Gastrulae were obtained in which the future crescentic blastopore was indicated only by heavier pigmentation or by a slightly puckered appearance of the cells. After removal of the jelly layers, but not the chorion, the embryos were placed in wax-bottomed watch glasses, with the blastopore uppermost. Bits of agar impregnated with Nile blue sulphate according to the method of Stone[4] were placed on the embryo and held firmly in place by a celluloid bridge. After half an hour the agar was removed and the embryo transferred to a dish of pond water. Two days later the animal was either dissected to locate the stain or was fixed by Yntema's method[5] for future sectioning. A total of 40 animals was thus studied, of which 27 were dissected, 8 were sectioned and stained, and 6 were discarded because the stain was too faint for accurate observation.

Results of the study show that the cells which later form the thyroid lie in the median plane and *within* the early blastoporal groove, slightly more toward its ventral lip. The location was clearly demonstrated in 14 embryos. Stain placed on either side of the midline (5 embryos) is found, if at all, only on one side of the thyroid bud. Stain placed dorsal to the groove (5 embryos) later lies in the anterior wall of the foregut, and stain placed ventral to the groove (8 embryos) is pushed caudad by the ventral union of the heart anlagen.

To Vogt's classic map it is therefore possible to add, within the early crescentic blastopore and in the midline of the embryo, the location of the small area which in *Hyla regilla* will give rise to the thyroid gland.

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Effect of Low Body Temperature on Toxicity of Drugs.

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Low body temperature is known to produce a stuporous condition in different animals, accompanied by extremely slow respiration and pulse rate, and a decrease in the basal metabolism. Little work has been done on the effect of toxic doses of drugs in animals whose body

[4] Stone, L. S., *Anat. Rec.*, 1932, **51**, 267.

[5] Yntema, C. L., *J. Exp. Zool.*, 1937, **75**, 75.

temperature has been artificially lowered. Dworkin *et al.*[1-3] injected convulsive doses of insulin in cats, woodchucks and dogs previously cooled by means of amytal and ice bath, and found that cooling inhibited convulsions. Koeninck[4] injected strychnine, picrotoxin, muscarine, pilocarpine, caffeine, apomorphine and tetanus toxin into a few *hibernating* bats. All drugs acted slowly, but produced typical symptoms and—except pilocarpine—deaths. Blanchard[5] injected cobra venom and some toxins into hibernating marmots and did not obtain any significant change of toxicity during hibernation. Pfeiffer, Foster, and Slight[6] found in hibernating animals a marked increase in the toxicity of strychnine and caffeine, 10-8 times respectively. Coramine and picrotoxin were twice as toxic, while the lethal doses of cocaine, metrazol and ephedrine remained unchanged; other sympaticomimetic drugs showed a decrease of toxicity in hibernation. It seemed of interest to study the effect of toxic doses of convulsant and other drugs on animals with body temperature artificially lowered by cooling.

Method. Mice were wetted with ice water or 95% alcohol and then placed in front of an electric fan. The body temperature, thus rapidly lowered to 18-20° C, remained between 18 and 22° for the duration of the experiment (rectal temperatures were determined by a thermocouple). The lethal dose of each drug used was first determined on non-cooled mice and this dose was injected into the cooled animals. The mortality ranged in the controls from 33 to 86% (see Table).

TABLE I.
Toxicity of Drugs for Cooled Animals.

Drug*	No. of animals		Survival time		Mortality	
	Cooled	Control	Cooled Min.	Control Min.	Cooled %	Control %
Strychnine sulfate, 3 mg/K	6	7	17	5	100	72
Metrazol, 100 mg/K	6	7	55	5	83	86
Nicotine, 4 mg/K	12	6	24	4	100	33
Amphetamine sulfate, 150 mg/K	6	5	29	16	100	40
Morphine sulfate, 350 mg/K	7	8	105	62	72	62
Cocaine HCl, 200 mg/K	6	6	11	5	100	67
Procaine HCl, 250 mg/K	9	8	17	8	100	63

*All injections for experiments listed in this table were made intraperitoneally.

1 Dworkin, S., and Finney, W. H., *Am. J. Physiol.*, 1927, **80**, 75.

2 Finney, W. H., Dworkin, S., and Cassidy, G. S., *ibid.*, 1927, **80**, 301.

3 Cassidy, G. S., Dworkin, S., and Finney, W. H., *ibid.*, 1925, **73**, 417.

4 Koeninck, A., *Arch. f. Physiol.*, 1899, p. 389.

5 Blanchard, R., *Compt. R. de la soc. de biol.*, 1903, **55**, 734.

6 Pfeiffer, C., Foster, M. A., and Slight, D., *J. Pharm. and Exp. Therap.*, 1939, **67**, 307.

Results. Cooling depressed convulsions in all animals. The toxicity of the drugs, with the exception of metrazol, was greater in cooled mice than in the corresponding controls, the average total mortality being 95% in the cooled as against 60% in the controls. The slight increase in percentage mortality with morphine is probably not significant. However, the survival time was markedly prolonged in all cooled animals (from about 2 to 11 times). That these changes were independent of the route of administration was indicated by experiments with metrazol and strychnine where intravenous as well as subcutaneous and intraperitoneal injections were made.

Discussion. Our results on toxicity of strychnine and metrazol corroborate those of Pfeiffer *et al.*[6]; however cocaine and amphetamine (benzedrine)* showed in contrast to their effect on hibernating squirrels an increased toxicity in cooled mice. This may be taken as an indication that drugs producing cerebral stimulation are unlike in their effects on hibernating and artificially cooled animals. A further pharmacological analysis may be able to throw some light on similarities and differences in the mechanisms involved in hibernation on one side and artificial cooling on the other.

The prolongation of life in cooled animals may be partly due to delayed resorption of the drug. The general depression of the rate of metabolism probably contributes another important factor, by decreasing the demands of the tissues for vital substances. A similar prolongation of life was observed by one of us in cooled rabbits and cats[7] after lethal doses of insulin. That the depression of convulsions through cooling may be an important mechanism, is suggested by the fact that the most strongly convulsant drugs (strychnine, metrazol, nicotine) show the greatest prolongation of life.

Summary. Artificial cooling of mice resulted in increased mortality after injection of lethal doses of strychnine, nicotine, amphetamine, cocaine, and procaine; no marked difference was observed after metrazol and morphine. Prolongation of surviving time was observed with all animals which succumbed, most markedly after strongly convulsant drugs.

* Supplied through the courtesy of Smith, Kline and French Laboratories.

7 Tyler, D. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 278; *ibid.*, 1940, **45**, 117.