

ABSENCE OF ALTERATIONS IN GROSS MORPHOLOGY OR DEVELOPMENTAL RATES OF *DAPHNIA* *MAGNA* SUBJECTED TO ANTUITRIN-S¹

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During the course of a series of investigations on the physiology of mammalian reproduction it became desirable to ascertain whether or not any readily detectable changes in gross morphology or developmental rates might be elicited in parthenogenetic female *Daphnia magna* subjected to chorionic gonadotropins. This extremely transparent crustacean exhibits usually 17 instars. The first five to seven instars occupy an average of one day each, and parthenogenetic reproduction commences between the fifth and seventh instars. The remaining instars occupy an average of two days each. Unfertilized eggs develop rapidly in the brood pouch, with a brood of usually three young females being produced before each molt.

A culture medium for maintaining the animals was prepared by mixing 5 gms. of horse manure and 25 gms. of sandy muck in one liter of Standard Reference Water. The infusion was allowed to stand 2-3 days, after which it was passed through coarse filter paper. The filtrate was allowed to stand 3-4 additional days before being used.

Effects on Gross Morphology. Twenty-seven pairs of mature, non-gravid females from the 5th to the 14th instars were placed singly in test tubes holding 1 cc. of the culture medium at 8°C.² To the test tube containing one of the members of each pair was added 75 IU of Antuitrin-S (Parke-Davis), an amount equivalent to the average minimum concentration of chorionic gonadotropin in 1 cc. of a 24-hour sample of human pregnancy urine at the peak of excretion. The remaining member of each pair served as a control. At the end of 24 and 48 hours test and control animals were, for practical purposes, identical, the Antuitrin-S having had no effect on the gross morphology of the test specimens.

Effects on Developmental Rates. Eighty-seven pairs of gravid females were placed in one of four groups in accordance with the following criteria:

Group I. Specimens with developed ovaries with eggs soon to be deposited.

¹The initial animals were supplied by Edward Fairchild, Jr., Senior Industrial Fellow of the Louisiana Petroleum Refiners' Waste Council.

²At room temperature (30°C at the time of the experiment) the death rate was about twice as high as the birth rate during preliminary observations when the population density was 3 specimens per milliliter of culture medium (30 specimens in 10 cc.), whereas at the reduced temperature there were no deaths during two weeks of preliminary observation when the population density was 5 organisms per cc. (50 specimens in 10 cc.).

- Group II. Specimens containing early embryos in the brood sacs.
- Group III. Middle embryo stages.
- Group IV. Advanced embryo stages.

Experimental and control specimens were placed singly in 1 cc. of culture medium at 8°C. At this temperature the time between molts was doubled, thus reducing the margin of observational error, an advantage if the developmental rates were to be revealed as a variable in the presence or absence of Antuitrin-S. One member of each pair was subjected to 75 IU of Antuitrin-S, and the other was utilized as a control. At the end of 24 hours, any increase in size of the ovaries (Group I), or of the embryos (Groups II, III, and IV), was recorded subjectively for each experimental and control organism according to the following scheme: 1.00, no change or slight development; 2.00, moderate development; 3.00, considerable development. When the subjective data were tested for significant differences none was indicated (Table I). Only in Group IV does the *P* value nearly approach the limit of the accepted standard of probability. On cursory examination it was apparent that the developing young in the brood pouches of the experimental and control animals had attained essentially the same developmental stages.

TABLE 1. MEAN CHANGES AT THE END OF 24 HOURS

Group	No. Initial Pairs Less Number Dying	Mean Change		<i>P</i> Values
		Experimental	Control	
I	12-2	1.60 ± .698*	1.50 ± .528*	.75
II	20-2	2.22 ± .732	1.77 ± .731	.099
III	20-2	1.28 ± .842	1.28 ± 1.030	infinity
IV	18-3	1.60 ± .976	2.27 ± .795	.065

*Standard Deviation

1.00 No change or slight development

2.00 Moderate development

3.00 Considerable development

Conclusion. Within the limitations of the present experiment, Antuitrin-S had no effect on the gross morphology or the developmental rate of *Daphnia magna*. Therefore, from the standpoint of practicability, this organism is valueless for the detection of chorionic gonadotropin in physiological quantities.