Technical Papers

Effects of Temperature, Light, and Nutrition on the Sensitivity of the Male *Rana pipiens* to Exogenous Chorionic Gonadotropin

Nicholas E. Kontaxis and John R. Maher*
Chemistry Branch, Sixth Army Medical Laboratory, Fort Baker, California

Donald E. Pickering
Pediatric Endocrine and Metabolic Laboratory, University of California Medical Center, San Francisco

A variable decrease in the sensitivity of male *Rana pipiens* to exogenous chorionic gonadotropin during the summer months has limited their use in the bioassay of this hormone (*1–6*). The decrease in sensitivity has been variously attributed to temperature (*2–4*), breeding period (*3–5*), and illumination (*6*). Cutler (*7*), experimenting during the summer with male *R. pipiens* that were kept in a refrigerator, observed no seasonal breeding period variation.

This paper reports the results of a series of experiments designed to evaluate the effects of temperature, light, and nutrition on the sensitivity of the male *R. pipiens* to exogenous chorionic gonadotropin (*8*) and thereby to establish the optimum environment for the use of this amphibian in the bioassay of this hormone.

After receipt, the frogs were stored for 1 wk in an outdoor cage with running tap water and an environmental temperature ranging from 15° to 19°C. All frogs were then screened with 20 and 40 IU of chorionic gonadotropin. Those that reacted positively were given an additional 1-wk resting period in the same environment (*9*). The screening process was designed to eliminate refractory male frogs or females not detected previously by their sexual characteristics (*2, 10*). Only male frogs that reacted positively to 40 IU of chorionic gonadotropin and with body weights ranging from 35 to 40 g were selected for this investigation. Of the 760 frogs received from October 1953 through May 1954, 623 (82 percent) reacted positively to 40 IU of chorionic gonadotropin. The fact that 137 frogs (18 percent) of those screened reacted negatively emphasized the importance of screening all animals before using them experimentally. On two occasions during the 2-wk period of screening, all frogs were force-fed 1 to 2 g of whole

Table 1. Summary of results of all experiments. Light was supplied from a 100-w bulb. Day of injection was computed from the first day on which environment was imposed.

Group	Environmental conditions			Hormone injected (IU)	Day of injection	No. of frogs injected	No. of frogs positive	Percentage positive
	Temp. (°C)	Light	Nutrition					
1	28	Illuminated	Fed	20	13	39	7	18
				30	32	38	12	53
				40	45	37	29	78
2	21	Illuminated	Fed	20	13	38	14	37
				20	49	34	12	35
				30	33	34	22	65
				40	44	34	31	91
3	6	Illuminated	Fed	20	13	36	19	53
				30	33	34	34	100
				10	49	31	8	26
4	28	Total darkness	Fed	20	20	36	6	17
				30	38	36	4	11
				30	65	36	2	6
				40	74	30	0	0
5	5	Total darkness	Fed	10	14	28	7	25
				20	29	27	9	33
				30	36	15	4	27
6	20	Illuminated	Starved	20	60	20	3	15
				30	75	20	6	30
				40	92	20	9	45
7*	28	Illuminated	Fed	30	17	29	0	0
				30	49	26	0	0
				40	31	27	0	0
				40	65	26	0	0

* This group consisted of the same frogs as group 4.

liver. "Red-leg" infection was prevented by the use of sulfathiazole.

Eight to 10 frogs were placed in 8- by 8-in. glass jars that contained about ½ in. of tap water, which was changed weekly or as required. The temperature was recorded daily. At the time intervals indicated in Table 1 the frogs were injected in the dorsal lymph sac with 1 ml of an aqueous solution of chorionic gonadotropin containing 10, 20, 30, or 40 IU. Before each injection the urine of the frogs was checked to insure that it was sperm-free. Each frog's urine was checked for the presence of sperm at 1, 1½, and 3 hr after the injection.

The positive frogs in groups 1, 2, 4, 6, and 7 were returned to their respective experimental environments after the results of their injection were established; the positive frogs in groups 3 and 5 were kept at room temperature, with and without illumination, respectively, for approximately 5 days to insure elimination of all liberated spermatozoa (*9, 11*). The negative frogs of groups 3 and 5 were kept at room temperature for about 6 hr before they were returned to the refrigerator.

The frogs in groups 1, 4, and 7 were kept in an incubator and those in groups 2 and 6 were kept in an unheated, unventilated room.

Illumination was supplied from a common, unfiltered, 100-w light bulb. Feeding was accomplished by forcing a small piece of beef liver (1 to 2 g) down the animal's esophagus.

Table 1 summarizes the results of all the experiments performed. The concentration is expressed in International Units (1 IU = 0.1 mg). The day of injection was computed from the first day on which the experimental environment was imposed. The frogs in group 7 were the same as those in group 4. After these animals had been maintained in total darkness for 74 days, they appeared to be permanently sterile, for they failed to respond positively to 40 IU of chorionic gonadotropin after 65 days of exposure to constant illumination (group 7).

Figure 1 presents the dose-response curves for groups 1, 2, 3, 4, and 5. The percentage responding positively is plotted against the concentration of chorionic gonadotropin injected. It is evident from this figure that light has a more pronounced effect on the sensitivity than temperature.

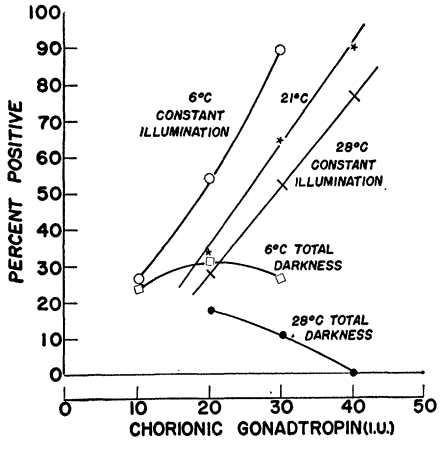

Fig. 1. Dose-response curves for groups 1, 2, 3, 4, and 5.

Comparison of groups 2 and 6 in Table 1 reveals that nutrition also affects the sensitivity. However, work in progress here indicates that nutritional effects are negligible when the animal is maintained at refrigerator temperature.

The following recommendations are made on the basis of these findings: (i) For maximum sensitivity to exogenous chorionic gonadotropin, male *R. pipiens* should be maintained at refrigerator temperature, under constant illumination, and in a state of good nutrition. (ii) All frogs should be screened, after having been thus maintained for a 2-wk period, to establish their sensitivity to the hormone and to eliminate females and refractory males. (iii) Under these conditions we have observed that 50 and 100 percent respond to 20 and 40 IU of chorionic gonadotropin, respectively.

References and Notes

* Present address: Research and Development Service, Fitzsimons Army Hospital, Denver 8, Colo.

1. M. Samson, *Science* **111**, 231 (1950); J. B. Holyoke and E. E. Hoag, *Am. J. Clin. Pathol.* **21**, 1121 (1951); R. H. Fischer, *Med. Ann. Dist. Columbia* **22**, 62 (1952).
2. J. E. Hodgson, *J. Am. Med. Assoc.* **153**, 271 (1953).
3. R. Lehman, *Am. J. Med. Technol.* **18**, 298 (1952).
4. J. V. Thorberg and K. Hansen, *Acta Endocrinol.* **6**, 51 (1951).
5. O. J. Pollak, *J. Lab. Clin. Med.* **36**, 127 (1950).
6. C. Galli-Mainini, *J. Am. Med. Assoc.* **138**, 121 (1948).
7. J. N. Cutler, *J. Lab. Clin. Med.* **34**, 554 (1949).
8. The chorionic gonadotropin used in this study was kindly supplied by the U.S. Pharmacopoeia (U.S.P. International Standard) and Parke, Davis and Co. (Antuitrin "S," dry form in steri-vials). The activity of the hormone was checked with mice and frogs.
9. J. V. Thorberg, *Acta Endocrinol.* **6**, 373 (1951).
10. S. L. Robbins and F. Parker, Jr., *New Engl. J. Med.* **241**, 12 (1949).
11. P. B. Wiltberger and D. F. Miller, *Science* **107**, 198 (1948).

26 January 1955.

Unique Electron Exchange Polymer

Saul Soloway and Leonard Schwartz

Department of Chemistry,
The City College, New York, N.Y.

The field of electron exchange resins was initiated by Cassidy when he showed that polyvinylhydroquinone had reversible redox properties (*1*). His first product was readily soluble in many common organic solvents, indicating a low degree of polymerization. However, on copolymerization of vinylhydroquinone and divinylbenzene, he obtained a cross-linked, insoluble, infusible redox resin, which was shown to be useful for electron exchange experiments in columns (*2*).

Manecke measured the oxidation potentials of the redox polymers resulting from the condensation of