sant action is presumably unrelated to GABA levels of brain cortex, whereas the later anticonvulsant action may be associated with increased cortical GABA. In our own experiments the same dosage of drug reported to have a late anticonvulsant effect for monkeys proved to have an early convulsant action in epileptic monkeys, so that the method of administration and dosage of drug were probably not significant factors in this regard. The early convulsant effects, moreover, are usually associated with obvious physiological changes such as hyperpnea and methemoglobinemia which were shown in rats to be unrelated to brain GABA levels(4).

Summary. 1) Chronic epileptic monkeys (alumina cream) showed greater susceptibility than normal or non-epileptic brain-operated monkeys to the early convulsant effects of hydroxylamine. 2) Rats made epileptic (intracerebral cobalt) showed a similar increased susceptibility to hydroxylamine compared to unoperated controls.

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Effect of Oxidative Phosphorylation Inhibitors on Synthesis of Liver Mitochondria Phospholipids.* (28025)

JOHN N. YOUNGS AND W. E. CORNATZER
Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota School of Medicine, Grand Forks

Liver mitochondria are composed of 21% phospholipids on a dry weight(1,2). Purified cytochrome oxidase contains phospholipids (3) and is reactivated by purified phospholipids(4). Recently a number of other enzymes, DPNH-cytochrome c reductase, succinic cytochrome c reductase, succinic coenzyme Q reductase and DPNH diaphorase have been shown to contain 26-83% lipid and the lipids are predominantly phospholipids (5). All of these enzymes are involved in the electron transfer system. Mitochondria(6) and the electron transport particles(7) are very active in phosphorylation. Kennedy has demonstrated that synthesis of phospholipids takes place in isolated mitochondria and requires the maintenance of oxidative phosphorylation(8). Bilirubin(9), dinitrophenol(10), arsenate(11,12) and oligomycin (13,14) have been shown to inhibit oxidative phosphorylation. In view of these observations, experiments were undertaken to determine the effect of inhibition of oxidative phosphorylation on synthesis of liver mitochondria phospholipids.

Methods. Male albino rats of Sprague-Dawley strain, weighing 100-140 g, were divided into 4 groups and received a single daily injection of the following compounds. The animals in Series I were injected intraperitoneally with arsenate (14 mg/kg), bilirubin (3.3 mg/kg), in Series II rats were injected with oligomycin (0.83 mg/kg), in Series III, dinitrophenol (30 mg/kg) and in Series IV, dinitrophenol (30 mg/kg for 3 days). Control rats for each group received intraperitoneal injections of saline except the control animals for Series II which received

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propylene glycol:water (1:1, v/v). Intraperitoneal injection of 200 μc of P^{32} as NaH_2PO_4 was administered one hour following injection of above compounds. Four hours later the animals were killed by decapitation. This time interval corresponds to the ascending part of the specific activity time curve, where synthesis of phospholipids occurs to a degree greater than breakdown (15,16). The liver was removed, rapidly weighed, homogenized in a Potter-Elvehjem tissue grinder with Teflon pestle in ice-cold 0.25 M sucrose containing 0.00018 M $CaCl_2$. The homogenate was divided into 2 fractions. From one fraction acid-soluble phosphorus was removed by extracting with 10% TCA and radioactivity(17) and total P(18) were determined. The other homogenate fraction was separated into cellular fractions by the method of Hogeboom *et al.*(19). The homogenate was centrifuged at 600 $\times$ *g* for 15 minutes to remove the nuclei. The mitochondria were separated from the supernatant by centrifugation at 10,000 $\times$ *g* for 15 minutes. The mitochondria were washed with 0.15 M NaCl and recentrifuged at 10,000 $\times$ *g* for 15 minutes. Lipids were extracted one time with methanol and 4 times with chloroform: methanol (2:1, v/v) by heating the mitochondria in the solvent on a waterbath 40°C for 15 minutes. The sample was centrifuged at 1500 rpm for 3 minutes after each extraction period to facilitate removal of the solvent. The pooled lipid extracts were evaporated to dryness with Rinco Rotating Vacuum Evaporators (Rinco Instrument Co.), the water removed with Freeze-Dryer (VirTis Co., Inc.) and residue taken up in chloroform. On aliquots of the chloroform solution radioactivity(17) and total phosphorus(18) were determined. The phospholipids were chromatographed on silicic acid impregnated glass filter paper(20). The solvent system was diisobutyl ketone:acetic acid:water:benzene (160:50:8:7, v/v). The chromatograms were dried, stained with Rhodamine 6G and the phospholipids identified with ultraviolet light. The individual phosphatides were hydrolyzed and extracted with 3 N methanolic HCl. The hydrolysate was evaporated to dryness, and phosphorus(18) and radioactivity(17) determined. From these results and the radioactivity measurements, relative specific activities of the individual phospholipids were calculated(21). Relative specific activity expresses the relationship between isotopic concentration in a compound and in its precursor(22). Under the conditions of our experiments an increase in formation of phospholipids may be reasonably assumed when higher values for relative specific activities are found. The relative specific activity is the ratio of the specific activity of the phospholipid P to that of the acid soluble P. The specific activity is defined as counts per minute per aliquot (% of dose injected)/mg of phosphorus per aliquot.

TABLE I. Composition of Individual Phospholipids of Rat Liver Mitochondria.*

	% of total lipid P
Phosphatidyl inositol	7.0 $\pm$ 1.3†
Sphingomyelin	3.5 $\pm$ .7
Lecithin	49.8 $\pm$ 2.1
Phosphatidyl serine	4.8 $\pm$ .7
" ethanolamine	29.5 $\pm$ 1.5

* Avg values of 10 animals.
† $\pm$ standard dev.

Results. Table I shows the composition of the individual phospholipids of rat liver mitochondria. The predominant phospholipid is lecithin. The percentages of total phospholipid phosphorus for the various phosphatides of liver mitochondria are very similar to those of whole liver(20). Table II shows the effect of various agents which inhibit oxidative phosphorylation on uptake of radioactive phospholipid into the various phospholipids of liver mitochondria. To evaluate the significance of results the *t* test of significance(23) was applied to the difference between mean of control and experimental values. The data show that administration of bilirubin, oligomycin and dinitrophenol produces a statistically significant decrease in the total phospholipids in liver mitochondria. There is a statistically significant decrease in phospholipid synthesis of phosphatidyl inositol, sphingomyelin, lecithin, phosphatidyl serine and phosphatidyl ethanolamine following administration of a single dose of dinitrophenol or when 3 daily doses are given. Dinitrophenol has

TABLE II. Relative Specific Activity of Mitochondria Phospholipids of Liver Following Single Dose of Various Agents.

Material administered	No. of animals	Total phospholipids	Phospholipid fractions: Phosphatidyl inositol	Sphingomyelin	Lecithin	Phosphatidyl serine	Phosphatidyl ethanolamine
Series I							
Saline	5	.064 ±.005	.043 ±.005	.036 ±.004	.075 ±.005	.065 ±.011	.068 ±.008
Arsenate	6	.043 ±.006*	.020 ±.005†	.018 ±.008	.047 ±.009*	.021 ±.009*	.036 ±.006*
Bilirubin	5	.019 ±.005*	.048 ±.028	.044 ±.020	.029 ±.013*	.051 ±.014	.038 ±.007*
Series II							
Propylene glycol	6	.077 ±.007	.054 ±.012	.051 ±.009	.078 ±.010	.065 ±.002	.064 ±.005
Oligomycin	5	.056 ±.008†	.053 ±.008	.018 ±.008*	.059 ±.008†	.036 ±.006†	.055 ±.006†
Series III							
Saline	3		.031 ±.014	.058 ±.012	.084 ±.015	.051 ±.009	.078 ±.008
Dinitrophenol	3		.021 ±.007†	.035 ±.005*	.046 ±.013*	.035 ±.006*	.063 ±.009*
Series IV							
Saline	5	.062 ±.007	.039 ±.004	.040 ±.008	.067 ±.006	.058 ±.010	.064 ±.004
Dinitrophenol	5	.033 ±.004*	.021 ±.002*	.017 ±.004*	.037 ±.004*	.051 ±.008†	.055 ±.009†

Numbers preceded by ± are standard dev. Test of significance was applied to difference between mean of control and experimental values. The P probability for chance occurrence of this difference was:

* $<.01$ † $<.05$

been shown to inhibit incorporation of the isotope into phospholipids in cat brain slices (24). Kennedy(8), using isolated rat liver mitochondria, has shown that incorporation of P^{32} into total phospholipids was reduced by addition of dinitrophenol. Administration of single dose of arsenate, bilirubin and oligomycin inhibited incorporation of the isotope into all phospholipid fractions except sphingomyelin and phosphatidyl inositol. However, oligomycin inhibited incorporation of the isotope into the sphingomyelin fraction and arsenate inhibited the phosphatidyl inositol fraction. It is apparent from the data that compounds which inhibit oxidative phosphorylation decrease the synthesis of phospholipids in mitochondria. Since ATP is needed for synthesis of phospholipids, this inhibition of oxidative phosphorylation where ATP is made may be a direct reflection of lipid phosphorylation. The role of these lipids in the mitochondria is unknown. However, it is of interest that phospholipids are not known to be involved in oxidative phosphorylation but make up approximately ⅓ of the dry weight of mitochondria(1,2) being found in membrane of the mitochondria(25) where oxidative phosphorylation takes place and are chemically part of enzymes involved in this process(3,5).

Summary. 1. The effect of single doses of arsenate, bilirubin, dinitrophenol and oligomycin on synthesis of mitochondria phospholipids of the liver of rats was studied. 2. A statistically significant decrease occurred in synthesis of phosphatidyl inositol, sphingomyelin, lecithin, phosphatidyl serine and phosphatidyl ethanolamine of liver mitochondria following administration of dinitrophenol. 3. Administration of arsenate, oligomycin and bilirubin inhibited statistically the incorporation of the isotope into all the phospholipid fractions of liver mitochondria except sphingomyelin and phosphatidyl inositol. However, oligomycin inhibited incorporation of the isotope into the sphingomyelin fraction and arsenate inhibited the phosphatidyl inositol fraction.

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Centrally Mediated Inhibition of Gastrointestinal Propulsive Motility By Morphine over a Non-Neural Pathway. (28026)

S. MARGOLIN*

Schering Corp., Bloomfield, N. J.

The constipating action of morphine in man and in laboratory species is well known, but the mechanism of this action upon the intestine has remained obscure(1,2,3). A generally accepted view attributes this phenomenon primarily to a local direct action upon the intestine(4) and derives from the observation that morphine causes an inhibition of intestinal propulsive motility of both normal and extensively denervated dog intestine *in situ*(5,6). Nevertheless, Krueger (1) suggested "a central effect on gastrointestinal motility might be unmasked by craniad injections into the carotid artery . . . and related devices." Recent reviews(3,7) have reemphasized the possibility of an inhibitory action of morphine upon intestinal propulsion mediated centrally. Margolin(8) inferred and Green subsequently confirmed (9), that a primary route by which morphine causes a suppression of intestinal propulsive activity is indirect *via* the central nervous system. The present study based upon experiments with over 2,000 mice was designed to consider the possibility that morphine was causing a suppression of intestinal propulsive activity over a non-neural pathway.

Methods. The bioassay of drugs by intracerebral injections into mice has been reported(10), and our laboratory applied this technic to studies on the mechanism of the action of morphine and other drugs upon the central nervous system. The procedures for administering the drugs intracerebrally to mice, rats and guinea pigs and assaying effects upon intestinal propulsion according to the method of Macht-Barba-Gose(11) have been presented(8,12).

Results. Intracerebral injection of morphine sulfate into unanesthetized albino mice (22 g) reduced the distance traversed by a charcoal suspension through the small intestine. By bioassay in 50 experiments, a 50% reduction of the distance traveled by the

* Present address: Wallace Laboratories, Cranbury, N. J.