White Carneau pigeons for 20 days without eliciting crop milk secretion. It appears therefore, that a species difference exists in the ability of these drugs to influence prolactin secretion and perhaps other hormones which regulate mammary growth and lactation.

Summary. 1. A single intravenous injection of 1 mg reserpine kilo body weight into 5 mature female rabbits, followed by sacrifice 1 week later, resulted in at least a 5-fold increase in pituitary prolactin content over 5 controls. There was no evidence that reserpine induced mammary growth or lactation. 2. Subcutaneous injections of 0.2 mg estradiol daily for 10 days into 6 rabbits elicited 1 week later at least a 10-fold increase in pituitary prolactin content, a slight growth of the mammary duct system and no milk secretion. 3. When estradiol treatment was followed by a single intravenous injection of reserpine (above dose) and the rabbits killed 1 week later, pituitary prolactin content was at least 16 times greater than in controls. This was accompanied by a considerable degree of lobule-alveolar development and a limited amount of milk secretion in all 6 animals.

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Potentiation of Barbiturate Hypnosis by Certain Uncoupling Agents.*†‡ (23867)

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The hypothesis that barbiturates produce their depressant effects by uncoupling oxidative phosphorylation has been proposed(1-4). An objection to this premise is the observation that other uncoupling agents are not hypnotics or anesthetics in the intact animal. The possibility that many uncoupling agents fail to depress *in vivo* because of limited penetrability into the central nervous system has been discussed(4). While most uncoupling agents possess little or no hypnotic properties *per se,* the present study will demonstrate that they potentiate hypnosis induced by the barbiturates. The uncoupling agents studied were: 2,4-dinitrophenol, (DNP) 2,4-dichlorophenoxyacetic acid (2,4-D) and chlorotetracycline (Aureomycin).

Methods. Sleeping time was measured in white mice of the Harlan strain of both sexes weighing 18 to 25 g. The test drugs or saline solutions were made up in such a manner as

* This study was initiated at Department of Pharmacology, University of Illinois, Chicago, and a preliminary report presented at Fall Meeting of Am. Soc. for Pharmacol. and Exp. Therap., Madison, Wisc., 1952.

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to administer the same volume/unit weight to all animals. All test drugs or saline were administered to mice 30 minutes prior to the challenging dose of the barbiturate with the exception of the adrenergic blocking agents which were administered 1 hour prior to the barbiturate. The "sleeping time" was designated as the interval between the loss and return of the righting reflex, or more specifically, the "on side time." To minimize variations in sleeping time from external stimuli, a regularly intermittent jet of air was played over all animals. The sequence of injections for each treatment time was randomized. In each experiment, injections were made by one person and observations performed and recorded by another. Separate additional control groups were sometimes run at the end of an experiment (Table I). The statistical significance of the differences between various groups was analyzed by the conventional "t" test in Tables I and II. In the case of Table III the experimental design was arranged so that the results could be statistically evaluated by the use of factorial coefficients in the analysis of variance. Specifically, equal numbers of animals were used in all treatment groups, and each experiment contained representative cells from each treatment group. Sprague-Dawley rats were used in studies designed to determine whether or not the uncoupling agents interfered with mechanisms for barbiturate degradation. The animals in this study were pretreated with 20 mg/kg I.P. of DNP or saline in equal volume/unit weight. Forty minutes after pretreatment, 40 mg/kg of secobarbital was administered intravenously. Blood samples were obtained from tail vein at 5, 10, 20, 40 and 60 minutes after administration of barbiturate and analyzed. Blood secobarbital levels were determined by a modification of the method of Brodie *et al.*(5). Similar experiments were carried out in mice except in this instance serial samples from a single animal could not be used because of blood volume required for analysis. Consequently, groups of animals were sacrificed at each time point and comparison between groups was made rather than utilizing a given animal as its own control. Mice were given various doses of DNP and sacrificed by decapitation at various times as indicated in Table IV. The brains were rapidly removed, weighed, and homogenized in water. Blood was collected in heparinized tubes and centrifuged at 8000 x g for plasma recovery. Suitable aliquots of homogenate and plasma were taken for DNP estimation using the analytical method of Mudge and Taggart(6).

TABLE I. Potentiation of the Hypnotic Effect of Barbiturates by Certain Uncoupling Agents.*

No. animals	Uncoupling agent	Dose, mg/kg	Sleeping time, min.†	"P"‡
Exp. 1				
20	None		24.4 ± 3.3	
20	Dinitrophenol	20	46.0 ± 2.6	<.001
20	Chlorotetracycline	50	38.8 ± 4.7	<.05
Exp. 2				
25	None		25.0 ± 3.4	
25	Dinitrophenol	20	45.8 ± 2.2	<.001
25	Chlorotetracycline	50	43.2 ± 5.6	"
25	2,4-dichlorophenoxyacetate	50	63.9 ± 5.1	"
10	None	50	23.8 ± 1.6	n.s.
Exp. 3				
16	None		31.6 ± 3.8	
8	Dinitrophenol	20	51.2 ±10.6	<.05
8	Chlorotetracycline	25	28.4 ± 5.6	n.s.
8	"	50	73.4 ±13.7	<.01
16	2,4-dichlorophenoxyacetate	50	71.3 ± 6.0	<.001
8	None		49.3 ± 7.4	<.05
Exp. 4				
10	None		0 (ataxia only)	
20	Dinitrophenol	20	5.2 ± .44	

* All animals were given a challenging dose of 50 mg/kg secobarbital (amobarbital in Exp. 4) 30 min. after pretreatment with the indicated uncoupling agent as described under Methods.

† Expressed as mean ± stand. error.

‡ 1st set of barbiturate controls *vs* the indicated group.

Results. Under the experimental conditions described and the dosages employed, all uncoupling agents used, 2,4-dinitrophenol (DNP), chlorotetracycline and 2,4-dichlorophenoxy acetic acid, (2,4-D) significantly increased sleeping time of mice treated with secobarbital. Sleep also was observed in animals which were pretreated with DNP and subsequently challenged with a dose of 50 mg/kg of amobarbital injected intravenously. This dose of amobarbital produced only a slight ataxia in a group of mice pretreated with isotonic saline. These data are shown

TABLE II. Effect of Various Doses of DNP on Hypnotic Effect of Secobarbital,* 11 Animals per Series.

DNP, mg/kg	Sleeping time, min. (mean ± S.E.)	"P"†
None	13.0 ± 2.33	
5	29.0 ± 5.67	<.01
10	35.0 ± 5.71	<.001
15	36.0 ± 5.71	"
20	49.0 ± 5.02	"
None	19.0 ± 3.12	

* All animals were given a challenging dose of 40 mg/kg secobarbital 30 min. after pretreatment with the indicated dose of DNP as described under Methods.

† Combined secobarbital control groups *vs* secobarbital + DNP.

in Table I. In Exp. 3 the control group run at end of the day differed significantly from control group run earlier. Also this control group did not differ significantly from the DNP treated group although it did differ significantly from the chlorotetracycline and 2,4-D treated groups. Since this happened on only one occasion we have not given this result much weight. Pretreatment with DNP at 5, 10, 15 and 20 mg/kg and subsequent administration of a standard dose of secobarbital progressively increased duration of sleeping time (Table II). At the higher doses of DNP, some animals were hyperthermic, and a slight depression of normal activity could be observed prior to administration of the barbiturate.

Pretreatment of animals with the adrenergic blocking agents, dibenamine and yohimbine, failed to antagonize the increase in sleeping produced by the uncoupling agent. The adrenergic blocking agents *per se* significantly prolonged the duration of sleeping time when compared with saline-pretreated mice. In combination with DNP both dibenamine and yohimbine were apparently additive in enhancing these depressant effects (Table III).

Side effects noted with administration of the blocking agents were hyperactivity with yohimbine and slight depression with dibenamine. In combination with DNP, neither agent modified the hyperthermia produced by the administration of DNP. The gross effects on motor activity produced by the blocking agents were unchanged by the administration of DNP.

With both rats and mice no significant difference was noted in the disappearance of barbiturate from the blood of control animals and animals previously treated with DNP. The variability was quite high in mouse experiments, but in rat experiments where each animal was his own control more consistent results were obtained.

Measurable amounts of DNP reach the brain, maximum concentration observed 30 minutes after i.p. administration (Table IV). Since we did not obtain very good recoveries (perhaps because we did not use mechanical shaking for extractions) the values given in Table IV must be minimal. In animals sacrificed by cardiac puncture the blood content of brain was approximately 0.02 ml/g(7). Thus 5.8 of the 17.9 millimicromoles DNP observed in brain at a 20 mg/kg dose might be from the blood content. However, correction for recovery would just about balance correction for blood content and we conclude that the brain concentration would be about 1.5×10^{-5} M at 20 mg/kg.§

Discussion. It has been shown that bar-

TABLE III. Combined Hypnotic Effect of DNP, Adrenergic Blocking Agents and Secobarbital.

No. animals	Adrenergic blocking agent, mg/kg I.P.	DNP, mg/kg	Secobarbital, mg/kg	Sleeping time, min., mean ± S.E.
	Dibenamine			
27	50	None	40	30.5 ± 9.6
27	50	15	"	39.8 ±12.7
27	None	None	"	17.9 ± 5.4
	Yohimbine			
27	15	None	40	26.0 ± 7.9
27	15	15	"	37.8 ± 6.8
27	None	15	"	28.7 ± 9.1

DNP *vs* no DNP, "P" = <.001.
Adrenergic blocking agent *vs* no adrenergic blocking agent, "P" = <.001.
Yohimbine *vs* dibenamine—not significant.

§ Goldman, Laatsch, Molison, Urata and Hunter at Washington University in St. Louis observed blood levels of about 80 millimicromoles/ml and brain levels of about 3.5 millimicromoles/g in rats after 40 mg/kg i.p. doses (personal communication). We have no explanation for the difference between our results and theirs.

TABLE IV. 2,4-Dinitrophenol in Mouse Brain and Plasma (Effect of Dose and Time).

Values expressed as millimicromoles/g tissue or/ml plasma and are averages of determinations on No. of animals given in parentheses.
Avg recovery from tissue in 10 experiments was 65% (range: 51-86%); from plasma in 3 experiments was 80%. Tabular values are not corrected.

	Dose†			
	10 mg/kg	20 mg/kg	40 mg/kg	60 mg/kg
Brain	7.0*(4)	17.9(8)	56.4(8)	128.6(2)
Plasma	139 (2)	290 (6)	425 (8)	822 (2)

	Time‡			
	15′	30′	45′	60′
Brain	47.7 (2)	51.3(2)	32.6(2)	28.0(2)
Plasma	518 (2)	440 (2)	417 (2)	272 (2)

* Method will detect approx. 8 mμmoles; tissue and plasma blanks are essentially zero. However, the 10 mg/kg tissue value is not reliable.

† Animals were given the doses of NaDNP indicated intraper. 30 min. prior to sacrifice by decapitation.

‡ Dose, 40 mg/kg.

biturates uncouple oxidative phosphorylation *in vitro*, and it has been postulated that this uncoupling is a basis of the central nervous system depressant action of the barbiturates. 2,4-dinitrophenol also uncouples oxidative phosphorylation *in vitro*, but when given alone *in vivo* shows very little depressant activity. Only a small portion of a given dose of DNP reaches the central nervous system. The concentration observed at 20 mg/kg (1.5×10^{-5} M) is sufficient to produce a pronounced uncoupling effect *in vitro*(3). The effectiveness of this concentration on metabolic processes *in vivo* is a matter for conjecture. However, if an animal is under the influence of a barbiturate, then the small amount of DNP which does reach the brain may exert a noticeable depressant effect by the addition of the uncoupling effects of these two agents. This explanation could account for the present findings. DNP is a somewhat more potent uncoupling agent *in vitro* than the other two uncoupling agents hence we used somewhat higher doses of chlorotetracycline and 2,4-D in the present experiments. Our data are not sufficiently extensive, however, to permit any correlation of potentiation potency *in vivo* with uncoupling potency *in vitro*.

A large number of compounds has been shown to potentiate barbiturate anesthesia. These include: glucose(8); potassium ion (9); epinephrine, lactate, glutamate and other carbohydrate intermediates(10,11); SKF 525 A(12); dimercaprol(13); antabuse(14); and isoniazid(15). The mechanisms by which the above compounds potentiate barbiturate anesthesia do not include the uncoupling of oxidative phosphorylation. The potentiation of barbiturate anesthesia by the carbohydrate intermediates may be mediated by epinephrine. Dimercaprol, SKF 525 A and isoniazid apparently interfere with systems concerned with barbiturate detoxication, while potentiation with antabuse can be demonstrated for many depressants unrelated to the barbiturates(16). We have attempted to rule out the possibility that the uncoupling agents are potentiating anesthesia *via* an epinephrine release by use of adrenergic blocking agents. The latter compounds failed to prevent the potentiating effects of the uncoupling agents and actually increased barbiturate sleeping time, an interesting finding in itself. Our studies on blood barbiturate levels after DNP would also seem to negate the possibility that an inhibition of barbiturate degradation is involved. Chlorpromazine, a compound which will also potentiate barbiturate anesthesia, has been reported to uncouple oxidative phosphorylation(17), although the potentiation observed with this compound might obviously be attributable to its adrenergic blocking properties.

The authors recognize that explanations other than addition of uncoupling effects are possible to account for the observed potentiation. We have attempted to eliminate effects of detoxification and epinephrine release. Studies on possible potentiation of the anesthetic effects of classes of drugs other than the barbiturates and the use of other uncoupling agents may yield data germane to the general problem. We are further investigating metabolic changes brought about by these drugs both singly and in combination to obtain direct data bearing on the question of uncoupling *in vivo*.

Summary. It has been demonstrated that certain uncoupling agents increase the hypnotic effects of certain barbiturates. Potentiation by uncoupling agents is not the result

of inhibition of detoxication mechanisms nor is epinephrine release implicated since the effect is not prevented by adrenergic blocking agents. The nature of this effect and its relationship to the mechanism of action of the barbiturates is discussed.

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Turnover of 5-Hydroxytryptamine (Serotonin) in Tissues.* (23868)

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Development of sensitive and specific analytical methods for serotonin (5-hydroxytryptamine)(1,2) has made it possible to demonstrate its presence in many tissues. Thus, although the bulk of serotonin is found in mucosa of gastro-intestinal tract, significant amounts are also found in blood platelets, brain, spleen and lung. Enzymes involved in serotonin formation and destruction are present in all depots, except blood platelets, indicating that the amine is apparently made, released and destroyed for specific functions in these depots. It is hoped that these studies on turnover of serotonin in various tissues may be helpful in elucidating the nature of these functions.

Materials and methods. A solution of serotonin hydrochloride was prepared in the following manner. Serotonin creatinine sulfate was dissolved in water adjusted to pH 10 and the solution saturated with NaCl. Serotonin was extracted into n-butanol, and after addition of heptane it was reextracted into dilute HCl. The serotonin concentration was standardized colorimetrically. *DL*-tryptophan 2-C^{14} (specific activity 0.3 $\mu c/\mu M$) was obtained from Tracerlab Inc. Radioactive 5-hydroxytryptophan (5HTP) was synthesized in this laboratory following procedure outlined by Ek and Witkop(3). The amino acid was labelled in the β-carbon of the side chain and had a specific activity of 0.1 μc μM. Radioactivity measurements were made with Robinson gas flow proportional counter having background of 2 counts/minute. Counts were corrected to constant weight, based on experimentally determined self-absorption curve, and expressed as counts/minute/μmole of compound. Serotonin in tissues was isolated by previously

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