and that this effect is very strongly dependent on dose rate.

The most plausible explanation of direct relation between dose rate and damage to oocytes is that some repair is occurring. The mechanism by which radiation kills oocytes is not known. Since the cells do not divide, chromosome imbalance cannot be the cause of death. Oakberg(3) finds the early oocytes to be killed very rapidly. Our experiments, reported elsewhere(16), indicate that the sensitive system, whatever its nature, is not markedly protected by hypoxic conditions during irradiation.

Summary. 1) In an attempt to contribute to an understanding of unusual radiation sensitivity exhibited by the mammalian oocyte, a non-dividing cell, fertility experiments were carried out to determine the effect of dose rate (fractionation, different intensities of continuous radiation). 2) Fractionation markedly reduced damage to fertility; and division of dose into 10-r fractions was more effective in this respect than division into 25-r fractions. Continuously exposed females were even less affected in breeding performance than females which had received fractionated irradiation, and the lower the dose rate the smaller was the deleterious effect on fertility. In all groups, production, in terms of number of females casting litters, remained at maximum level until beginning of a sudden steep decline that ended in sterility. It is for this reason that some experiments of other investigators, who have measured performance only in terms of the first postirradiation litter, have failed to show similar dose-rate effects. 3) The results indicate that some repair of radiation damage to oocytes can occur, and that repair is greater at lower dose rates.

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Comparison of Physostigmine and Amphetamine in Antagonizing the EEG Effects of CNS Depressants.* (25289)

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Many investigators have shown in animals that cholinergic drugs activate EEG by exciting subcortical centers(1-3). Other experiments reveal that adrenergic drugs also induce EEG activation by acting on the same areas of the brain(2-5). This indicates that the

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reticular activating system(6) contains both cholinergic and adrenergic sensitive neurons. The finding that chlorpromazine will block EEG action of amphetamine but not that of physostigmine(7) suggests that chlorpromazine inhibits adrenergic sensitive neurons but not cholinergic ones. It seemed of interest, to ascertain whether other neurosedatives share this property of chlorpromazine and to further study the effectiveness of physostigmine to antagonize EEG effects of CNS depressants.

Methods. Unanesthetized albino rabbits weighing 1.7 to 3.5 kg were employed. Gross behavioral and electroencephalographic changes were observed simultaneously. Electroencephalograms were obtained(3) by inserting steel electrodes into the cranium over the right motor, left motor, left limbic, and left occipital areas of the cortex and recorded by a Grass Polygraph. For brevity, only tracings from the left motor area will be illustrated. Blood pressure was recorded from one femoral artery by mercury manometer and the respiratory rate as well as other physiological phenomena were observed. Drugs in this study were chlorpromazine HCl, pentobarbital Na, meprobamate, phenaglycodol, physostigmine salicylate, and *d*-amphetamine sulfate. Meprobamate was administered as 2.5% supersaturated solution cooled to about 45°C before injecting. Phenaglycodol was dissolved in 50% polyethylene glycol 400 vehicle. Other drugs were dissolved in distilled water or 0.9% saline. All were injected into marginal ear vein, and those that produce EEG synchronization were usually administered first. Five to 10 minutes subsequently a single injection of either physostigmine or *d*-amphetamine was made. Doses of drugs are shown in Table I. In another series of animals, physostigmine or *d*-amphetamine was administered alone and their effects noted.

TABLE I. EEG Changes Produced by Physostigmine and *d*-amphetamine on the Sleep Pattern Induced by Pentobarbital, Chlorpromazine, Phenaglycodol, and Meprobamate.

Neurosedative		Physostigmine and *d*-amphetamine		
Drug	Dose, mg/kg	Drug	Dose, mg/kg	No. activated per total
Pentobarbital	5	Amph.	1–2	0/3
		"	3–4	2/2
		Phys.	.1–.2	2/2
	10–20	Amph.	2–7	2/10
		"	10–20	7/8
		Phys.	.2–.3	14/18
Chlorpromazine	1–2	Amph.	3–5	4/9
	3–5	"	5	2/6
		"	10	3/3
	5–10	Phys.	.1–.2	8/8
Phenaglycodol	25–50	Amph.	1–3	0/4
		"	5	3/3
		Phys.	.1–.2	4/4
	60–80	Amph.	2–4	1/4
		Phys.	.1–.2	2/2
Meprobamate	40–70	Amph.	3–5	5/7
	80–140	"	2–3	0/6
		"	4–10	6/6
	100	Phys.	.2	3/3

Results. EEG and behavioral effects of neurosedatives. Activation EEG pattern was manifest in control records of most animals (Fig. 1, 2). All CNS depressants changed this alert tracing to a synchronous one (Fig. 2). Gross behavioral effects of these drugs were similar, since, as the dose increased, signs of depression became progressively more marked. Pentobarbital was more predictable in this regard than were other CNS depressants studied but even with this agent variations occurred from animal to animal. A few animals were given 25-30 mg/kg doses but an isopotential EEG resulted. They are, therefore, not included in Table I. Occasionally a low dose of chlorpromazine (1-2 mg/kg) would induce an EEG synchronization without concomitantly causing flaccid paralysis observed with higher doses (3-10 mg/kg) and in 2 animals given 60 mg/kg of meprobamate, no behavioral or EEG changes were seen until an additional 40 mg/kg was injected. These descriptions illustrate variations in response occasionally observed. There was no correlation between central effects of these drugs and changes in blood pressure nor were these changes significant enough to induce EEG changes in the rabbit(8,9). Furthermore, subsequent administration of amphetamine or physostigmine produced changes in blood pressure and respiration independent of EEG effects observed.

Amphetamine. Behavioral and EEG antagonism to effects of neurosedatives. When given alone, small doses (0.5 mg/kg) of *d*-

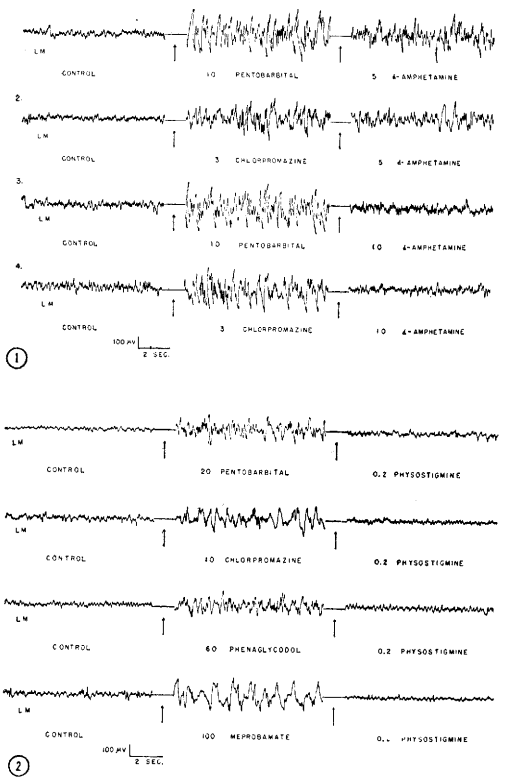

FIG. 1. Tracings 1 and 2 show that both pentobarbital and chlorpromazine can abolish the usual EEG effect of a 5 mg/kg dose of *d*-amphetamine. Tracings 3 and 4 show, however, that in higher doses amphetamine can reverse the EEG pattern induced by these agents. Numbers below records are mg/kg inj. Leads: left motor cortex (LM).

FIG. 2. Fig. shows that physostigmine in small doses (right tracings) can antagonize the EEG effects of high doses of pentobarbital, chlorpromazine, phenaglycodol and meprobamate (center tracings). Numbers below records are mg/kg inj. Leads: left motor cortex (LM).

amphetamine increased irritability of animals to stimuli, and changed a predominantly synchronous control EEG tracing in 7 to an alert one and in 3 mg/kg doses or more produced hyperactivity. Neurosedatives, in doses employed, prevented EEG (Fig. 1, Table I) and behavioral effects of small doses of amphetamine. Amphetamine if given in large enough doses, however, antagonized the EEG effect of CNS depressants (Fig. 1) and concomitantly awakened the animal. The dose of amphetamine necessary to reverse central effects of CNS depressants, paralleled the dose of the latter given (Table I). This was most evident with pentobarbital, which in higher doses increased the dose of amphetamine necessary to produce EEG activation from 20 to 40 times above that required in animals given amphetamine alone.

Physostigmine. Behavioral effects and antagonism of EEG effects of neurosedatives. When physostigmine was given alone in 0.1 mg/kg doses, it evoked EEG activation pattern in 6 of 7 rabbits which predominantly manifested a synchronous EEG control record, though otherwise they looked normal. Smaller doses produced this EEG change less consistently. In low doses (0.1 to 0.2 mg/kg) physostigmine rarely (15% of animals) produced peripheral effects such as fasciculation. In contrast to amphetamine, physostigmine (Fig. 2) in low doses antagonized the EEG patterns produced by high doses of meprobamate, phenaglycodol, chlorpromazine and pentobarbital. In some pentobarbital injected animals an additional injection was necessary to completely reverse the EEG synchronization. Physostigmine slightly elevated muscle activity in animals previously given meprobamate, phenaglycodol, chlorpromazine and low doses of pentobarbital although unlike amphetamine never provoked hyperactivity. Following higher doses of pentobarbital (15 to 20 mg/kg), physostigmine did not produce overt effects though EEG activation pattern was present for approximately 30 minutes.

Discussion. Either physostigmine or amphetamine (in sufficient amounts) antagonized the synchronous EEG pattern produced by meprobamate, phenaglycodol, chlorpromazine and pentobarbital in rabbits. However, when given after CNS depressants, amphetamine usually produced significant behavioral effects concomittant with EEG arousal, whereas physostigmine evoked EEG activation without necessarily inducing behavioral changes. Apparently physostigmine can excite corticipetal pathways of the reticular activating system(6) with little or no effect on lower parts of the neuraxis. Similarly, physostigmine does not induce signs of behavioral excitement (e.g., struggling) in cerveau isolé animals whereas amphetamine does (3). Interestingly, the physostigmine antagonists atropine and scopolamine have the

opposite effect in rabbits; they block corticipetal activation pathways but not centrifugal ones (which provoke motor responses (10)).

Our findings suggest that EEG alerting mechanism is more sensitive to physostigmine than to amphetamine. Thus, in high doses, the neurosedatives increased the minimal dose of amphetamine necessary to produce its usual EEG effect by at least 4 times and with pentobarbital from 20 to 40 times (Fig. 1). In contrast, physostigmine in low doses antagonize the electrocortical effects of CNS depressants (Fig. 2). Such observations support the conclusion of Rinaldi and Himwich(1) that the reticular formation EEG activating system is fundamentally cholinergic in rabbits. The fact that low doses of atropine will block the EEG effect of adrenergic drugs but not that of physostigmine(3,11) further supports that conclusion. Higher doses of atropine will, however, prevent the EEG arousal caused by physostigmine(3,11), DFP and Ach(9).

EEG and behavioral antagonism between i.v. administered amphetamine and pentobarbital is in agreement with the use of the former as an analeptic in barbiturate poisoning(12) but is in contrast to the findings of Bradley and Elkes in cats(11). Moreover, these investigators did not find that physostigmine antagonized the EEG synchrony induced by pentobarbital. Their experiments differed from ours, however, in dosage and, in one instance, the kind of barbiturate employed and also in the form of amphetamine, route of administration (i.p.) and species employed. The observation that EEG effects of meprobamate(13) and chlorpromazine(7) can be reversed by physostigmine has been confirmed and extended to include higher doses of these drugs. The report(7) that chlorpromazine can block the EEG effect of amphetamine was also confirmed but our findings further reveal that this phenomenon is dose dependent since in higher doses amphetamine will break through this blockade. In this regard chlorpromazine resembles pentobarbital (Fig. 1).

Since all of CNS depressants (especially pentobarbital) reduced effectiveness of amphetamine to activate the EEG alerting mechanism, it seems unlikely that the property of chlorpromazine to block central effects of epinephrine(4,14) and amphetamine(7) is related to its peripheral adrenolytic action as suggested by several workers(4,7). Also it is reported that atropine can block EEG activation normally produced by amphetamine(3, 11) and other adrenergic drugs including epinephrine(3) so that chlorpromazine is not unique in this regard. Lastly, both atropine (1,10) and chlorpromazine(8,14) can abolish EEG activation resulting from direct stimulation of the midbrain reticular formation. The last cited workers(14) concluded that their electrophysiological observations did not support the interpretation of Hiebel *et al.*(4) that the blockade of the central excitatory effects of epinephrine account for the action of chlorpromazine. Thus experiments other than those based on EEG responses are apparently necessary to define adequately whether a drug acts as an adrenolytic agent centrally.

Summary. 1) Pentobarbital, chlorpromazine, phenaglycodol and meprobamate when injected i.v. into rabbits produced a synchronous (high voltage low frequency) EEG pattern. Physostigmine or *d*-amphetamine, in suitable doses, antagonized this pattern and evoked an alert (low voltage high frequency) EEG tracing. The dose of amphetamine necessary to antagonize synchronous EEG record generally paralleled the dose of neurosedative administered, especially in the case of chlorpromazine and pentobarbital. In contrast, low doses of physostigmine usually exerted its EEG effect regardless of dose of CNS depressant employed. This indicates that the EEG activation mechanism is more sensitive to cholinergic than to adrenergic drugs and supports the conclusion of several authors that the EEG alerting mechanism is fundamentally cholinergic in rabbits. 2) The evidence indicates that the EEG effects of chlorpromazine are not necessarily related to its peripheral adrenolytic actions.

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Effect of Microbial Antigens on Irradiation Mortality in Mice.* (25290)

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Boivin-type endotoxins from *Proteus morganii* decrease mortality in x-irradiated mice (1,2). It is also known that endotoxins from *Salmonella typhosa, Escherichia coli,* and *Serratia marcescens* increase the survival of irradiated mice, rats, and hamsters(3,4). Since the chemically isolated lipopolysaccharide substances called endotoxins or toxic somatic antigens are thermostable antigenic components associated with the bacterial surface, bacterial vaccines and boiled culture supernatants, both of which would be expected to contain these somatic antigens, should also be effective in decreasing irradiation mortality. For this reason, the present preliminary investigation was initiated. We have found that mortality is definitely decreased by injection of Gram-negative antigens. Gram-positive antigens, however, appear ineffective in this respect. Other attempts to decrease mortality by means of bacterial antigens other than purified endotoxins have been unsuccessful (5).

Materials and methods. Thermostable antigens were prepared from *Klebsiella pneumoniae, Proteus morganii, Escherichia coli,* and an α hemolytic Streptococcus by heating saline suspensions of each organism for 1 hr in a boiling water bath. Following clarification, toxicity tests were performed on each of the sterile antigen solutions using non-irradiated mice. Groups of mice were injected intravenously with 0.1 ml of a sublethal dilution of each antigen 24 hr before irradiation. The following commercial (Parke, Davis) antigens were used: Streptococcus (Bio. 327), each ml containing saline extractable substances from 2×10^8 hemolytic and non-hemolytic Streptococci; Staphylococcus (Bio. 385), each ml containing soluble antigens from 1×10^9 *Staphylococcus aureus* and *Staphylococcus albus* mixed with Staphylococcus toxoid; Typhoid-Paratyphoid vaccine (Bio. 446), each ml containing 1×10^9 killed *Sal. typhosa,* 250×10^6 *Sal. paratyphi,* and 250×10^6 *Sal. Schottmulleri.* All of these antigens were used undiluted and given intravenously in a volume of 0.1 ml. A concentrated saline suspension of zymosan was heated in a boiling water bath for 1 hr, diluted as required with buffered saline (pH 7.0), and injected intravenously in a volume of 0.2 ml. Zymosan was obtained from Immunological Specialties, Los Angeles. Total body x-irradiation was delivered from a 200 kv Picker-Waite X-Ray therapy machine operated at 20 ma and a distance of 20 cm. Added filtration consisted of 0.5 mm Cu and 1 mm Al. Mice used were

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