added lecithin can substantially relieve this inhibition. As the reconstituted cytochrome *P*-450 system still contains deoxycholate (Lu *et al.*, 1969), lecithin may be essential here to overcome similar deoxycholate inhibition.

Treatment of microsomal fraction with phospholipase C (*Clostridium welchii*) exerts several effects on cytochrome *P*-450 system activities. At 5° or 37°C (about 95% lecithin hydrolysis) aminopyrine demethylase is inhibited by 50–75%. At 37°C phospholipase C inhibits aniline hydroxylase by about 30%, but this inhibition is not due to lecithin depletion because defatted serum albumin prevents inhibition. Further, at 5°C phospholipase C does not inhibit aniline hydroxylase but slightly enhances it, though 95% lecithin hydrolysis can still be obtained.

Recent investigations may explain these findings. When microsomal fraction is treated with phospholipase C, a highly active endogenous acylhydrolase generates fatty acids from the diglyceride products even at 5°C (Cater & Hallinan, 1971). Fatty acids are high-affinity substrates for the cytochrome *P*-450 system and at low concentration selectively inhibit other type I metabolism (confirmed in this investigation) without inhibiting type II (aniline) metabolism (Orrenius & Thor, 1969; Di Augustine & Fouts, 1969).

Finally, added sonicated lecithin does not completely restore the aminopyrine demethylase activity lost by 5°C-phospholipase C-treated microsomal fraction. Phospholipids reactivate many other phospholipid-depleted enzyme systems reportedly phospholipid-dependent, and Fleischer *et al.* (1962) maintain that reactivation is an essential criterion that must be met before an enzyme can be considered to be phospholipid-dependent.

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The Interaction of Glutamate Dehydrogenase and Malate Dehydrogenase with Phospholipid Membranes

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The phenomenon of allotopy has been clearly described for the *F*₁ coupling factor (Racker, 1967). The present report describes a search for allotropic effects involving the NAD-linked dehydrogenases of the mitochondrion. Such effects would complement the solution variables that are known to affect structural transitions in some of these enzymes (Dodd & Radda, 1969). The interaction between sonicated dispersions of purified phospholipids, corresponding to those found in the inner mitochondrial membrane, and the enzymes were studied by kinetic and spectroscopic methods.

With glutamate dehydrogenase the charge type of the lipid head group determines the extent of lipid-protein complexing. Phosphatidylcholine did not significantly alter the activity of the enzyme at lipid concentrations up to 1 mM, and the n.m.r. spectrum of the lipid (1%, w/v) was not altered in the presence of enzyme (up to 2%, w/v). Phosphatidylethanolamine did not affect the activity in the oxidation of glutamate, but interfered with the assay in the reverse direction, possibly owing to Schiff-base formation with 2-oxoglutarate. The negatively charged cardiolipin, which is found uniquely in the inner mitochondrial membrane, strongly and reversibly inhibited the enzyme (50% inhibition with 0.1–20 μM-lipid, depending on conditions). Another anionic phospholipid, phosphatidylserine (which does not occur in mitochondria), also inhibited the enzyme, but less effectively (50% inhibition at about 8 μM). The lipid-enzyme interaction decreases with increasing ionic strength, indicating an electrostatic contribution to the binding. N.m.r. studies suggest that there is also an apolar interaction involving the lipid chains.

The studies with malate dehydrogenase concentrated on detecting possible stabilization of this enzyme through interaction with lipids. A clear-cut stabilization was found with lysophosphatidylcholine. Under conditions such that the enzyme alone lost 50% of the initial activity in about 2 h, lysophosphatidylcholine gave a slight (20%) activation and this activity remained constant for several hours. N.m.r.

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studies indicated that, as with the related enzyme 2-hydroxybutyrate dehydrogenase, both the polar choline head group and the hydrocarbon chains are involved in the binding of the lipid to the protein.

These specific lipid-protein complexes are probably examples of allotropic interactions. This form of complexing may explain aspects of the behaviour of these enzymes *in vivo*.

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Biological Activity of Double-Stranded Ribonucleic Acid Fragments

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Fungal viruses containing double-stranded RNA have been detected in a number of *Penicillium* species, and all of them tested as yet have been found to possess the capacity to induce interferon in animals and to give protection against infection by different viruses (for review see Colby, 1971). The double-stranded RNA responsible for these effects has been studied with respect to the importance of molecular weight for biological activity.

Double-stranded RNA isolated from *Penicillium chrysogenum* virus (Lemke & Ness, 1970; Buck *et al.*, 1971) has been fragmented in three different ways: (a) by digestion with ribonuclease III; (b) by digestion with high concentrations of pancreatic ribonuclease A in high salt concentration; (c) by ultrasonic disintegration.

Degradation was followed by viscosity measurements, polyacrylamide-gel electrophoresis and sucrose-density-gradient analysis. With the exception of some special mild conditions, breakdown was always random, producing a heterogeneous mixture of fragments of a rather wide size range. Optimal conditions for obtaining relatively large fragments with no traces of intact material have been worked out. The 'melting' temperature, T_m , was practically unchanged after degradation, suggesting that the breakdown products were still double-stranded.

The capacity of the RNA to induce interferon was diminished even after mild degradation, though at the same time the toxicity increased as compared with that of the intact RNA. (These changes were observed in a virus-sensitive strain of mice, BSVS.) As similar results were obtained whichever method of degradation was used, but the results varied with the degree of degradation, it would seem that biological activity is dependent on molecular weight rather than on the intact structure of any particular site in the RNA molecule.

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Resolution of Supercoiled Deoxyribonucleic Acid Structures by Light-Scattering

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There is, at present, considerable interest in and uncertainty about both the exact tertiary structure of supercoiled DNA and the possible transitions occurring on alteration of the secondary structure by, for example, dye titration (Bauer & Vinograd, 1968; Wang, 1969; Revet *et al.*, 1971; Dean & Lebowitz, 1971). Previous techniques used have been mainly hydrodynamic, requiring small amounts of material, but providing rather ambiguous answers to the problem of tertiary-structure determination.

Light-scattering requires larger amounts of material, but supplies a direct numerical parameter of tertiary structure, the root-mean-square radius, and also a complete simple interference curve on which further calculations can be made.

We have undertaken a light-scattering study of bacteriophage ϕ X174-RFI DNA in attempts to differentiate between different possible structures, such as (a) the straight interwound model, (b) the toroidal model (Gray, 1967) and (c) the interwound Y-shaped model as seen in electron micrographs.

The experiments were performed on pure bacteriophage ϕ X174-RFI DNA in BPES buffer (Krasna & Harpst, 1964), pH 6.8, at 25°C by using a refractive-index increment of 0.166 ml/g (Krasna, 1970).

Results were as follows. The number of superturns/molecule was -11.8 ± 1.6 ; in further calculations it was assumed to be an integer, 12. The molecular weight of bacteriophage ϕ X174-RFI DNA was $3.17 \times 10^6 \pm 0.19 \times 10^6$, significantly lower than the value of twice 1.7×10^6 previously reported for single-stranded bacteriophage ϕ X174 DNA (Sinsheimer, 1959). The root-mean-square radius was 103.5 ± 12 nm.

To extract further information from the scattering experiment, we predicted reciprocal particle scattering factors, $P(\theta)^{-1}$, for each of the models mentioned above, having dictated their dimensions with the experimental root-mean-square radius and molecular