integration of the cell's metabolic activities and into mechanisms used by cells in their response to environmental hazards.

## CELLULAR RESPONSE TO SELECTED BIOCHEMICAL LESIONS

### ATP Deficiency and the Liver

*Ethionine.*—Ethionine, the ethyl analogue of methionine (5), induces a variety of pathologic changes in animals including fatty liver, pancreatitis, and renal lesions in short-term experiments, and liver cancer in chronic ex-

periments (6–8). In the rat, the species studied most, this methionine analog has at least four metabolic effects in the liver: (*a*) rapid induction of an ATP deficiency, (*b*) ethylation of RNA (9–13), of highly selective proteins especially nuclear proteins (14), and of several other cellular components (see 6, 7), (*c*) inhibition of methylation of RNA and of other cellular components utilizing methionine as the donor (15, 16), and (*d*) incorporation into protein in place of methionine (17).

The available evidence strongly suggests that most of the acute hepatic effects of ethionine are due predominantly or exclusively to the rapid induction of a deficiency of cellular ATP. These effects include fatty liver (8), major shifts in the polysome-ribosome equilibrium toward the ribosomes and ribosomal subunits (18–20) accompanied by marked inhibition of protein synthesis (21), a variety of ultrastructural alterations in the cytoplasmic organelles of the liver cell (19, 22, 23), and characteristic lesions in the nucleolus of the same cell (24) accompanying severe inhibition of RNA synthesis (25).

The decrease in hepatic ATP level following ethionine administration was originally suggested by Stekol (cf. 7) but was first clearly shown by Shull (26). Basically, the lowering in ATP concentration is the result of the formation of S-adenosylethionine (SAE) at a rate faster than the liver cell can regenerate the adenosine moiety through transethylation and faster than it can synthesize adenine nucleotides *de novo* from available precursors (27). Such an "adenine-trapping effect" of ethionine was clearly shown first in yeast by Schmidt et al (28) and suggested by Stekol (cf. 7). With saturating doses of ethionine (0.75 to 1 mg per g body weight), the liver ATP concentration remains at about 15 to 20 percent of the control values for up to 36 hours after a single dose (23, 27, 29). The ability to induce different degrees of ATP deficiency in the liver with different doses of ethionine is sufficiently reproducible to make this a versatile system for the study of many aspects of ATP metabolism in an intact animal.

In view of the mechanism of genesis of the hepatic ATP deficiency, it is not at all surprising that it can be readily prevented or easily reversed, by supplying adenine or other adenine nucleotide precursors such as inosine or 5-amino-4-imidazole carboxamide (26, 30–32). Thus, one can, at will, turn the basic biochemical lesion on or off in the intact animal. This illustrates an important aspect of the analytical approach to biochemical pathology, reversibility, a feature that has also been stressed by Peters (3).

The key property of ethionine that enables it to induce such marked changes in ATP levels with the many evident consequences is its ready conversion to SAE and the imbalance thus created between the rate of utilization of ATP for SAE synthesis and the rate of regeneration of the adenosine moiety through utilization of the SAE. In the rat, with methionine, these two overall reactions are quantitatively so balanced that even large doses of methionine given over a short period of time do little to disturb the balance. However, at least one species, the guinea pig, does not appear to

possess such a fine balance and this species shows some of the same consequences with large doses of methionine that the rat shows with ethionine (see below).

An interesting feature of the ethionine-treated animal is the peculiar specificity with respect to the liver. Although other organs [e.g. pancreas (33)] do show a decrease in ATP level, this occurs to a much lesser degree and much more slowly than in the liver. This is most probably due to the fact that the liver contains much more of the methionine-activating enzyme (ATP:L-methionine S-adenosyl-transferase) than the other organs examined (34).

Many of the consequences of the low ATP level in the liver are those that might well be expected in view of the important role that ATP plays in cellular metabolism. The dominant and most evident consequence is a rapid and progressive increase in triglyceride content (TG) beginning within a few hours after the administration of ethionine and continuing for about 48 hours (cf. 8). This fatty liver, although a striking response, appears to be a secondary consequence of the interference with protein metabolism and will be discussed more fully later.

However, an interesting feature of this lesion is the ready ability of the liver to esterify free fatty acids coming from the adipose tissue with glycerophosphate, despite the low level of ATP in the liver (8). The rate of formation of TG is linear for about 48 hours and is approximately 17 mg per hour per 100 g body weight (8). This suggests that the ATP-dependent reactions essential for this synthesis, namely the formation of fatty acid-coenzyme A and glycerophosphate molecules, have high affinities for ATP (low $K_m$ values) and are able to function well at the low ATP concentrations present. There is considerable evidence that the generation of ATP from ADP via mitochondrial oxidative phosphorylation continues at essentially a normal rate in the ethionine-treated rat (*27*).

It is clearly evident that the ratio of ATP to ADP to AMP and the number of anhydride-bond phosphates per adenosine moiety (35) are probably far more important to the function of the cell than is the absolute value of any single adenine nucleotide (see 36, 37). In view of the many single enzymes that are regulated by ATP, ADP, and AMP, and that require ATP as substrate, it is to be anticipated that the severe perturbation in adenine nucleotide metabolism induced by ethionine would have effects on many metabolic pathways in the liver. As pointed out by Sauer & Sarkar (38), the ethionine-treated animal is rather a special model from the point of view of adenine nucleotide metabolism. With most systems (e.g. anoxia, diabetes, etc.) the ratios of the different adenine nucleotides to each other change but the total nucleotide concentration remains pretty much in the normal range. In the ethionine-treated animal, the levels of all three adenine nucleotides decrease, although that of ATP drops more than that of ADP or AMP (38). This makes the ethionine model an interesting one from the point of view of the control of cellular metabolism by adenine nucleotides, es-

pecially using the approach of Atkinson (35–37) and his concepts of energy change of the adenylate pool.

The few studies on the cellular consequences of the adenosine trapping induced by ethionine indicate some over-all response patterns. RNA synthesis and protein synthesis in the liver in vivo are particularly susceptible to inhibition by ethionine. In both instances, the degree of inhibition parallels closely the magnitude of the decrease in ATP concentration (21, 25). Although adenine nucleotide precursors readily prevent or reverse the inhibition of synthesis of each of these macromolecules (21, 25), the exact mechanism whereby the low ATP concentration inhibits the synthesis is not yet clear. In the case of protein synthesis, there is some effect of low ATP on the integrity of the polysome, presumably on the formation of the initiation complex between messenger RNA and the ribosomal subunits. For RNA synthesis, it has been suggested that the effect is a more direct one on the supply of an essential substrate, ATP (25). The observation that liver nuclear ATP concentration decreases in the ethionine-treated animal (39) is consistent with this suggestion.

As already indicated, TG synthesis continues unabated. Oxidative phosphorylation (cf. 8) and gluconeogenesis from pyruvate (40) also appear to be intact and functioning normally. The latter finding is somewhat surprising, since it has been reported that the blood sugar decreases considerably in animals given ethionine (41, 42). Liver glycogen is rapidly lost (43) while fatty acid synthesis from acetate is severely compromised (44). Fatty acid oxidation is decreased but only many hours after considerable accumulation of TG has occurred (see 8). The total liver NAD concentration as well as the redox state of NAD, as indicated by the $NAD^+/NADH$ ratio, show little or no change, at least for 5 hours (45–47). In contrast, the total NADP concentration shows a 50 percent decrease within the same time interval and the ratio $NADPH/NADP^+$ decreases significantly. The bulk of the decrease in NADP is in the reduced form (NADPH).

The decrease in liver ATP induced by ethionine is also accompanied by the appearance of acute porphyria (48) and a striking loss of liver $K^+$ and an increase in liver $Na^+$ (49, 50) but has no apparent influence upon hepatic sulfobromophthalein (BSP) uptake, conjugation with glutathionine, or excretion as free or conjugated BSP in the bile (51). Thus, the adenine nucleotide disturbances induced by treatment with ethionine lead to rapid and reproducible metabolic aberrations that form a distinctive pattern. However, it is also clear that a final clear-cut correlation between the presence and degree of alteration in ATP metabolism and the metabolic aberrations seen in the liver of the ethionine-treated animal is not possible because of the complexity of the response pattern and because of the presence of other interactions between the analog and the cell constituents.

*Methionine.*—Unlike the rat, the guinea pig does develop a syndrome with methionine resembling in part that seen with ethionine (52). The liver

ATP concentration shows a fairly rapid decrease with a concomitant increase in S-adenosylmethionine (SAM) (52). However, unlike the rat with ethionine and SAE, the liver of the guinea pig with methionine does not accumulate an amount of SAM equivalent to the decrease in ATP concentration. There does occur a considerable accumulation of S-adenosylhomocysteine (SAH), a metabolite normally found in the guinea pig liver (52). The decrease in ATP level and some other effects such as hypoglycemia and fatty liver are prevented by the administration of adenine along with methionine (52). Also, the nucleolus of the liver cells in the methionine-treated guinea pig shows a rapid disorganization and fragmentation of its components, indistinguishable from that seen in the rat liver after ethionine (24).[1] In both systems, the lesion is readily reversed by the administration of adenine (24)[1]. Thus, there is increasing evidence that at least some of the acute effects of methionine in the guinea pig are related to the drop in ATP concentration.

As pointed out by Hardwick et al (52), the syndromes in the ethionine-treated rat and the methionine-treated guinea pig resemble, at least superficially, methionine intoxication in some humans (53–55) and some patients with hypermethioninemia (56). Conceivably, some part of the human response may be a reflection of an acute hepatic ATP deficiency. It will be interesing to compare the response to methionine of several mammalian species, especially since it appears that another species, the rabbit, may resemble the guinea pig more than it does the rat (57). The clarification of the position of the human vis-a-vis this spectrum may be of great practical importance in the interpretation and treatment of some selected human diseases.

*Fructose.*—The intravenous administration of D-fructose to rats leads to a very rapid fall in liver ATP concentration, followed by a decrease in total adenine nucleotide level and an increase in plasma uric acid and allantoin (58). Protein synthesis, as measured by DL-leucine-1-$^{14}$C incorporation, was severely depressed during the period of low ATP. Blood glucose concentration was also decreased. The major effects of fructose administration lasted only a short while (up to 30 minutes). It is considered likely that the basis for the drop in ATP concentration is the rapid phosphorylation of fructose to fructose-1-phosphate coupled with the much slower splitting of the fructose-1-phosphate into glyceraldehyde and dihydroxyacetone phosphate via aldolase. The latter step appears to be rate limiting (59). The liver cell nuclei appear to show some of the early changes seen with ethionine and with methionine in the guinea pig (60).

The findings with fructose in the rat appear to resemble those seen in some patients with hereditary fructose intolerance (61). It has been suggested that part of the symptomatology may be due to hepatic ATP defi-

[1] H. Shinozuka, personal communication.

ciency. Conceivably, the judicious administration of adenine nucleotide precursors might have beneficial effects in such patients.

*General.*—It is becoming evident that "hepatic ATP deficiency" may be of more general interest than was considered early in the study of ethionine. Certain signs seem to stand out as possible more or less specific markers of such a pathologic process. Among these are fatty liver, depletion of liver glycogen, hypoglycemia, and characteristic changes in liver cell protein synthesis, polysomes, and nucleoli. It may be possible in the foreseeable future that a few selected chemical determinations will become of diagnostic value in indicating the existence of such a cellular disorder in an intact animal or in the human. The quantitative analysis of the response patterns to various combinations of changes in ATP, ADP, and AMP and inorganic phosphate might help to lay a much more basic foundation for our understanding of this type of cellular biochemical disease.



## Inhibition of Protein Synthesis

The analysis of the cellular consequences of inhibition of protein synthesis would be most meaningful in terms of the response to deficiencies of single discrete molecular species of proteins. However, such an approach is not possible in higher organisms at this time except in a very few instances of clear cut genetic deficiency. Therefore, as a first approximation, it is important to delineate the reaction pattern of each type of cell in an intact animal to overall inhibition of protein synthesis.

Numerous alterations in the subcellular organization and function of rat liver parenchymal cells, induced by a wide variety of toxic agents, have been attributed to inhibition of protein synthesis (62). However, the majority of toxic compounds studied have many biochemical effects in addition to inhibition of protein synthesis. Under such circumstances, it becomes difficult to correlate structural changes with a specific biochemical lesion. It is important, therefore, to study known highly specific inhibitors of protein synthesis which act directly at some locus on the protein synthetic apparatus and which hopefully have one or very few biochemical effects.

*Ethionine.*—This compound has been widely used, especially in previous years, as an inhibitor of protein synthesis. Although new insight into some reaction patterns of liver cells to protein synthesis inhibition was derived from these studies, such as the correlation between disturbed protein metabolism and fatty liver (8), the known complexity of the interactions between this analog and liver cell components makes it difficult to derive a clear cut conclusion with this compound. The fact that it inhibits protein synthesis indirectly, probably via the induction of an ATP deficiency, is also an unsatisfactory property of ethionine.

*Puromycin.*—This antibiotic was one of the first compounds shown to act with specificity on protein synthesis (cf. 63). The mechanism of action of puromycin in inhibiting protein synthesis is its substitution of charged transfer RNA at the P site on the ribosome with premature termination of polypeptide chain growth and release of peptides and incomplete proteins containing a terminal puromycin molecule.

Puromycin appears to be a "universal" inhibitor of protein synthesis acting in prokaryotes as well as eukaryotes. In the intact animal, it inhibits protein synthesis in every cell studied. Its administration leads to acute necrosis (cell death) in the pancreas, salivary glands, gastrointestinal tract and lymphoid tissue (64–67) and to selected damage to neurons in the brain (68, 69). In the latter, the organelles showing the most damage are the mitochondria (68, 69). In the pancreas and stomach, the endoplasmic reticulum appears to be the site of obvious maximal damage (66, 67). In the liver, puromycin induces a fatty liver with a periportal distribution not unlike that seen with ethionine (70) (see below).

However, it is extremely doubtful whether many of these lesions, other than fatty liver and possibly lymphoid damage, are due to inhibition of protein synthesis per se. Almost complete inhibition of protein synthesis by other agents such as cycloheximide (see below) does not lead to the same cellular alterations, and a form of cycloheximide, acetoxycycloheximide, is quite effective in decreasing or preventing the cellular changes induced in the brain by puromycin (69). It is therefore likely that many of the lesions induced by puromycin are related not to the inhibition of protein synthesis but to the released peptides and the possible damage of such potent pharmacologic agents (65, 69). The possible role of inhibition of cyclic-3′-5′-AMP phosphodiesterase and the concomitant increase in tissue concentration of the cyclic AMP (71, 72) also remains to be studied. A valuable control for studies with puromycin is the use of puromycin aminonucleoside, which has no inhibitory effect on protein synthesis.

*Cycloheximide.*—This compound is another highly specific inhibitor of protein synthesis. However, unlike puromycin, cycloheximide acts only in eukaryotic cells and not in prokaryotes, and even in the former, it inhibits general cytoplasmic ribosomal but not mitochondrial protein synthesis (see 73). Recent evidence strongly suggests that cycloheximide, in the usual low dosage used in vivo, acts almost exclusively as an inhibitor of peptide chain termination or release (74). Almost complete inhibition of protein synthesis in the rat for many hours leads to only a few cellular changes in selected organs (75, 76).

*Tenuazonic acid.*—This tetramic acid derivative is another inhibitor of protein synthesis which acts in intact animals (77). Although studied much

less extensively than other inhibitors, it appears that the cellular response to this agent in rats is similar to that with cycloheximide.[2]

Although the study of the response of different cells to inhibition of protein synthesis is very young, it appears from this vantage point that a certain pattern begins to emerge. This relates to lymphoid and related tissues, the cell cycle of proliferating cells and fatty liver.

*Lymphoid and related tissue.*—The lymphoid tissue in lymph nodes, Peyer's patches of the intestine, and the spleen show extensive cell death within two to three hours after the injection of cycloheximide (75) or some other inhibitors of protein synthesis (78). The cells affected are in the germinal centers. The small lymphocytes seem to be unaffected. With one dose, the lymphoid tissue recovers within 24 hours, in contrast to the persistence of irreversible cell damage with alkylating agents or X-irradiation (78). The exact nature of the cells damaged with inhibitors of protein synthesis and the relation of such cell loss to antibody synthesis and to so-called "immunosuppression" awaits clarification.

*Interruption of cell cycle.*—The administration of cycloheximide (79, 80) or tenuazionic acid[2] causes the complete disappearance of mitoses in intestinal crypt epithelium, regenerating liver, and other somatic tissues or organs. As anticipated from in vitro studies, inhibition of protein synthesis in vivo inhibits the cell cycle at various points late in G-1, during the S-phase and very early in G-2 but not in late G-2 or in M. The cells beyond the early G-2 block continue to progress through G-2 and mitosis, thus accounting for the virtual complete disappearance of cells in mitosis. The S-phase block may be due to well-known inhibition of DNA synthesis that accompanies inhibition of protein synthesis in all proliferating cells, both prokaryotes and eukaryotes. Recently, it has been found that a degree of inhibition less than 80 to 85 percent has no perceptible influence on the progression of cells through the G-2 and M phases.[2] This system may now become useful in studying the isolation and nature of the protein or proteins that appear to be essential for progression of the cells through G-2 and M. The possible importance of such a study for cell synchronization in vivo in cancer chemotherapy is evident. In fact, recent work in the intact animal has already led to a synchronization of proliferating cells greater than 75 to 80 percent.[2]

*Fatty liver.*—Inhibition of protein synthesis appears to be an important biochemical lesion for several types of fatty liver—e.g. $CCl_4$, ethionine, puromycin (8, 81, 82). The liver plays a major role in the conversion of free

[2] R. S. Verbin and E. Farber, unpublished results.

fatty acids from the blood to TG which is put back into the blood as plasma lipoproteins. The real biological significance of this physiologic function is not yet completely clear. For this lipoprotein output, a rapidly turning-over protein, phospholipid, cholesterol, and perhaps polysaccharide as well as TG are required. The inhibition of synthesis of protein (or of other components such as phospholipid in choline deficiency) cuts off the supply of the essential protein with a concomitant accumulation in liver TG. Apparently, no feedback inhibition of TG synthesis occurs under such conditions, such as has been found for cholesterol (83) and for some of the carbohydrate moieties of plasma glycoproteins (84).

Recent work with cycloheximide (85) suggests that the picture may be more complex than just outlined. Despite a high degree of inhibition of protein synthesis (over 95%) for many hours, only slight increases in liver TG are seen with this inhibitor. Also, the male rat given ethionine shows considerable inhibition of protein synthesis in vivo without a large increment in TG concentration such as seen in the female with ethionine (86–88). These puzzling features remain to be explained. One aspect of importance is the supply of free fatty acids in the liver. As has been emphasized by Lombardi (81, 82), the level of TG in the liver at any one time is dependent not only upon the rate of output as lipoprotein but also on the rate of supply and uptake of the precursor fatty acids. Another feature that may be of significance is the state of aggregation of the ribosomes. Normally, the liver under resting physiologic conditions contains the bulk of the ribosomes as polysomes. With ethionine and puromycin, the equilibrium is markedly shifted toward ribosome monomers and subunits. With $CCl_4$, the ribosomes also accumulate as polysome precursors. In contrast, with cycloheximide, and in the male rat treated with ethionine, the inhibition of protein synthesis is not associated with any major shift toward polysome precursors (88, 89). The bulk of the TG in the liver is synthesized in the endoplasmic reticulum, a structure also associated with the majority of the liver polysomes. Is it possible that the maintenance of intact polysomes in close association with the endoplasmic reticulum may allow a regulatory control of TG synthesis which is lost when the polysome is no longer *in situ* on the membrane? The absence of significant TG accumulation in the liver of animals treated with actinomycin D could conceivably fall into the same class, since recent work has shown that this antibiotic affects only free and not bound polysomes (90).

## Inhibition of RNA Synthesis

The problems mentioned above concerning inhibition of protein synthesis are equally applicable to the analysis and interpretation of response patterns to interference with RNA synthesis. Ultimately this will have to be understood in terms of specific molecular species of RNA, of which there must be thousands. Presumably, each peptide chain or protein has a unique messenger RNA. In addition, there are at least three main types of ribosomal RNA (28S, 18S, 5S) and perhaps 50 to 60 tRNA's. Also, there is evi-

dence that the nucleus may contain many species which never reach the cytoplasm and perhaps are degraded soon after synthesis (see 91, 92). However, despite these complexities, limitations, and reservations, it still becomes important to study the basic response patterns of different cells to inhibition of synthesis of classes or groups of RNA molecules.

Of the numerous agents that interfere with RNA synthesis, many are as effective as inhibitors of DNA synthesis as they are for RNA synthesis. For proliferating cells, this offers great difficulty in the interpretation of the results, since inhibition of DNA synthesis by itself leads to profound changes (see below). This discussion, therefore, is limited to those agents which appear to be more or less specific for RNA synthesis or are active in resting as well as proliferating cells.

*Actinomycin D.*—This is by far the most specific as well as the most important of the known compounds. Although hundreds of studies of diverse kinds have used this compound, there is still little evidence that it has any major effects on cells in the intact organism other than through its selective interaction with DNA. It appears to combine predominantly or almost exclusively with the guanine of DNA (93) and possibly with that cellular DNA that is currently active in RNA synthesis (e.g. 94). This antibiotic shows a progressive spectrum of action on RNA related to dosage. At low doses, actinomycin D has virtually a selective effect on ribosomal RNA synthesis and on the labeling of the nucleolus by precursors of RNA (95). This may be related, at least in part, to the high cytosine or guanine plus cytosine content of ribosomal RNA. As the dose is increased, more and more species of RNA are affected until with high doses (1 and 2 mg/Kg. body weight) over 95 percent of RNA synthesis is affected.

*Alpha-amanitin.*—This is one of a group of toxic compounds found in the true poisonous mushrooms *Amanita phalloides* called amatoxins (96). Another group of toxins from the same organism is the phallatoxins. Both groups are complex cyclic peptides (96). One of the most interesting from the point of view of toxicology and biochemical pathology is $\alpha$-amanitin, a cyclic octopeptide. $\alpha$-Amanitin induces a variety of severe lesions in several organs (kidney, liver, etc.) in experimental animals. In the mouse, it induces cell death (necrosis) in liver and kidney. However, in the rat, it has reversible effects on the liver. It selectively inhibits the RNA polymerase activated by $Mn^{++}$ and ammonium sulfate (97) both in vitro and in vivo in the rat and mouse without any apparent binding or interaction with DNA (98).

*Ethionine.*—Both male and female rats given ethionine show a rapid and striking inhibition of RNA synthesis in the liver (25, 88). It appears that this is related to the drop in ATP concentration rather than to other effects of ethionine, since adenine, inosine, 5-amino-4-imidazolecarboxamide can prevent or reverse the inhibition essentially completely without any decrease in the concentration of SAE.

*Overall effects.*—The only specific inhibitor of RNA synthesis so far studied in any systematic way, which acts on virtually all mammalian cells is actinomycin D. The most extensive study has been reported by Schwartz et al (99, 100). Cell death in lymphoid tissues, bone marrow, and intestinal crypt epithelium is a rapid response (a few hours). The changes in the lymphoid tissue and bone marrow are reflected in lymphocytopenia and thrombocytopenia. Commonly neutrophilic granulocytosis also occurs. The cell death leads to gross involution of lymph nodes, thymus, and spleen. The crypt cell damage leads to atrophy of the crypts with nuclear atypia and swollen cells (101–103). With sublethal doses, the epithelial cells recover by 4 days. The intact and regenerating liver shows some minimal cytoplasmic loss of basophilic bodies and nuclear changes (see below), along with evident decrease in the number of mitoses (100). However, unlike the crypt cells, no significant degree of irreversible hepatic cell damage is observed.

The overall cellular reactions to actinomycin are by no means confined to proliferating cells. Sensory neurons of the posterior spinal ganglia (99), and all the salivary glands and the exorbital lacrimal glands (99, 104–106) are severely damaged. The neurons and the acinar cells show extensive cell death with inflammatory infiltration. In the nervous system, this leads to myelin degeneration in the dorsal roots and peripheral nerves. The ventral roots remain unaffected.

The metabolic basis for all of the above lesions is still not understood. Since actinomycin D has such a specific effect on DNA and on RNA synthesis, it is presumed that the cellular responses to this antibiotic are related to the inhibition of RNA synthesis. However, whether they are the direct consequence or remote consequence of such biochemical lesions is not known.

*Nuclear alterations.*—The cell organelles most extensively studied in regard to disturbances in RNA metabolism are the nuclei, especially the nucleoli and the cytoplasmic ribosomes.

From the relatively large number of studies that have been done on the response of many different cells to a variety of agents (physical, chemical, and viral—see 107–109), it is becoming apparent that there are at least two general response patterns to inhibition of RNA synthesis—(*a*) a rapid separation and dissociation of the normal nucleolar components (fibrils, granules, chromatin) leading to an organelle that has its three major component parts virtually completely segregated (Figures 2 and 3); (*b*) a progressive fragmentation of the nucleolus with apparent scattering of "pieces" of the nucleolus in the nucleus (Figure 1).

(*a*) Nuclear segregation is associated classically with actinomycin (110, 111) (Figures 2 and 3) although it was first clearly described with another compound that combines with DNA, 4-nitroquinoline-N-oxide (112). A similar effect is seen with many different compounds that combine with DNA and presumably inhibit RNA synthesis through this mechanism. Some typical other examples are aflatoxin (108, 113), proflavin (114, 115), daunomycin (108), ethidium bromide (108), nogalamycin (107, 108), and pyr-

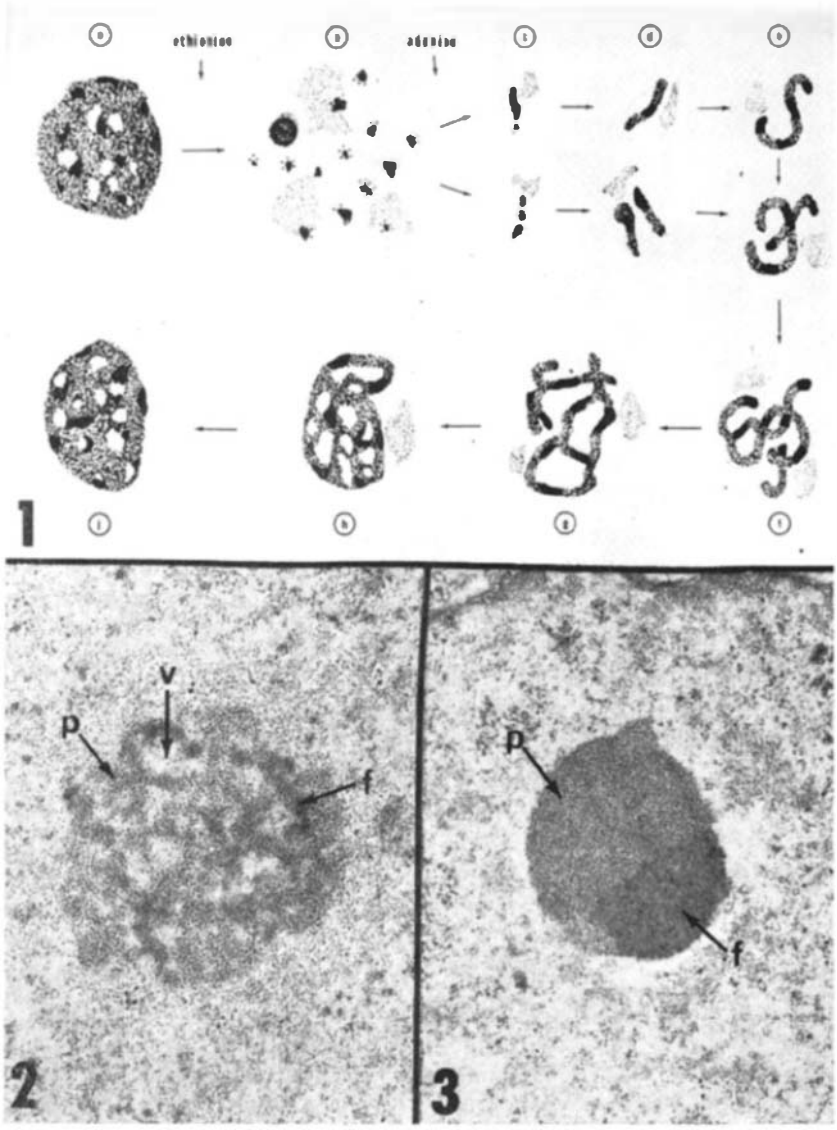


FIG. 1. Schematic illustration of the sequential changes of the nucleolar reformation after adenine administration. a, normal nucleolus. b, disorganization and fragmentation of nucleolus after the ethionine injection. c-h, various structural forms encountered two hours after adenine administration. i, structure close to normal nucleolus encountered 4 hr after adenine administration (24). Reproduced by courtesy of the *Journal of Cell Biology*.

FIG. 2. Hepatic cell nucleolus of control rat. Particulate (P) and fibrillar (F) components are intermingled and are arranged in skein-like pattern enveloping small nucleolar vacuoles (V). ×17,000. (Courtesy of Dr. Shinozuka)

FIG. 3. Typical nucleolar segregation observed in the hepatic cell nucleolus of rat 2 hours after the administration of antinomycin D (1 μg/gm body weight). With the disappearance of nucleolar vacuoles nucleolus becomes compact. Particulate (P) and fibrillar (F) components are separated into two distant zones. ×18,000. (Courtesy of Dr. Shinozuka)

rolizidine alkaloids (116). It is also seen with physical agents such as ultraviolet irradiation and several different viruses and mycoplasma (107–109).

(*b*) Nucleolar fragmentation is seen with ethionine (24, 117), α-amanatin (118, 119), methionine (in the guinea pig) (H. Shinozuka, personal communication), and perhaps azaserine in part (108).

With neither pattern is the intimate biochemical mechanism of nucleolar alteration understood. However, it appears that the reaction of the cell is related more to the mechanisms of inhibition of RNA rather than to the inhibition per se (108, 109). With both groups of compounds the maximum degree of inhibition of RNA synthesis is often the same—sometimes well over 90 to 95 percent. With such extensive inhibition, it is doubtful whether there is much difference in the spectrum of RNA moieties affected (120).

The first group of compounds, those inducing segregation of dissociation, have been shown to react directly with DNA. Of these, actinomycin D is by far the best understood. It is now known that this antibiotic has at least four metabolic effects in eukaryotic cells including liver or other organs in the intact organism: (*i*) noncovalent binding to some guanine moieties of DNA (93), (*ii*) inhibition of RNA synthesis (93), (*iii*) rapid acceleration of breakdown of some fraction of recently synthesized nuclear RNA (120–124), and (*iv*) interruption of the normal transfer of ribosomal and transfer RNA from the nucleus to the cytoplasm (120, 121, 125). The latter three are probably secondary to the first. It is also probable that the interference with nuclear-cytoplasmic flow is not an important factor in the genesis of the nucleolar reaction. The available evidence from comparative studies with actinomycin and ethionine supports the hypothesis that nucleolar segregation is a reflection of the ability of the agent to bind to DNA and possibly to trigger the breakdown of some moiety of nuclear RNA (45S?) (126) as a consequence (109, 124). It does not favor the hypothesis that inhibition of RNA synthesis by itself or the interruption of transfer are related (127). This tentative conclusion is consistent with the results of studies on aflatoxin (128–132), 4-nitroquinoline-N-oxide (133–136), proflavin, known to react directly with DNA through intercalation (137), nogalamycin, which binds to adenine and thymine of DNA (138), and many other compounds (107, 108) all of which bind to DNA. In the case of aflatoxin, inhibition of RNA synthesis (128, 131) and RNA polymerase (130) and a rapid loss of nuclear RNA (132) have been reported.

It is conceivable that DNA in the nucleolus plays a central role in RNA metabolism not only in its template function but also in regulating the further metabolism and transformation of certain molecular species (120). It could "protect" the newly-synthesized RNA from attack by contiguous nucleases until suitably packaged by further metabolism and combination with other constituents such as protein (120). Alternatively, it might play an important role in controlling the activity of ribonuclease (120). In either case, compounds which bind directly with DNA could disrupt these hypothetical functions of DNA and thereby lead to the disorganization of the nucleolus.

The second group of compounds, those producing fragmentation of the nucleolus, appears to inhibit RNA synthesis more directly and not via a primary attack on DNA as template. The best known of these is ethionine which appears to act on RNA synthesis, not through DNA but by other as yet unknown mechanisms (120). Ethionine has been reported to inhibit RNA polymerases (139). It would appear that both RNA polymerases (140, 141) are affected (139). Alpha-amanitin has been found to be a strong inhibitor of RNA polymerase II, the polymerase located in the nucleoplasm, but does not affect the nucleolus-associated enzyme, polymerase I (140–143). Since this interesting peptide does not induce cell death in liver in the rat, it purports to become one of the most specific and useful compounds in the analysis of RNA metabolism and its disturbances in disease. Another potentially useful compound in this area is toyocamycin, an antibiotic which appears to have a highly selective inhibitory effect on the normal transformation of 45S nuclear RNA to ribosomal RNA, presumably by being incorporated into the 45S moiety (144, 145). This compound induces a fragmentation of the nucleolus in myeloblasts from chickens with myeloblastic leukemia, not unlike that seen in the liver with ethionine (146). Another interesting compound which appears to have similar effects on nucleoli in cells in culture is adenosine (147, 148). Thus, several different drugs or toxic compounds and two normal constituents of mammalian cells, adenosine and methionine (in the guinea pig), all of which inhibit RNA synthesis but by mechanisms other than through a primary effect on DNA, cause nucleolar fragmentation of a similar kind. The discovery of the common denominator in all of these compounds may well give new insight into how a vital cell organelle, the nucleolus, is organized for its function in ribosome biogenesis. There is one other aspect of this which should be pointed out. With ethionine, methionine, or $\alpha$-amanitin, the nucleolar changes have been shown to be reversible. With ethionine or methionine, this is readily induced by reversing the ATP deficiency. A unique series of transformations have been seen which appear to represent the way in which the nucleolus is packaged from simpler precursors (24) (Figure 1). This offers an interesting model for the study of the genesis of this organelle in a resting cell, especially since it apparently does not require new protein synthesis but does require RNA (149).

*Cytoplasmic ribosomes.*—The effects of the various inhibitors of RNA synthesis on cytoplasmic polysomes, ribosomes, and protein synthesis is still poorly understood. Again, the most straightforward appears to be actinomycin D. This antibiotic induces a slow progressive inhibition of protein synthesis in the liver accompanied by a disaggregation of polysomes (150–153). Recent results have shown that only the free polysomes are affected within a 12 hour period (90). The effects of aflatoxin on liver cells are much more complex than are those of actinomycin since aflatoxin induces necrosis while actinomycin D does not (109, 153).

## Inhibition of DNA Synthesis and Cell Death

It has generally been considered that interference with DNA synthesis or metabolism in proliferating cells leads to death of the affected cell (154). This has been one of the major guiding principles in the design of chemical agents for cancer chemotherapy. In the same general context, it is frequently assumed that interruption of the cell cycle by whatever means is also an effective way to compromise the viability of proliferating cells.

Recent results on the cellular response to inhibitors of protein and DNA metabolism raise doubts concerning the validity and adequacy of this concept (78, 155). As already discussed above, interruption of the cell cycle of regenerating liver and crypt epithelial cells by inhibitors of protein synthesis produces no apparent effect on cell viability (79, 80). Also, inhibition of protein synthesis is consistently accompanied by inhibition of DNA synthesis in virtually all prokaryotes and eukaryotes studied. Yet inhibitors of protein synthesis, such as cycloheximide and tenuazonic acid, do not lead to cell death in many epithelial cells such as crypt epithelial cells of the intestine which are sensitive to inhibitors of DNA synthesis such as 1-$\beta$-D-arabinofuranosylcytosine ("cytosine arabinoside", ara-C) or to alkylating agents or X-irradiation which have as known major targets DNA. This apparent contradiction led to experiments on the effects of inhibitors of protein synthesis, such as cycloheximide and tenuazonic acid on cell damage induced by ara-C, nitrogen mustard (HN2), or X-irradiation (78). The results clearly show that inhibition of protein synthesis of about 75 percent or more (but not less) will selectively prevent cell damage of intestinal crypt cells induced by one of these three methods and that the inhibitor can be given even after the maximum initial biochemical lesion has occurred (up to 30 or 45 minutes) and still be effective. It thus appears that, at least insofar as the crypt epithelial cells are concerned, protein synthesis, perhaps as enzyme induction, may be necessary for the cell damage induced by interference with DNA metabolism. The nature of the protein(s) required and of the possible coupling between disturbances in DNA metabolism and protein synthesis seem to be potentially important areas for further exploration of this phenomenon.

These findings in the intact animal seem to support, in principle, the concept of unbalanced growth or thymineless death first presented by Cohen & Barner (156, 157) in bacteria and subsequently studied also in eukaryotic cells in vitro by several investigators (e.g. 158, see 78). These studies indicate that protein synthesis and possibly also RNA synthesis play important roles in the response of various types of cells to perturbations in DNA metabolism associated with loss of viability.

*Cell specificity.*—It must be emphasized that the response of cells to interference with DNA synthesis or metabolism is very much a function of the type of cell studied (see 78, 155). Lymphoid tissue cells (in germinal

centers) stand at one end of the spectrum in that they are rapidly killed by inhibitors of the synthesis of protein, RNA, or DNA. Cells programmed for only occasional (reparative?) proliferation, such as liver after partial hepatectomy, salivary glands after isoproterenol administration (159, 160), or the kidney after the injection of folic acid (161), are at the other end of the spectrum in that inhibitors of DNA synthesis as well as of RNA and protein, alkylating agents, or irradiation have no obvious acute damaging effect (see 78, 155). Cells that are continuously dividing under the usual range of physiological activity are in the middle of the spectrum in that they are rapidly damaged by inhibitors of DNA synthesis, alkylating agents, or irradiation (78) but not by inhibitors of protein synthesis.

These findings seem to point to the conclusion that the effect of an agent inducing rapid damage to some cells via a presumed action on DNA metabolism is as much a function of the metabolic organization of the cell as it is of the nature of the initial biochemical lesion. If this conclusion proves valid, it becomes obvious that a detailed knowledge of the steps between the initial biochemical effect and the final death of the cell might open up an opportunity for the development of new approaches in the chemotherapy of cancer.

## BIOCHEMICAL ANALYSIS OF BIOLOGICAL EFFECTS OF TOXIC AGENTS

The first part of this review was concerned with the analysis of how different cells respond to a few selected biochemical lesions. This second part will concern itself with the inverse—the analysis of the biochemical basis for a biological effect as a part of the action of a toxic compound. Needless to say, both approaches are essential for an eventual cellular understanding of pathology and toxicology and should ultimately give results which overlap to such a degree as to lay a firm foundation for new insights into mechanisms of disease and their control.

### Carbon Tetrachloride

This agent has been selected for review because it still commands an unusual degree of interest on the part of pharmacologists and pathologists and because the "life history" of its study offers so many lessons in the cell biology of disease. Since this compound was reviewed last in Annual Review of Pharmacology in 1963 (162), considerable progress has been made in the analysis of the pathogenesis of lesions induced in experimental animals. This progress is of two kinds—negative and positive. An extensive review (164) and some shorter critical reviews (163, 165–169) have appeared during the past several years. Therefore, only a brief discussion of some key problems and some critical comments seem justified at this time.

On the negative side, it is now quite clear that the sympathetic nervous system plays at most only a minor role in the genesis of liver cell death and fatty liver induced by $CCl_4$. The marked effect attributed to the nervous

system seems to be related in large measure to the progressive decrease in body temperature (168). Reversal of this, simply by warming the animal, restores the hepatotoxicity of $CCl_4$. Similarly, the role of central ischemia in the generation of necrosis has received anything but support from the work of Stoner (see 169) on measurement of oxygen tension and of Brauer (170) on the isolated perfused liver. In addition, there is little support (164, 166, 171–174) for the hypothesis (175) that solution in essential lipid components of cells is the major mechanism of $CCl_4$ hepatotoxicity. Also, the primary lysosomal hypothesis does not appear to hold up to continual scrutiny (see 164–166).

On the positive side, there is increasing evidence that $CCl_4$ must first be metabolically converted to an active derivative, possibly a free radical (see 164, 166), before it initiates its damaging effects. The interaction of $CCl_4$ with the liver is a complex one and appears to consist of at least three major steps—(*a*) destruction of important constituents of microsomal membranes such as cytochrome P450 (171, 176), (*b*) activation to metabolically potent derivative(s), and (*c*) subsequent damage to endoplasmic reticulum and other cell constituents. The evidence at present favors the following time sequence—(*b*), then (*a*), then (*c*). According to this view, resistance to $CCl_4$ of the newborn rat (177), of animals treated with $\beta$-diethylaminoethyl diphenylpropylacetate (SKF-525-A) (178), of animals fed low protein or protein-free diets (179), and reversal of the latter protective effects by 1,1,1-trichloro-2, 2-bis-(p-chlorophenyl) ethane (DDT) or phenobarbital (179) are best explained by the large changes in the activities of key microsomal enzymes associated with each of these conditions (180). Thus, it appears that certain, as yet unknown, enzymic components of the microsomal mixed-function oxidases convert $CCl_4$ to an active toxic agent which in turn rapidly destroys some of the components of the same system, such as cytochrome P-450 (171, 176), and also initiates a destructive effect on the endoplasmic reticulum and the cytoplasmic ribosomal protein synthetic system (164, 166). Many of these latter effects can be diminished or prevented by the use of a variety of antioxidants (e.g. 181, see also 164, 166). The effect of the active metabolite on microsomal enzyme activities is quite specific, in that other constituents of the same organelle, such as NADP-cytochrome C reductase, cytochrome $b_5$, or microsomal neotetrazolium reductase are unaffected (171, 176, 182).

The essential validity of this view also appears to apply to the toxicity of $CS_2$. Conversion to an active metabolite, destruction of certain key components in the microsomal enzyme complex, and the induction of liver cell necrosis with the combined use of phenobarbital and $CS_2$ but not with $CS_2$ alone seem to parallel in general the experience with $CCl_4$ (183, 184).

An interesting byproduct of these studies is the development of a new model for cirrhosis of the liver which should prove useful (185). Rapid and highly reproducible liver cirrhosis is easily induced by the use of phenobarbital along with $CCl_4$.

A critical phase of these studies now becomes the mechanism of the induction of fatty liver and necrosis. A primary prerequisite is the identification of the form or forms of the active metabolite(s). In addition, methods must be found for the dissection of the number of steps in each sequence leading to the gross end results. It is already clear that at least some dissection of this is now possible. For example, (*a*) antioxidants protect against both fatty liver and necrosis and yet do not appear to diminish the destructive effect on cytochrome P-450 (171), (*b*) phenergan and other antihistamines protect to a considerable degree against the necrosis but not against the fatty liver induced by $CCl_4$ (e.g. 186), and (*c*) cycloheximide protects completely against the disaggregation of polysomes and inhibition of protein synthesis in $CCl_4$-treated animals (187)[3] but has no effect on the membrane changes, the labeling of liver lipid and protein with $^{14}CCl_4$, or necrosis.[3]

One of the suggested possible key reactions in the sequence is lipid peroxidation (164, 166, 167). This occurs rapidly and regularly. However, whether it can account for the total picture in the hepatotoxicity of $CCl_4$ remains to be established (166, 180).

*Cell death and necrosis.*—At the heart of the problem of the toxicity of $CCl_4$ in experimental animals is the question "what do you have to do to a cell, metabolically, to kill it?" As recently pointed out by Judah (163), a pioneer in the field of biochemical pathology and $CCl_4$ hepatotoxicity, we now are acquiring considerable knowledge which tells what one can do to a cell metabolically and *not* kill it. For example, marked inhibition of protein and RNA synthesis, severe depression in ATP and adenine nucleotide concentrations, large shifts in $K^+$ and $Na^+$, to name but a few, all disturb the cell in various ways but do not lead to cell death in most cells, even when they persist for 24 hours or longer. The further pursuit of this important phase of $CCl_4$ hepatotoxicity might lead to new clues to the solution of this important problem.

## Lathyrogens and Connective Tissue

A fascinating example of the biochemical analysis of the pathogenesis of a toxic reaction concerns the osteolathyrogens and their effects on bone and connective tissue. This study was initially undertaken at the University of Wisconsin in the early 1930s in the attempt to isolate the toxic factor in the chick pea, *lathyrus sativus* which induces disease of the nervous system in humans who resort to eating this in times of starvation. A factor was isolated from the related sweet pea, *lathyrus odoratus* which induces severe and crippling disease of the bones and connective tissue in growing animals (see 188 for brief historical review). A crystalline substance was subsequently isolated in 1954 that reproduced the disease. This material proved to be $\beta$-(N-$\gamma$-L-glutamyl)-aminopropionitrile (189). It was soon shown by several investigators that the toxic properties resided in the $\beta$-aminopro-

[3] E. Farber, H. Liang and H. Shinozuka, unpublished results.

pionitrile (BAPN) portion of the molecule (see 188). Studies in the chick embryo with BAPN showed no change in total collagen content but a large increase in cold saline soluble collagen (190). All tissues showed the same phenomenon. Normally, collagen consists of three supercoiled peptide chains with cross linking. Piez and coworkers found that collagen from lathyrogen-treated animals consisted of immature $\alpha$-chains and suggested that BAPN somehow inhibited the intramolecular and intermolecular cross linking in collagen (191, 192).

Other nitriles such as aminoacetonitrile were also found to be active lathyrogens. The nitrile group was essential and this led to the finding that many other classes of carbonyl blocking agents are also effective. They could be arranged in a spectrum of activities—nitriles as the most potent, then ureides, hydrazides, and hydrazines (188, 193). These observations suggested the hypothesis that the lathyrogens were active by blocking carbonyl groups, postulated to play an essential role in normal polymerization of collagen. This prediction has subsequently proved to be valid for collagen, mainly through the work of Piez and Gallop and their respective coworkers (see 188 for references). Collagen does possess both free aldehydes and aldehydes bound stably in aldol condensation and less stably in Schiff bases. Elastin also has been found to possess two new amino acids, desmosine and isodesmosine, derived from lysine aldehydes (194). The collagen isolated from lathyrogen-treated animals is deficient in aldehydes. Whether BAPN and other lathyrogens act only on the aldehydes of developing collagen and elastin or on the enzymatic generation of cross-links or by both mechanisms remains an interesting phase of the problem.

It appears that there are many similarities between the effects of lathyrogens, copper deficiency, and pyridoxine deficiency on collagen and elastin (see 188). The elucidation of the biochemical pathology of these diseases and their interrelationships may well give exciting new insights into the structure and function of connective tissue. Conceivably, such insight might even lead to a rational control of fibrosis in many disease states.

## TRIETHYLTIN

It is widely appreciated that drugs and toxic chemicals have played a major role in the study of respiration and oxidative phosphorylation. Since many of the known components of these metabolic systems are in mitochondrial membranes, it has not been easy to dissect them apart by isolation and purification procedures so successful for more soluble enzymes.

A toxic agent which is proving to be interesting and useful for the analysis of oxidative phosphorylation is triethyltin. This metabolically stable compound induces extensive cerebral edema in the white matter of the brain and spinal cord, associated with progressive weakness, paralysis, and convulsions (195–197). The edema appears to be intracellular in glial cells (197).

Triethyltin has a marked inhibitory effect on the coupling between mitochondrial respiration and phosphorylation in the brain and in other organs (198). It binds in a highly selective manner to mitochondria by two classes of binding (199, 200). The one with the highest affinity, probably located in the inner mitochondrial membrane, is related to the effects on oxidative phosphorylation. These binding sites are almost certainly histidines. A quantitative analysis of the binding in relation to metabolic effects has suggested a new orientation of the organization of the major mitochondrial energy generating system which accounts for many of the known properties of oxidative phosphorylation including the mechanism of action of uncouplers (199). This hypothesis, derived from the mechanism of action of a toxic agent, offers new approaches to the understanding of ATP generation in mitochondria coupled to respiration.



## General Considerations

The cellular analysis of the toxic manifestations of drugs and chemicals is now becoming possible because of the growth of knowledge in biochemistry and cell biology. Even from the few areas covered in this review, it is evident that this field offers one of the new challenges in pathology and pharmacology. An important prerequisite for the growth of this field is the development of models that have the properties necessary for this type of analysis. It is already clear that the identification of the primary biochemical lesion, although essential, is insufficient by itself if we are to understand how the living cell responds to a toxic chemical. Cells, like whole organisms, have acquired in their evolutionary history, ways to respond to and to counteract perturbations in their homeostases. The elucidation of the nature and mechanism of these response patterns and the orientation of thinking along such lines in cell biology could lay a new foundation for a more dynamic understanding of how a toxic agent evokes the manifestations it does in an intact organism. This knowledge in turn would almost certainly have a major impact on our concepts of disease in general.

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