are those due to CH stretching at 2915 cm^{-1}, CH_2 and CH_3 bending between 1456 to 1459 cm^{-1}, P=O stretching between 1350 to 1250 cm^{-1}, P−O−C vibrations about 1050 to 980 cm^{-1}, and the amide II band near 1540 to 1550 cm^{-1}.) A reduction of the amide I band is observed only in the spectrum of films prepared from sonic aqueous membrane dispersion from which the lipid has been removed. It is possible that some sphingomyelin, which also contributes to absorption in the amide I and amide II regions, is less easily removed from the larger membrane fragments.

Heating the films, after removal of lipids, to temperatures as great as +150°C does not produce a detectable transition to a β-conformation. This may indicate that some residual lipid tightly bound to protein can protect the membrane protein from transition by thermal denaturation to a β-conformation. Bulk extraction of membrane fragments with a mixture of chloroform and methanol (1 : 1), according to the method of Reed *et al.* (*8*), leaves a residue which, when examined by infrared spectroscopy, shows a broad asymmetrical amide I band with peaks at 1652 cm^{-1} and a shoulder at 1628 cm^{-1}. Membrane films cast from organic solvents, for example, 2-chloroethanol, pyridine, or dimethyl formamide (Fig. 4), show a marked splitting of amide I band with peaks at 1652 and 1628 cm^{-1}, indicating a transition of a great portion of membrane protein to a β-configuration. A similar effect is observed with dimethyl sulfoxide or formic acid.

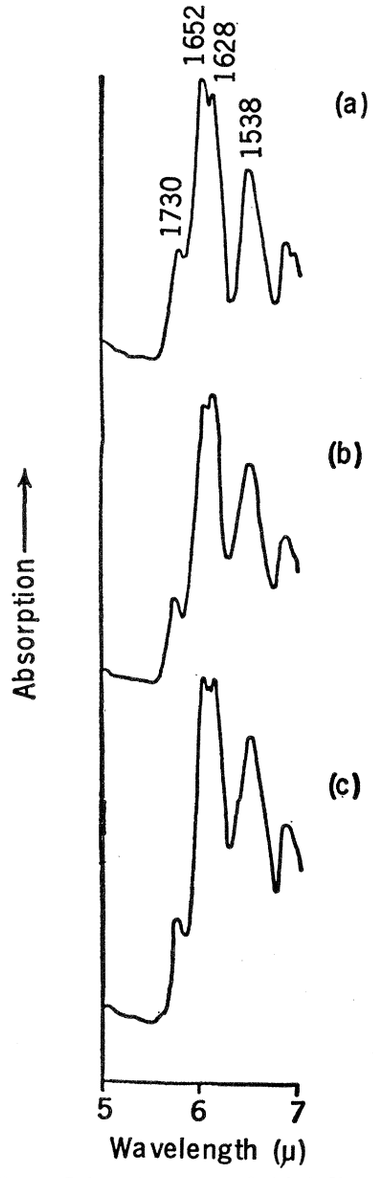

Fig. 4. Infrared spectra of dried films of erythrocyte membranes cast from 80 percent 2-chloroethanol (a), dimethylformamide (b), and pyridine (c). All were recorded at room temperatures.

Another feature of the infrared spectrum of the membrane is a shoulder at 1711 cm^{-1} associated with a lipid carbonyl group. The infrared spectrum of the lipid extract does not show this band. The presence of this band may be associated in some way with the interaction of lipid and protein. Further studies of the infrared spectra of other membranes will be useful in providing information about their structure.

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Actinomycin D: Inhibition of Phospholipid Synthesis in Chick Embryo Cells

Abstract. *When chick embryo fibroblasts are exposed to actinomycin D for 30 minutes to 3 hours, there is progressive inhibition of phospholipid synthesis, so that by 3 hours the inhibition is 40 percent. All phospholipids are affected. Since this inhibition is twice as great as the inhibition of protein synthesis, some effects of actinomycin D previously ascribed to decreased protein synthesis must be reevaluated.*

Although widely used as an inhibitor of DNA-dependent RNA synthesis (*1*), actinomycin D has many effects which are not clearly related to this inhibition. These include inhibition of the replication of the RNA viruses poliovirus (*2*), influenza virus (*3*), and reovirus (*4*); inhibition of the replication of the RNA bacteriophage MS2 (*5*); interference with the assembly of the DNA bacteriophage T4 (*6*); inhibition of intestinal amino acid transport (*7*); and stimulation of the breakdown of messenger RNA (*8*). The rate of protein synthesis falls in cells treated with actinomycin D (*9*); this decrease is thought to be secondary to inhibition of RNA synthesis. Thus, the effects of actinomycin D on viral replication and amino acid transport have been ascribed to decreased protein synthesis. We now report that actinomycin D also inhibits phospholipid synthesis, and that the inhibition of phospholipid synthesis greatly exceeds the inhibition of protein synthesis in our system.

Chick embryo fibroblasts were prepared as previously described (*10*). They were exposed to actinomycin D (0.5 μg/ml) for 30 minutes to 3 hours, washed with Eagle's medium, and incubated with various radioactive substrates for 30 minutes; the cells were then scraped from the glass. The phospholipids were extracted by the method of Folch *et al.* (*11*). The incorporation of uracil-H^3 into RNA, and of leucine-H^3 into protein, was measured by the Schmidt-Tannhauser procedure (*12*).

Actinomycin D inhibited the incorporation of $^{32}P_i$ into phospholipid (Fig. 1). The magnitude of the inhibition increased with time of exposure to actinomycin D. Phospholipid synthesis was decreased by 10 percent in cells treated with actinomycin D for 15 minutes and by 25 percent in cells treated for 30 minutes, whereas protein synthesis was

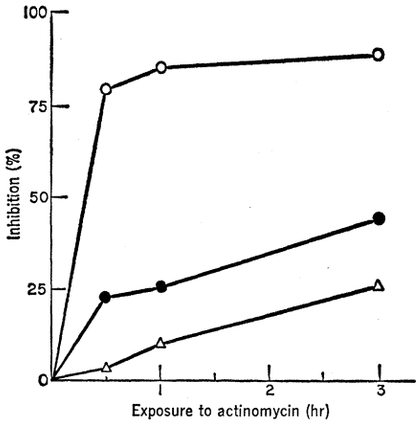

Fig. 1. Inhibition of phospholipid (●), protein (△), and RNA (○) synthesis by actinomycin D. Chick embryo fibroblasts were exposed to actinomycin D (0.5 μg/ml) for 30 minutes to 3 hours, washed, and incubated with $^{32}P_i$ (1 μc) for 30 minutes; the phospholipids were extracted, and the radioactivity was measured. Each value is the mean from three experiments.

barely affected. In cells treated with actinomycin D for up to 3 hours, phospholipid synthesis continued to be inhibited more than protein synthesis.

The phospholipids of treated and control cells were extracted and then separated by thin-layer chromatography (*13*); the concentration of each phospholipid and its content of ^{32}P were determined. Treatment with actinomycin decreased the incorporation of $^{32}P_i$ into all phospholipids; sphingomyelin was most affected (Table 1). There was no detectable effect on the phospholipid composition of the cells.

It seemed possible that the decrease in the incorporation of $^{32}P_i$ into phospholipid might not represent a decrease in net phospholipid synthesis, but might be due either to an inhibition of phosphate uptake from the medium or to a decrease in the specific activity of intracellular adenosine triphosphate secondary to inhibition of RNA synthesis. We therefore investigated the incorporation of other precursors into phospholipid. In cells treated with actinomycin D for 4 hours, the incorporation of choline-C^{14} into phospholipid was decreased by 30 percent. When the phospholipids were separated by thin-layer chromatography, both sphingomyelin and lecithin were affected. Actinomycin D also caused a 35 percent decrease in the incorporation of glucose-C^{14} and glycerol-C^{14} into lipid-soluble material. With these precursors, neutral lipids which account for about one third of the total tissue lipid were present in the extract and were not separated from the phospholipid.

The mechanism by which actinomycin D inhibits phospholipid synthesis is unclear. Chick embryonic fibroblasts are rapidly differentiating into adult cells with extensive internal membranes, and actinomycin D may inhibit membrane formation. Alternatively, actinomycin D may inhibit the activity of an enzyme required for phospholipid synthesis.

In tissues treated with actinomycin D, a decrease in cellular function which is not directly related to a decline in RNA synthesis is often ascribed to a fall in protein synthesis subsequent to decreased RNA synthesis (*2–4, 7, 8*). Our finding that actinomycin D inhibits phospholipid synthesis in chick embryo fibroblasts more than it inhibits protein synthesis offers an alternative explanation for such findings.

Table 1. Effect of actinomycin on the incorporation of $^{32}P_i$ into various phospholipids. Cells were exposed to actinomycin D (0.5 μg/ml) for 2 hours, washed, and then incubated with $^{32}P_i$ (10 μc) for 30 minutes at 37°C. The cells from three 100-mm plates, each containing about 10^8 cells, were pooled for each analysis.

Phospholipid	Phospholipid concentration (nmole phospholipid per mg protein)		Specific activity (count/min per nmole phospholipid)	
	Control	Actinomycin	Control	Actinomycin
Sphingomyelin	72	81	0.55	0.12
Lecithin	368	362	38	19
Phosphatidylserine	20	22	140	77
Phosphatidylinositol	66	59	345	162
Phosphatidylethanolamine	174	154	14	9.4
Unknown	29	16	78	64
Total	729	694		

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14. We thank R. Katzen and Mrs. D. Hughes for their technical assistance.

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Dimethyl Sulfoxide: An Inhibitor of Liver Alcohol Dehydrogenase

Abstract. *Dimethyl sulfoxide inhibits horse liver alcohol dehydrogenase. In the direction of aldehyde reduction, this inhibition is competitive with aldehyde, with an inhibition constant of* 5×10^{-3}M. *Dimethyl sulfoxide reacts with the binary complex consisting of enzyme and the reduced form of nicotinamide-adenine dinucleotide to form a highly fluorescent ternary complex, with a dissociation constant similar to the inhibition constant. The inhibition of aldehyde reduction can be interpreted as due to competition between aldehyde and dimethyl sulfoxide for the carbonyl binding site of the above-mentioned binary complex.*

Dimethyl sulfoxide (DMSO), a by-product in the manufacture of paper, has recently gained notoriety as a potentially useful agent in the treatment of rheumatoid arthritis and scleroderma, and as a solvent for the topical administration of other drugs (*1*). Although DMSO has numerous biological activities, the chemical bases of these activities are unknown, and the specific interaction of DMSO with tissue constituents has not previously been reported. In the course of studies on the inhibition of dehydrogenases by thyroxine, we found that DMSO is an inhibitor of liver alcohol dehydrogenase (ADH). In this report we present some kinetic studies of this inhibition

Table 1. Fluorescent properties of free and bound NADH. All data were obtained in 0.1*M* tris HCl, *p*H 8.6, at 25°C.

Fluorescent species	Excitation maximum (nm)	Emission maximum (nm)	Relative quantum yield*
NADH	340	465	1
ADH-NADH	325	435	3
ADH-NADH-DMSO	325	435	10

* Quantum yields are expressed relative to the quantum yield of free NADH, which is taken as 1.