μ can be estimated from Eq. 1 and 2. It is found that $\mu \leqq 10^{10}$ dyne/cm^2, which is a reasonable limit for a protein crystal.

When a structural element adds to the lock washer, as in Fig. 2, the configuration is stabilized by the amount of the binding energy. The stability continues to increase significantly until the length of the rod approximates the rod diameter. In the presence of the nucleic acid core, the stability would be further increased.

Qualitatively, the binding energy at the edges must be sufficient to give the screw-dislocated configuration a sufficient life-time to stabilize itself by further growth. Since the crystal ring with an edge dislocation offers no easy growth mechanism, it is only temporarily stable at a *p*H of 5, where tobacco mosaic virus rods grow.

If it is postulated that the dimensions of the structural elements are a function of *p*H, the formation of closed disks at a *p*H of 6 becomes understandable. If the closure gap in a single ring (Fig. 1, top) decreased, the elastic energy of forming an edge-dislocated structure would decrease as the square of the closure distance. As the gap decreased in width, or the circumferential dimension of the structural element increased, the strain energy would become unimportant with respect to bonding energy at the edges. The lifetime of the edge-dislocated structure would become very long, and almost all crystals should form as disks.

The characteristic rodlike morphology becomes a necessary consequence of the physical laws governing the growth behavior. It is of interest to proceed one step further—that is, to obtain a reasonable model by which the reproduction of tobacco mosaic virus rods in living plant cells can be accounted for by purely physical laws.

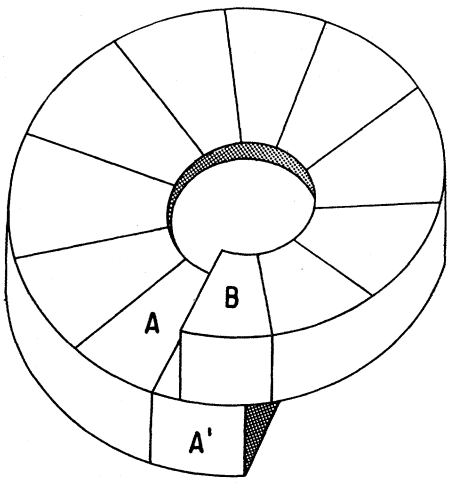

Fig. 2. Temporarily stabilized screw dislocation.

It is emphasized that this model is based on a simple and reasonable hypothesis, but that direct evidence as to its validity is unavailable.

It is well known that chemical reactions may either proceed heterogeneously or homogeneously. Recent studies of whisker growth (*6, 7*) have further clarified the role of surface structures in chemical reactions. It has been shown that aluminum oxide (*7*) can form from the reaction of water and a suboxide on a perfect surface of an alumina crystal but not in the vapor phase. On the other hand, copper (*6*) can form from the hydrogen reduction of cuprous iodide only at growth steps on a copper surface. It seems a fair extrapolation to suggest that in many organic reactions a preferential yield of a desired product may be obtained only in the presence of the crystalline product phase.

To account for the synthesis of tobacco mosaic virus in plant tissue, it is postulated that native tobacco mosaic virus elements are synthesized from simpler molecules only at the growth steps of the original rods. If the new structural units are synthesized faster than the core is built in, the solution becomes saturated with respect to the unstabilized tobacco mosaic virus rod phase. Since this solution would be supersaturated with respect to nucleic acid-stabilized rods, it is proposed that nucleation of new stabilized rods could occur at the available supersaturation.

Since tobacco mosaic virus particles are only synthesized in plant cells in the presence of infecting particles, it must be concluded that the structural elements are not synthesized homogeneously in the cell solution. The reproduction process must involve nucleation. Nucleation requires a finite critical supersaturation of the cell solution with respect to nucleating phase. The production of the critically supersaturated solution must be thermodynamically possible. Kinetically this reaction only occurs in the presence of infecting particles. Thus, it does not occur homogeneously.

A detailed mechanism (*8*) has been described for the growth of tobacco mosaic virus particles involving the screw dislocation growth mechanism. A reasonable mechanism has been offered to account for the reproduction of tobacco mosaic virus particles in living plant cells. These mechanisms are based on the physical chemical behavior of strictly nonliving substances.

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Induced Synthesis of Liver Microsomal Enzymes Which Metabolize Foreign Compounds

Abstract. The administration of 3,4-benzpyrene to rats markedly increases the activities of certain liver microsomal enzymes which metabolize foreign compounds. Evidence based on studies of enzyme induction is presented which suggests the presence in liver microsomes of several enzymes which can catalyze the same type of reaction.

Previous studies have shown that a variety of polycyclic aromatic hydrocarbons induce the synthesis of certain liver microsomal enzymes by a mechanism not involving the adrenal gland, the pituitary gland, or the hormone testosterone (*1,2*). For instance, the intraperitoneal injection of rats with 3,4-benzpyrene (BP), 3-methylcholanthrene (MC), or 1,2,5,6-dibenzanthracene (DBA) induces marked increases within 24 hours in the activities of the hepatic microsomal enzymes which reduce the azo linkage and N-demethylate aminoazo dyes (*1*), hydroxylate 3,4-benzpyrene (*2*), or N-2-fluorenylacetamide (*3*) and conjugate *o*-aminophenol as the glucuronide (*4*). These increases in enzyme activity are paralleled by marked increases in total liver protein (*1*). The purpose of the study reported here (*5*) was to determine the effect of 3,4-benzpyrene on various other liver microsomal enzymes which metabolize foreign compounds.

The results shown in Table 1 are typical of four similar experiments in each of which pooled livers from 10 to 30 animals were used. The intraperitoneal administration of 1 mg of BP to weanling rats resulted in increased activity of a variety of enzymes within 24 hours. The hydroxylation of 3,4-

Table 1. Effect of 3,4-benzpyrene on the metabolism of foreign compounds by liver microsomes. Holtzman rats (40 to 50 gm) were maintained on a synthetic diet high in all the known vitamins (*1*) and were injected intraperitoneally with 0.25 ml of corn oil or 1 mg of 3,4-benzpyrene in corn oil. The animals (30 in each group) were decapitated 24 hours after administration of benzpyrene. The livers were removed at a temperature of 2°C and pooled, and 33 percent liver homogenate was made in 1.15 percent potassium chloride solution. The homogenate was centrifuged, and all enzyme assays were carried out on the same 9000 *g* supernatant (20 minutes) or on washed microsomes obtained by centrifugation of the 9000 *g* supernatant for 30 minutes at 105,000 *g*. Acetanilide, naphthalene, quinoline, and 4-methoxyacetanilide metabolism were measured in a system containing microsomes, TPN, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase, and the metabolism of the remaining substrates was measured with the 9000 *g* supernatant fraction fortified with glucose-6-phosphate and TPN. In all cases the formation of reduced TPN was not a rate-limiting step for the enzyme reaction. References for the enzyme assays are given in column 1 in parentheses after the name of each substrate.

Substrate	Major product	Metabolism (μmole / gm of liver per hr)*		Activity ratio
		Control	Benzpyrene	
	Hydroxylation			
3,4-Benzpyrene (*2*)	Mixture of hydroxylated products	0.42	5.1	12
Acetanilide (*13*)	4-Hydroxy-acetanilide	0.48	3.1	6.5
Zoxazolamine (*14*)	6-Hydroxy-zoxazolamine	1.3	4.9	3.8
Chlorozoxazone (*14*)	6-Hydroxy-chlorozoxazone	2.2	3.0	1.4
Quinoline (*13*)	3-Hydroxy-quinoline	0.20	0.41	2.0
Naphthalene (*13*)	1-Naphthol	0.54	1.1	2.0
	Dihydro-diol formation			
Naphthalene (*12*)	Naphthalene-1,2-dihydro-1,2-diol	0.28	0.54	1.9
	N-Demethylation			
3-Methyl-4-monomethylaminoazobenzene (*1*)	3-Methyl-4-aminoazobenzene	1.9	10	5.3
N-Methyl-aniline (*9*)	Aniline	0.41	0.82	2.0
Monomethyl-4-aminoantipyrine (*15*)	4-Amino-antipyrine	0.061	0.20	3.3
Meperidine (*16*)	Normeperidine	0.38	0.12	0.32
Benadryl (*16*)	2-(Benzhydryloxy)-ethylamine	0.46	0.32	0.70
	Ether cleavage			
4-Methoxy-acetanilide (*13*)	4-Hydroxy-acetanilide	2.0	2.8	1.4
	Azo reduction			
4-Dimethylaminoazobenzene (*1*)	Aniline and N-dimethyl-*p*-phenylenediamine	4.3	8.6	2.0
	Sulfoxide formation			
Chlorpromazine (*17*)	Chlorpromazine sulfoxide	0.88	0.84	0.96
	Side-chain oxidation			
p-Nitrotoluene (*18*)	*p*-Nitrobenzoic acid	0.70	1.12	1.6

* The amount of microsomal protein per gram of liver is not altered by treatment with BP.

benzpyrene, acetanilide, and zoxazolamine and the N-demethylation of 3-methyl-4-monomethylaminoazobenzene are increased 4- to 12-fold by treatment with BP. Smaller increases are consistently observed for the hydroxylation of naphthalene, quinoline, and chlorozoxazone, the conversion of naphthalene to its 1,2-dihydro-1,2-diol, the N-demethylation of N-methyl aniline, the O-demethylation of 4-methoxy-acetanilide, the reduction of the azo linkage of 4-dimethylaminoazobenzene, and the side-chain oxidation of *p*-nitrotoluene. The N-demethylation of monomethyl-4-aminoantipyrine was increased in only two of four experiments. The sulfoxidation of chlorpromazine and the N-demethylation of Benadryl are either not affected or are slightly decreased by treatment with BP. In contrast, meperidine demethylation is markedly decreased.

Treatment with BP or MC does not affect several enzymes that metabolize naturally occurring substrates. Thus the activities of the liver microsomal enzymes glucose-6-phosphatase, TPN-cytochrome *c* reductase, DPN-cytochrome *c* reductase, Δ-4 cortisone reductase, and the soluble enzymes phenylalanine hydroxylase and tryptophan peroxidase-oxidase are not increased at 24 hours after treatment with hydrocarbon (*6*). However, it is of considerable interest that BP, MC, and DBA have a marked effect in stimulating biosynthesis of L-ascorbic acid in the rate (*7*).

The results presented here show that BP increases the activities of several microsomal enzymes that metabolize foreign compounds but that this hydrocarbon possesses some degree of specificity since the activities of some enzymes are either not influenced or are only slightly increased. Even among reactions of the same type it is found that BP is specific in stimulating certain enzymes to a much greater extent than others. The hydroxylation of 3,4-benzpyrene and of acetanilide are markedly increased while the enzyme(s) which hydroxylate naphthalene and quinoline are affected to a lesser extent. A similar selective action of BP on different N-demethylation enzymes is also shown in Table 1. It has previously been suggested that more than one liver microsomal enzyme may catalyze the same type of reaction —that is, ether cleavage (*8*), N-dealkylation (*9–11*) and hydroxylation (*12*). The studies of enzyme induction reported here provide further evidence for the presence in liver microsomes of several different enzymes which can catalyze the same type of reaction.

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