TABLE III. Motility of Stored Sperm Collected from 8 Dogs Prior to and During Oral Administration of Chlorpromazine.

Period	Days stored at 5°C	Period prior to treatment	Beginning of treatment period			End of treatment period		
			Group A	Group B	Avg of A, B	Group A	Group B	Avg of A, B
			(% motile sperm)					
I*	1	68	70	80	75	75	78	76
	4	65	70	68	69	70	70	70
	8	57	55	55	55	62	68	65
	12	30	38	35	36	40	48	44
	Mean	55	58	59	59	62	66	64
II	1	—	72	78	75	72	75	74
	4	—	68	72	70	70	65	68
	8	—	60	50	55	58	58	58
	12	—	42	35	39	32	38	35
	Mean	—	61	59	60	58	59	58

* See footnote, Table II.

of longer duration and at higher levels of chlorpromazine are necessary before it can be determined whether or not this drug can interfere with reproductive function in the male dog.

Summary. Additions of promazine or chlorpromazine in concentrations below 200 μg/ml of media suitable for prolonged storage of dog sperm were without effect on sperm motility. Higher levels of these drugs were spermicidal. Oral administration of chlorpromazine to 8 dogs (4.4 mg/kg of body weight) every other day or daily for 9 days did not reduce the libido of dogs trained to routine semen collection procedures. Motility of the sperm collected was unaffected, and sperm output was inconsistently higher in the treated group. It is concluded that the *in vivo* levels of chlorpromazine studied did not alter the normal hypothalamic-pituitary-gonadal relationship.

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Lethal Effect of Chlorpromazine on the Chick Embryo.* (28689)

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Chlorpromazine influences a variety of experimental biological systems: it inhibits a number of enzymes and cell respiration, and alters cell and mitochondrial permeability (1-22); it inhibits cell division in fertilized echinoderm eggs and regeneration in planaria and in the axolotl(23); it induces abortion in the mouse and rat, and is a teratological agent in the rat(24-27). The present report describes the lethal effect of this compound on the development of the chick embryo.

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TABLE I. Lethality of Chlorpromazine to Developing Chick Embryos.

Time (hr) after incubation	No. of exp	CPZ (mg)	Living embryos at 17 days	% surviving at 17 days	Avg wt (g)
0	3	0	27/42	64	17.0
		1.8	12/38	32	16.1
		.9	16/41	39	16.9
24	3	0	26/42	62	17.2
		1.8	4/45	9	17.0
		.9	23/47	49	17.3
48	3	0	32/39	82	16.7
		1.8	8/38	21	13.3
		.9	13/39	33	16.0
72	3	0	38/42	90	18.0
		1.8	28/44	64	17.1
		.9	25/26	96	17.8
96	3	0	32/42	76	17.1
		1.8	34/40	85	17.3
		.9	31/42	74	17.6

0.2 ml sterile saline ± CPZ injected into the yolk sac at time indicated.

Materials and methods. Chlorpromazine (CPZ)† was dissolved in 0.9% saline, filtered through a millipore filter, and carefully neutralized with sterile 0.3 N NaOH to a pH of approximately 7.0. Two-tenths ml solution, equivalent to 0.9 or 1.8 mg CPZ, was injected with sterile precautions into the yolk sac of fertile White Leghorn eggs just prior to or at various intervals after incubation of the eggs at 39°C. Control eggs were injected with 0.2 ml sterile saline. The eggs were candled on the fifth and tenth day, and the dead embryos were examined for malformations. All eggs were opened on the seventeenth day of incubation; the embryos were weighed and examined for gross defects.

Results. Table I indicates that the chick embryo is most sensitive to CPZ 24 hours after the start of incubation: only 9% of the embryos treated at this time with 1.8 mg CPZ survive to the seventeenth day, and most of the deaths occur within 24 to 48 hours after drug injection. 0.9 mg of the drug is also toxic. Drug treatment prior to and 48 hours after incubation is almost equally destructive to the embryo. At 72 hours, however, CPZ at the concentration employed in these experiments has considerably less effect; it has no effect at 96 hours. Higher concentrations were not studied. Only injection of 1.8 mg at 48 hours significantly affected embryo weight: the average weight of the treated embryos at 17 days was 13.3 g, and the corresponding figure for the controls was 16.7 g.

Herniation of the viscera and eye defects, including complete absence of eyes or decreased pigmentation, were seen somewhat more frequently in embryos from treated eggs than in the controls. No other gross defects were noted.

Discussion. The vast literature on chlorpromazine and other phenothiazine derivatives indicates that these valuable psychotropic drugs have diverse pharmacological effects. However, the precise mechanism or mechanisms of action of the drugs remain obscure. The changes in membrane properties induced by phenothiazines may explain many of their properties, including the lethal effect on chick embryos. The special affinity of the bacterial membrane for CPZ has been demonstrated in experiments by Hancock and Miller(22) with S^{35}-labelled CPZ: 80% of the CPZ adsorbed by *B. megaterium* is localized in the membrane. The drug may possess a similar high affinity for membranes of other cells.

The destructive action of CPZ on the chick embryo is particularly surprising in view of the fact that the yolk contains approximately 10% phospholipids. In experiments with bacteria(28), lecithin was found to prevent the anti-bacterial effects of CPZ at lecithin/CPZ ratios of approximately 5/1, possibly by direct combination with the cationic portion of the CPZ molecule. A similar reaction may occur in the cell membrane.

Borg and Cotzias have recently reported that phenothiazines in pure solutions react with Mn^{++} and Fe^{+++} to form a semiquinone radical and have suggested that this reaction might explain some of the biological effects of the phenothiazines(29). The formation of a semiquinone radical at the expense of metallo-enzymes could, for example, seriously affect cell metabolism by interfering with electron transport and oxidative phos-

† Obtained through the courtesy of Smith, Kline and French Co., Philadelphia, Pa.

phorylation, or by altering the local availability of ions that are rate-limiting factors. The relevance of this attractive hypothesis to biological systems has not yet been adequately demonstrated.

Summary. Chlorpromazine, injected into the yolk sac of fertile hens' eggs, is lethal to a high percentage of embryos. At dosage levels of 0.9 and 1.8 mg the compound is destructive if it is administered within the first 48 hours of incubation; it shows little effect at 72 hours, and none at 96 hours.

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Urinary Excretion Pattern of Injected H³-Testosterone.* (28690)

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The 17-ketosteroids in urine reflect primarily the production of weak androgenic steroids from the adrenal cortex. It is not surprising that levels of 17-ketosteroids may be quite normal in the presence of various virilizing disorders. Because of this lack of correlation, greater interest has recently been focused on the metabolism of the potent androgen, testosterone. Hollander(1) identified testosterone in the spermatic vein of adult males and Finkelstein *et al*(2) were able to measure free testosterone in the plasma of normal subjects. Camacho and Migeon (3) and Rosner *et al*(4) have found small but measurable amounts of testosterone glucuronide in normal male urine.

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