

Effect of pH on Biological Activity of Chorionic Gonadotropin

Abstract. Increasing the pH from 3.0 to 10.8 increased the uterine weight three-fold and the number of positive vaginal smears from 0 to 78 percent in immature rats injected with chorionic gonadotropin in aqueous solution. Activity of a solution held at pH 2.6 and 5°C for 24 hours was restored by neutralization.

Recently this laboratory received for assay a commercial sample of chorionic gonadotropin which consisted of a vial containing the dry chorionic gonadotropin powder and a second vial containing an aqueous diluent in which was incorporated, among other things, 25 mg of thiamine hydrochloride per milliliter. In accordance with the usual practice, the powder was first dissolved in the diluent and was then further diluted with 10-percent alcohol in order to obtain the proper dosage range for the bioassay. For comparison, similar solutions of International Standard chorionic gonadotropin in 10-percent alcohol only were prepared. The sample failed to elicit a uterine weight response when injected into immature female rats in total doses of 0.3 and 0.6 international unit per rat, whereas the International Standard chorionic gonadotropin at these doses showed the activity expected. The injections were made subcutaneously in two equal daily injections for 3 days, with sacrifice of the animals on the 5th day.

The assay was repeated, but the sample diluent was omitted and the sample powder was dissolved directly in 10-percent alcohol. The powder now showed the full labeled potency.

When the International Standard chorionic gonadotropin was dissolved in the sample diluent or in 10-percent ethyl alcohol containing an equivalent amount

of U.S.P. thiamine hydrochloride, the International Standard chorionic gonadotropin showed no activity at the usual doses administered. However, when a solution of the International Standard chorionic gonadotropin was injected at one site and the thiamine hydrochloride was injected at another site simultaneously, there was no inhibition of activity.

Since it has been reported that acid solutions of chorionic gonadotropin are unstable (1), International Standard chorionic gonadotropin in 10-percent alcohol containing hydrochloric acid equivalent to that supplied by the thiamine hydrochloride (pH 2.6) was injected, and there was no response. However, when the solution of chorionic gonadotropin so prepared was neutralized with NaOH after standing at 5°C for 24 hours, full activity was restored; this indicated that stability was not a factor.

Table 1 shows the results of two experiments in which the pH was varied from 3.9 to 10.8. In these experiments all vaginas were opened with a cotton swab on the evening of the 4th day and vaginal smears were made on the 5th day at 96 and 100 hours after the first injection; following this the animals were sacrificed and their uteri were weighed. In the first experiment the pH of the injection medium was varied by the addition of either dilute HCl or dilute NaOH, the final volume of each group being kept constant. In the second experiment the pH was adjusted by means of McIlvaine's phosphate-citric acid buffer (2), and with dilute NaOH.

As Table 1 demonstrates, not only is the activity of chorionic gonadotropin inhibited at a low pH but it is considerably enhanced at a high pH. These observations are important in connection with the development of an official method of bioassay for this drug. It is also of interest to know whether the

effects noted here also obtain when the drug is used in human beings.

Recently Banik and Chakravarti (3) reported that the activity of human chorionic gonadotropin was inhibited when injected in the male toad in the same solution with quinine dihydrochloride, ergotoxine, ethanesulfonate, emetine hydrochloride, or atropine sulfate. It is possible that the inhibition noted was due to the low pH of the injected solutions rather than to an inhibitory action of the drugs themselves.

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Male Sterility Induced in Tomato by Sodium 2,3-Dichloroisobutyrate

Abstract. Spraying tomatoes with 0.3 percent sodium 2,3-dichloroisobutyrate (FW-450) at anthesis induced male sterility for 12 days, beginning 12 days after treatment. Only 20 percent fewer fruits were set on treated plants hand-pollinated with pollen from unsprayed plants than were set on untreated plants. The flowers again showed normal fertility 37 days after treatment.

The high cost of F₁ hybrid tomato seed has been one of the factors limiting commercial use of such seed. The use of male sterile mutants to eliminate the necessity for hand emasculating has been suggested as a means of reducing the cost of hybrid seed. Larson and Paur (1) described a functional male sterile mutant and suggested techniques of utilizing it. Many male sterile mutants have been identified, and some were listed by Rick (2). Before it would be possible to produce hybrid tomato seed commercially by means of these techniques, a breeding program of undetermined duration would probably be necessary, unless suitable male sterile mutants could be found by searching in large field populations, where, according to Rick (3), the normal incidence is about 0.05 percent. Alternatively, male sterility might be induced in one of the parents by irradiation, as discussed by Lesley and Lesley (4). If male sterility could be induced in tomatoes by means of a chemical, F₁ hybrid seed could be produced without hand emasculating and without

Table 1. Effect of the pH of the injection medium on responses of immature rats to 0.6 units of International Standard chorionic gonadotropin.

pH	Buffer	Uterine weight (mean ± standard error)	Vaginal smear		
			Positive (No.)	Animals (No.)	Positive (%)
Experiment A					
3.2	HCl	39.5 ± 4.5	0	11	0
6.0	None	60.2 ± 6.8	3	12	25
8.6	NaOH	71.5 ± 9.8	8	13	62
10.8	NaOH	98.6 ± 9.1	10	13	77
Control	None	42.0 ± 3.6	0	12	0
Experiment B					
3.0	Phosphate-citric acid	33.5 ± 8.5	0	18	0
5.0	Phosphate-citric acid	49.2 ± 6.5	2	18	11
7.0	Phosphate-citric acid	73.4 ± 6.7	6	18	33
9.6	NaOH	78.1 ± 4.9	9	18	50
10.8	NaOH	109.2 ± 6.1	14	18	78