Discussion. From the experiments described in the earlier part of this paper, it is evident that the thiosemicarbazones of nicotinaldehyde and of isonicotinaldehyde exerted a protective effect in the experimental hematogenous tuberculosis of mice. Levaditi's findings with the thiosemicarbazone of nicotinaldehyde were thus confirmed at least in the dosage of 50-100 mg/kg/day used by him. It seems, therefore, evident that the thiosemicarbazones of compounds which do not contain primary or secondary amino groups might exert an activity comparable or superior to Tibione.

Superior activity was particularly evident in the intranasal infection which was more resistant to such agents as para-amino-salicylic acid, niacinamide and also seemed to require considerably higher doses of Tibione than did the thiosemicarbazones of nicotinaldehyde and isonicotinaldehyde. No satisfactory explanation has as yet been found for this difference in activity.

Summary. (1) The anti-tubercular activity of the thiosemicarbazone of nicotinaldehyde in the intravenous infection of mice with *M. tuberculosis* as described by Levaditi was confirmed. (2) The thiosemicarbazone of isonicotinaldehyde was found as active as the thiosemicarbazone of nicotinaldehyde and the effect of both compounds was comparable to that of Tibione. Picolinaldehyde thiosemicarbazone was ineffective. (3) The thiosemicarbazones of nicotinaldehyde and isonicotinaldehyde also were found appreciably active in the therapeutically more resistant bronchogenic type of tuberculosis of mice produced by intranasal infection. Under these experimental conditions the compounds appeared superior to Tibione.

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Effect of Barbiturates on Oxidative Phosphorylation.* (18674)

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There is still much doubt as to the exact mechanism by which barbiturates produce their pharmacologic effects. Quastel and his associates(1) demonstrated that narcotics depress the respiration of brain tissue *in vitro* and postulated that the locus of the blocking action was at a flavoprotein step in the cytochrome chain. This supposition was further supported by the work of Grieg(2). However, both groups of workers used indirect means to demonstrate the postulated locus of action and did not specifically measure the step in question. Persky, *et al.*(3) have recently produced evidence which they interpret as showing that the site of action for the barbiturates is the dehydrogenase moiety of the pyruvic oxidase complex. Work in our laboratory has substantiated the overall inhibition of respiration in brain homogenates, but spectrophotometric measurements of DPN-cytochrome c reductase activity failed to show any unique sensitivity of this step to central nervous system depressants(4). Partial purification of the members of the cytochrome chain: DPN-cytochrome c reductase, cytochrome oxidase, and various dehydrogenases from brain, liver, and heart muscle did not disclose any enzyme which could be specifically inhibited by a reasonable

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concentration of the depressant drug(5). Furthermore, the recent work of Slater(6) subjects the earlier findings to reinterpretation. Using whole homogenates, no effect of narcotics on the efficiency of phosphorylations has been demonstrated(4,7), although such an effect could be shown with certain hypothermic drugs(8). The next logical step was the investigation of the effects of the narcotics on the phosphate:oxygen (P:O) ratios obtainable from the so-called particulate tissue preparations. This is a preliminary report on the effects of certain of the barbiturates on these preparations.

Experimental. Methods of tissue preparation and measurement of activity were based upon those now commonly used(9). A 10% homogenate of rat liver was prepared in 0.88 M sucrose with a Potter-Elvehjem homogenizer. The brain preparations were made in a similar manner except that 0.70 M sucrose, 0.20 M nicotinamide and 0.01 M sodium phosphate, pH 7.4, was used as a homogenizing medium. The homogenate was centrifuged (1500 x g for 3 minutes) and the resulting supernatant recentrifuged twice. The final supernatant was centrifuged (18,000 x g for 20 minutes), the residue resuspended in fresh homogenizing medium and recentrifuged at high speed as above. The final residue was resuspended in distilled water (2 ml/4 g tissue) and immediately pipetted into chilled Warburg vessels containing the materials as indicated in the legend of Fig. 1. All operations to this point were carried out at 5°C or below using cold reagents. The flasks were then placed on the manometers and equilibrated in the bath at 18°C for 10 minutes before starting to measure oxygen uptake. Enzymatic action was stopped by tipping in trichloracetic acid at the end of the desired period. Initial inorganic phosphate levels were obtained from vessels deproteinized when the oxygen uptake measurements were started (zero time). We were able to run experiments for as long as 70 minutes at 18°C without a decrease in the P:O ratios. Aliquots of the flask contents were analyzed for inorganic phosphate(10) and the net inorganic phosphate uptake determined by the difference between experimental flasks and the zero-time flasks. P:O ratios were calculated from these results and the concomitant oxygen uptake.

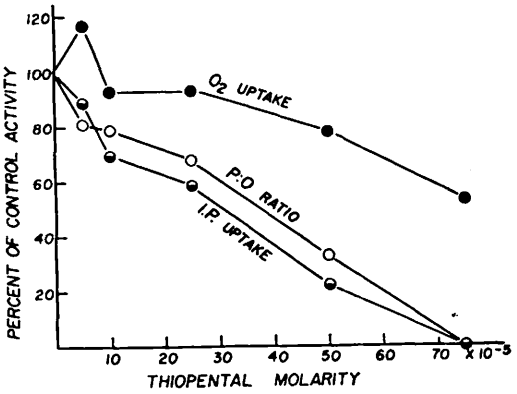

FIG. 1.

Effect of thiopental on oxidative phosphorylation in rat liver particulate preparations.

Vessel contents: 1.5 × 10^{-2} M Na-glycylglycine, pH 7.4, 2.0 × 10^{-2} M Na-phosphate, pH 7.4, 2.8 × 10^{-2} M glucose, 5.0 × 10^{-2} M KCl, 8.0 × 10^{-3} M $MgCl_2$, 2.5 × 10^{-3} M K-adenosine triphosphate, 8.3 × 10^{-6} M cytochrome c, 1.3 × 10^{-2} M Na-pyruvate, 4.3 × 10^{-3} M Na-fumarate, .1 ml yeast hexokinase (step 3a)(15), barbiturate concentration as indicated, .4 ml tissue preparation (see text), 1.2 × 10^{-2} M NaF, total volume 2 ml. Constituents added in the order given. .2 ml 50% trichloroacetic acid in sidearm. .2 ml 2 M KOH and filter paper in center well. Gas phase—air. Temperature—18°C. Duration of run 30, 40, or 60 min. (see text). Each point is the average of 3 separate experiments, percent activity for any individual vessel being calculated from its respective control. With regard to controls—from 13 separate experiments with a total of 24 individual vessels we obtained P:O ratios ranging from 2.29 to 3.12 with average of 2.65.

Results and discussion. We used thiopental, pentobarbital, amobarbital and phenobarbital and found that barbiturates are effective uncoupling agents. At a level of 1 x 10^{-4} M thiopental (Fig. 1) there is little or no decrease in the rate of respiration and yet the

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TABLE I. Effect of Barbiturates on Oxidative Phosphorylation in Particulate Tissue Preparations.*

Drug molarity	Rat liver								Rabbit brain		
	Pentobarbital			Amobarbital			Phenobarbital		Thiopental		
	O_2†	I.P.†	P:O†	O_2	I.P.	P:O	O_2	I.P.	P:O	O_2	I.P.	P:O
Control	6.2, 5.2, 7.0	14.4, 12.8, 18.8	2.3, 2.4, 2.4	5.8, 4.4	16.4, 13.6	2.8, 3.1	7.1, 4.6	19.2, 11.2	2.7, 2.5	4.8, 3.8	9.6, 8.0	2.0, 2.1
"	5.5	12.8	2.3	4.2, 4.5	11.6, 12.8	2.8, 2.8	6.2, 5.2	16.8, 12.8	2.7, 2.4	4.4, 3.2	8.8, 7.2	2.0, 2.3
1×10^{-5}	6.9	16.0	2.3									
5	7.2, 5.4	17.6, 13.6	2.4, 2.5	4.7	12.8	2.7	4.4	11.6	2.7	5.5, 3.2	8.0, 6.4	1.6, 2.0
1×10^{-4}	5.5, 5.4, 5.3	13.6, 13.6, 12.0	2.5, 2.5, 2.2	4.2	11.2	2.7	3.9	11.6	2.9	4.3, 3.2	8.0, 6.0	1.8, 1.9
2.5	6.7, 4.6	14.4, 10.6	2.1, 2.3	4.2	10.0	2.4	4.3	10.4	2.4	4.6, 3.1	5.6, 5.6	1.4, 1.8
5	2.9, 1.5	1.6, 0.8	0.6, 0.5	1.7	1.2	0.7	4.0	8.8	2.2	3.7, 3.2	2.4, 2.2	0.6, 0.8
7.5	1.4, 0.4	+2.4, +3.2	0.0, 0.0	0.6	+2.4	0.0	3.9	9.6	2.1	3.6, 1.8	0.0, 0.0	0.0, 0.0
1×10^{-3}	0.9, 0.4, 0.0	+4.0, +4.0, +2.4	0.0, 0.0, 0.0	0.0, 0.2	0.0 +2.4	0.0, 0.0	5.7, 3.5	9.2, 8.0	1.6, 2.2	2.9, 2.2	0.0, 0.0	0.0, 0.0

* Conditions and vessel contents same as indicated for Fig. 1, except in the case of the brain system which contained in addiiton 5.0×10^{-4} M diphosphopyridine nucleotide.

† Oxygen and inorganic phosphate uptakes expressed as microatoms.

‡ Phosphate:oxygen ratios calculated as described in text.

P:O ratio is depressed. With only moderately decreased (20-40%) respiration at concentrations of 5.0 to 7.5 x 10^{-4} M thiopental, the most potent of the barbiturates, almost completely inhibits phosphate uptake. For a lesser effect greater amounts (1.0 x 10^{-3} M) of phenobarbital were required (Table I). These effects were observed both in rat liver and in rabbit brain particulate preparations (Table I), but in the latter the P:O ratios were somewhat lower and consequently the results not optimal.

Like Persky *et al.*(3) we have tested the effect of the four barbiturates on the yeast hexokinase "trapping" system and find no inhibition at levels as high as 5 x 10^{-3} M. Hence, the uncoupling effect must be due to a depression of some prior transphosphorylation.

The chief objection to attributing the *in vivo* effects of narcotics to their inhibition of respiration detected *in vitro* is the relatively high concentrations of drug required in the latter case to produce effects of significant magnitude(3,12). From the minimum surgical anesthetic doses given by Gruhzit, *et al.*(13) for rabbits, assuming the distribution of an intravenous dose of barbiturate to be equal throughout the body, we have calculated the corresponding molar concentration in the tissue. For thiopental, amobarbital and pentobarbital these concentrations are 8.0 x 10^{-5} M, 2.5 x 10^{-4} M, and 2.0 x 10^{-4} M respectively. There is no great disparity between these values and those which gave a significant depression of the P:O ratio, namely: 1.0 x 10^{-4} M, 5.0 x 10^{-4} M and 5.0 x 10^{-4} M.

The uncoupling effects reported here are not nearly as striking as those which are observed with dinitrophenol and gramicidin(14) possibly because there is more overlap between the

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concentrations of barbiturates necessary to inhibit phosphorylation and those necessary to inhibit respiration.

McElroy(12) proposed that narcotics act not by merely depressing the respiratory rate of nervous tissue but by inhibiting some energy-yielding process, notably phosphate bond turnover, thereby depressing or abolishing neuronal activity. A recent paper by Westfall(15) indicates that small doses of barbiturates produce a small increase in the oxygen consumption of rat cerebral slices. Our data show a similar effect with particulate preparations (Fig. 1). Thus, barbiturates are no exception to the suggestion of Lardy and Elvehjem(16) that compounds which speed up the metabolic rate at the same time decrease the available energy by allowing respiration to take place without phosphorylation.

Further evidence that the mere depression of the respiration of the brain is inadequate to explain the pharmacologic effects of these compounds is shown by comparing the metabolic deficiencies of the brain in hypoglycemic coma and in thiopental anesthesia(17). In the former, metabolic rate and nervous activity fall equally; while in the latter, the fall in metabolic rate is small when the narcosis is deep. Larrabee, *et al.*(18) have also shown in studies on excised superior cervical ganglia that nervous activity may be depressed without a proportionate fall in oxygen consumption. All of which indicates that barbiturates may depress nervous activity *in vivo* by uncoupling oxidation from phosphorylation.

Summary. It has been shown that thiopental, pentobarbital and amobarbital lower the phosphate : oxygen uptake ratio obtained in particulate tissue preparations from liver and brain respiring on a pyruvate substrate. This depression is obtained at concentrations of the drugs which have only a slight effect on the oxygen uptake of the preparations and which approximate those which are necessary *in vivo* to produce surgical anesthesia. It is postulated that such an uncoupling of phosphorylation from oxidation may be one of the ways in which barbiturates act to produce anesthesia.

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Chemical Composition of Bovine Spermatozoa. (18675)

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Information on the chemical composition of spermatozoa is fundamental to a better understanding of the functional significance of the male gamete in the fertilization process as well as its contribution to subsequent developmental processes. There have been in the past several investigations dealing with various aspects of this problem. The work of Kossel(1) on the protamines of fish sperm in his researches dealing with the structure of basic proteins is an example. More recent-

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