for the difference in swimming time in both groups studied. It seems possible then that muscle paralysis and central sedation caused by both chlorpromazine and lower bath temperatures (28 or 19°C) become additive and explain the detrimental effect of chlorpromazine on animals swimming at temperatures lower than 32°C. At 9°C the effects of cold alone are so drastic and telescoped that chlorpromazine fails under these conditions to display its action.

Summary. When rats are swimming at 19 or 28°C, swimming time is markedly reduced by chlorpromazine (10 mg/kg) treatment. This difference is not due to differences in body temperature but is probably caused by sedation and muscular paralysis caused by this drug. At 9°C the detrimental effect of bath temperature on swimming time is so marked that chlorpromazine fails to display any action whereas at 32°C the stress is so small as to allow the animal to perform as well even when treated with chlorpromazine.

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Increase of Hexobarbital Sleeping Time by Certain Anticholinesterases.* (24139)

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Some organic phosphate insecticides, such as octamethyl pyrophosphoramide (OMPA) (1), p-nitrophenyl diethyl thionophosphate (parathion)(2) and ethyl-p-nitrophenyl thionobenzenephosphonate (EPN)(3) are converted by liver slices from relatively inactive to potent cholinesterase inhibitors. Conversion of OMPA(4), parathion(5), and hexobarbital(6) is an oxidative process, taking place in combined microsome and supernatant fractions of liver homogenates. Other organic phosphate anticholinesterases, such as S-(1,2-dicarbethoxyethyl)-O, O-dimethyl dithionophosphate (malathion), p-nitro-m-chlorophenyl dimethyl thionophosphate (chlorothion), and a mixture of bis-(dialkoxyphosphinothioyl) disulfides (phostex) are partially inactivated by liver slices(7). Since the alteration of these anticholinesterases by liver may involve the same enzyme systems as those detoxifying hexobarbital, their effects on duration of action of the latter were examined. Tetraethyl pyrophosphate (TEPP), and technical bis - dimethylamido - fluorophosphate (BFP) were used as examples of anticholinesterases not markedly activated nor rapidly inactivated by liver.

Methods and materials. Young adult male Swiss-Webster mice fasted 16 to 20 hours were used. Anticholinesterases† were given orally in corn oil 2 hrs before intraperitoneal injection of sodium hexobarbital in saline. TEPP was given to one group of mice 0.5 hr before the barbiturate. Sleeping time was the duration of loss of righting reflex from the side position.

Results. The highest doses of malathion and chlorothion and doses of other anticholinesterases used killed approximately 10% of the animals, all before injection of hexobarbital. Malathion, phostex and chlorothion produced the greatest prolongation of sleeping time (Table I). EPN and OMPA were also active, while BFP and TEPP were inactive.

Since malathion and chlorothion markedly prolonged sleeping time, it was expected that

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† Chlorothion, OMPA and BFP were supplied by Dr. K. P. DuBois of University of Chicago; EPN by E. I. duPont de Nemours Co.; malathion by Cyanamid Co.; phostex by Niagara Chemical Division, Food Machinery and Chemical Corp.; TEPP by Victor Chemical Works.

TABLE I. Effect of Anticholinesterases on Duration of Hexobarbital (80 mg/kg) Sleeping Time.

Anticholinesterase, mg/kg	No. of mice	Sleeping time (min.), mean ± S.E.		P
	11	26.5	5.0	
EPN-30	11	85.3	13.3	<.01
	5	14.2	2.9	
OMPA-10	9	41.1	7.1	<.02
	15	32.4	5.9	
Malathion-500	17	116.1	15.3	<.01
	5	35.4	7.0	
-125	9	90.6	17.9	<.05
	6	18.0	7.4	
- 60	6	28.5	4.7	> "
	15	40.0	9.2	
Chlorothion-500	20	218.9	23.4	<.01
	5	39.6	10.7	
-125	10	115.9	10.3	< "
	6	27.8	9.3	
- 60	6	69.0	15.0	<.05
	5	34.4	6.2	
Phostex-1000	6	137.5	18.0	<.01
	5	16.6	5.6	
BFP-0.5	9	22.2	5.5	>.05
	15	23.3	3.2	
TEPP-2	11	32.6	7.3	> "
TEPP-2*	10	29.9	7.2	> "

* TEPP inj. .5 hr before hexobarbital.

they would also increase toxicity of hexobarbital. In groups of 10 animals each, however, 500 mg/kg of malathion or chlorothion did not significantly alter the toxicity of 200 or 250 mg/kg, approximately the LD_5 and LD_{50} of this barbiturate. Nearly all deaths occurred within 30 minutes after injection of the barbiturate, although at these doses animals surviving hexobarbital alone slept an average of 2 hours.

In agreement with our findings that anticholinesterases which are not rapidly altered by liver do not prolong hexobarbital sleeping time, it has been reported that physostigmine does not affect hexobarbital sleeping time(8). However, administration of physostigmine to mice before injection of barbital hastens the onset of action of this barbiturate and increases the rate of its entrance into the brain, an effect attributed to inhibition of cholinesterase(9). If chlorothion has a similar effect, this might partly explain its prolongation of the action of hexobarbital, although increased rate of penetration may not necessarily be accompanied by an increased duration of action. To determine whether chlorothion and TEPP alter the rate of penetration of sodium barbital into the brain their effects on the time from its intraperitoneal injection to loss of the righting reflex was measured (Table II). Since chlorothion did not hasten the onset of action of barbital, it is evident that it did not increase the rate of entrance of barbital into the brain. TEPP had an effect (Table II) similar to that of physostigmine(9), indicating that TEPP may also increase penetration of barbital into the brain. The negative results obtained with chlorothion suggest, however, that factors other than cholinesterase inhibition may be involved in the effect of anticholinesterases on brain permeability to barbital.

Discussion. Since hexobarbital is oxidatively inactivated by liver the possibility is suggested that the anticholinesterases which are rapidly activated or inactivated by liver prolonged hexobarbital sleeping time by competing for the enzyme system responsible for oxidation of the barbiturate. Though the enzymatic characteristics of the liver inactivation of malathion, chlorothion and phostex are unknown the finding that these agents prolonged hexobarbital sleeping time, points to the possibility that they compete for the enzyme system which detoxifies hexobarbital. It is significant that TEPP and technical BFP, which are not markedly affected by liver, did not prolong the action of hexobarbital.

Failure of malathion and chlorothion to affect toxicity of hexobarbital might be attributed to failure to increase, within the first half hour, the maximum concentration of the barbiturate in the central nervous system, in

TABLE II. Effect of Chlorothion and TEPP on Time of Onset of Action of Sodium Barbital (300 mg/kg).

Anticholinesterase, mg/kg	No. of mice	Anesthetic lag (min.), mean ± S.E.		P
	10	32.4	1.7	
Chlorothion-500*	14	27.9	2.8	>.05
	8	40.0	7.5	
TEPP-2†	12	25.3	1.5	< "

* Inj. 2 hr before barbital.
† " .5 hr " " .

spite of inhibition of its oxidation by the liver. Since nearly all deaths from hexobarbital alone or combined with these agents occurred within the first half hour, it also appears that malathion and chlorothion did not maintain a sufficiently high concentration of barbiturate over a long enough time to be lethal.

The effect of these anticholinesterases on concentration of hexobarbital in some tissues of these animals, and their effect on duration of action of other barbiturates and other drugs which are oxidatively metabolized by liver are being investigated. In a preliminary test pentobarbital sleeping time in mice was also markedly prolonged by chlorothion. Such findings may form the basis for caution by those whose work cause exposure to this type of insecticide and for whom barbiturates or other drugs oxidized by liver are simultaneously prescribed.

Summary. Organic phosphate insecticides, OMPA, EPN, malathion, chlorothion and phostex, which are rapidly metabolized in liver, markedly increased hexobarbital sleeping time in mice, while BFP and TEPP did not. Malathion and chlorothion did not alter toxicity of hexobarbital. TEPP hastened onset of action of barbital while chlorothion did not. Certain anticholinesterases which are rapidly metabolized by liver may compete for enzymes which are responsible for destruction of hexobarbital.

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Alterations in Rat Serum Proteins in Single Deficiencies of Folic Acid and Vit. B_{12}.* (24140)

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The influence of a deficiency of folic acid (PGA) and Vit. B_{12} on rat serum proteins was reported(1). A lowering of total protein level was attended by reductions in serum concentrations of albumin, α_1-globulin and γ-globulin. An increase in proportion of β-globulin was interpreted as indicative of higher serum level of free Vit. B_{12} in the deficient state. In continuation of this work, the effects due to single deficiencies of Vit. B_{12} and PGA on serum proteins and Vit. B_{12} have been evaluated and are here reported.

Materials and methods. Young male rats (Wistar strain), 50 g weight, were depleted of their Vit. B_{12} and PGA reserves by maintenance on a deficient iodo-casein diet containing succinyl sulphathiazole(1) for 4 weeks. The animals were then divided into 4 comparable groups and were fed the following diets: (a) basal ration, (b) basal ration plus Vit. B_{12}, (c) basal ration plus PGA and (d) basal ration plus Vit. B_{12} and PGA. The basal ration consisted of (g/100 g of diet): Vit.-free casein, 10; succinyl sulphathiazole, 2; arachis oil, 6; shark liver oil, 2; salt mixture (U.S.P. No. 2), 4; sucrose, 10; and maize starch, 66, with vitamin additions as stated previously(1). Additions of Vit. B_{12} and PGA, where made, were at levels of 50 μg and

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