

that the salicylate of nicotine was much more toxic for plant tissues than other salts. This agrees with the findings of Macht and Livingston on the great toxicity of benzoates for living plants,¹¹ which observation was later found to hold good also for salicylates. The marked difference between the alkaloid nicotine and its salts is especially interesting when compared with the relative effects of certain other alkaloids and their salts, which were used as controls. We have made comparative pharmacological studies with solutions of atropine and atropine sulphate, cocaine and cocaine hydrochloride and strychnine (alcoholic solutions) and strychnine sulphate and found that those alkaloids did not differ substantially in toxicity from their salts.

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The Critical Period for Control of Sex in *Moina*.

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Banta and Brown¹ have shown that with crowding under favorable food conditions the critical period for sex control with *Moina macrocopa* is approximately 4 hours before the eggs are laid into the brood chamber. Stuart, Cooper and Miller,² while unable to affect control of the sex of the same Cladoceran species later than the fourth hour before egg laying, concluded that sex could be influenced by conditions prevailing previous to the fourth hour before egg laying. The following experiment was designed to show that the critical period for sex control is dependent to some extent upon the environmental conditions under which the animals are reared, *i. e.*, upon environmental conditions present earlier than, and interrupted before, the time of the critical period as described by Banta and Brown for crowding under good food conditions.

In 4 series of bottles, A, B, C and D, each series containing from 15 to 20 bottles, were placed 75 ml. of varying dilutions of manure infusion and 10 young *Moina macrocopa* females. The infusion in series A and B was diluted 12.5 times, in series C, 25 times, and in

¹¹ Macht and Livingston, *J. Gen. Physiol.*, 1922, 4, 573.

¹ Banta, A. M., and Brown, L. A., *Proc. Nat. Acad. Sci.*, 1929, 10, 71.

² Stuart, C. A., Cooper, H. J., and Miller, Rose, *Roux. Arch.*, Abt. D, 1932, 126, 325.

D, 100 times. (Dilutions were made with pond water.) All bottles were then incubated at approximately 25°C. Series A served as a control and was not manipulated in any way. Approximately 15 hours before the egg-laying period of the mothers in series B, C, and D the crowded mothers from one bottle in each series were isolated, each mother being transferred to a separate bottle containing 25 ml. of normal medium (concentrated manure infusion diluted 12.5 times). One hour later the same procedure was repeated with another bottle from each series and again another 1 hour later, and so on every hour until the mothers in the different series laid eggs.

By this procedure, in series B for example, mothers were subjected to a male-inducing condition (crowding) up to periods of time varying from 18 to 1 hour before egg-laying when the crowded mothers were transferred to a female-determining condition, *i. e.*, isolated in an adequate amount of fresh culture medium. Obviously then, the time before egg laying at which isolating the mothers failed to overcome the effect of crowding will constitute the critical period for sex control in the environment specified. In these experiments, which involved the sexing of more than 13,000 young, it was found that the critical period for sex control in these animals was different for the different environments. In series B, medium diluted 12.5 times, it was the same as that previously determined by Banta and Brown—approximately 4 hours before egg-laying. In series C, however, in which the medium was diluted 25 times, the critical period was found to be much earlier, namely from 7 to 8 hours before egg laying. The critical period for sex control in series D (medium diluted 100 times) appeared to be as early as 13 to 14 hours before egg laying. Furthermore, it was found in this series that while mothers isolated from 6 to 14 hours before egg-laying produced males, mothers isolated from 1 to 5 hours before egg laying produced, for the most part, female young. This is not surprising in view of the fact that Stuart, Tallman and Cooper³ have shown that *Moina macrocopa* mothers reared in very dilute medium up to the time of release produce only female young. The mothers in control series A (medium diluted 12.5 times as in series B) which remained crowded until releases occurred produced at least some males in every experiment, showing that the male production and the observed critical period in series B, C and D were the result of controlled environment rather than chance.

³ Stuart, C. A., Tallman, Juanita, and Cooper, H. J., *Physiol. Zool.*, 1931, **4**, 594.

The critical period for sex control in this animal requires further elucidation. As Banta and Brown¹ used the term it meant the time before egg laying at which a mother must be under a specific (crowded) environment in order to affect the sex of her young. This seems a legitimate use of the term, critical period. There is presumptive evidence that the actual *sex determination* is chromosomal. Allen and Banta⁴ obtained evidence that the nuclear spindle for the single maturation division begins to form roughly 1½ hours before the eggs are laid. It seems probable that the influential external environmental factors must have produced their effect within the egg at about that time. Whether this effective condition is a state of nutrition, accumulation or removal of some substance, or level of metabolism (or something else) is beside the present point.

Banta and Brown⁵ have reported that when temperature is used as the controlling factor the temperature condition must operate for a long period (during most of the previous life of the mother) in order to be most effective upon the sex of the offspring. The present data likewise indicate that environmental conditions operative many hours before egg laying are effective upon the sex of the young. If the term, critical period, is to continue to be used to designate the time at which a specific environmental condition must be operative, it, obviously, is not a fixed and definite time within the life of the individual mother but is the critical period for the operation of a particular external environmental condition.

On the other hand the critical period, theoretically, might be defined with reference to the immediate *internal* environment of, or the condition of, the egg itself, *i. e.*, as the time at which the effects produced by the specific external environmental conditions must have become built up (and remain potent) within or immediately about the egg itself.⁶ This period, as just defined, should theoretically be the same regardless of the influential environmental factors employed.

⁴ Allen, Ezra, and Banta, A. M., *J. Morph. and Physiol.*, 1929, **64**, 123.

⁵ Banta, A. M., and Brown, L. A., *Yearbook Carn. Inst. of Wash.*, 1924, No. 23, 36. Brown, L. A., and Banta, A. M., *Physiol. Zool.*, 1932, **5**, 218.

⁶ Banta, A. M., and Brown, L. A., *Yearbook Carn. Inst. of Wash.*, 1929, No. 28, 51.