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Effect of Certain Alkaloids Upon Early Cleavage in Arbacia Punctulata.

MARIE A. HINRICHS AND IDA T. GENTHER.

From the Department of Physiology, University of Chicago, and Washington University, St. Louis.

In order to study the physiological effects of certain alkaloids upon some of the lower organisms, and to determine, if possible, whether there was any evidence of specificity of action in these forms, the following experiments were planned: (a) A study of the effects of caffein on some of the physiological and morphological processes of the young and adult forms of *Planaria dorotocephala,* an organism with relatively labile tissue; (b) a study of the effects of caffein, atropine, and pilocarpine on cleavage and on the embryonic development of one of the lower organisms in which differentiation is rapid enough to permit of modification under the influence of agents acting only for a short period of time.

The first series of experiments, (a), concerned themselves with a study of the effects of caffein upon oxygen consumption, carbon dioxide production, and head frequency in *Planaria dorotocephala.* A record of differential disintegration in high concentrations of caffein also indicated a differential susceptibility to this alkaloid. No specificity of action was found for caffein in this form, and both stimulation and inhibition were recorded in the studies made with head frequency and with respiration, the effect depending upon the concentration used, and the length of the period of exposure.[1]

The experiments on early cleavage will be reported in this paper, and studies on differential modification of development in *Arbacia punctulata* will be reported in the following paper. In the study of the effects of various concentrations of alkaloids on the rate of early cleavage of *Arbacia* eggs, the following procedure was observed: Stock solutions of a 0.5% concentration were made up for each alkaloid. From these, further dilutions were made up such that concentrations ranged from 0.1 cc. to 10 cc. of stock solution per 100 cc. of sea water. The eggs of several females were mixed, fertilized, and washed, and then measured quantities of a uniform suspension of such eggs were placed in a series of varying concentrations of alkaloids, and studied at intervals to find out whether the rates of early cleavages could be modified as compared to the rate of cleav-

[1] Hinrichs, M. A., *J. Exp. Zool.*, 1924, xl.

age of a control lot of eggs kept in sea water. After such preliminary studies were made, the eggs were transferred to Erlenmyer flasks, and more alkaloid solution added. The original concentration was maintained.

Results: Counts recorded cleavage in 50% or more of each lot of eggs. It was found that with caffein, the first cleavage and second cleavage appeared at the same time as in the control in all dilutions up to 5 and 10 cc. per 100. (.025%-.050% concentration.) In these higher concentrations, cleavage was delayed to nearly twice the normal length of time in the first division, to one and a third times the normal period in the second division, and only slightly, in the third division. In Pilocarpine solutions, there was no apparent delay during the first cleavage. (In dilutions of Pilocarpine hydrochloride, there was even a suggestion of a slight stimulation in rate in concentrations as low as 0.2 to 0.6 cc. per 100. (.01-.06%) The nitrate solution was more strongly inhibitory than the hydrochloride of Pilocarpine, although there was no delay in the first cleavage. The second division in the nitrate series was delayed by concentrations as low as 0.3 cc. per 100, the time of cleavage appearing about 30 minutes later than the controls. The delay in cleavage rate was maintained in the Pilocarpine solutions through the fourth cleavage. Atropine showed by far the most rapid inhibitory effect on cleavage rate, *viz.*, 0.2 cc. per 100 (.01%), in the first cleavage. In the second and third cleavages, the delay did not appear until concentrations as high as 1 cc. per 100 were reached. In the fourth cleavage, concentrations up to 2 cc. per 100 showed no delaying effect. A slight stimulation in rate was recorded for the first cleavage in the 0.1 cc. per 100 concentration.

From the above data, it is evident that the effect on early cleavage is not marked until higher concentrations are reached with all alkaloids studied with the exception of Atropine. Also, the effect appears to "wear off" in later cleavages, again with the exception of higher concentrations of Atropine.

In order to study the effect of these various concentrations of alkaloids upon the rate of formation of top-swimming larvae, the eggs were now transferred to Erlenmyer flasks, and the time noted at which a ring of larvae formed at the top of the liquid. Fourteen series were studied with Pilocarpine nitrate, 7 series with Pilocarpine hydrochloride, 17 series with Atropine sulphate, and 6 with Caffein. Of the total number of 66 observations made with Pilocarpine nitrate, only 14% showed stimulation in speed of formation of top-swimming larvae, as compared to the seawater control.

All these were found in concentrations of from 0.4-3 cc. per 100, and from 7-9 hours after fertilization. 56% showed an inhibition in rate, and 30%, no effect. Forty-one observations were made with Pilocarpine hydrochloride, and of these, only 11% showed stimulation, 51% depression, and 38% no effect. This would seem to indicate that, in the concentrations used in these experiments, Pilocarpine is only slightly accelerative in its action on the rate of growth and division in this form.

Seventy observations were made with Atropine sulphate, and of these none were stimulative in rate of formation of top-swimming larvae, as compared to the control. Fifty-nine per cent showed inhibitory effects, and the rest produced no effect on rate of growth up to this stage. All inhibitory effects appeared within 7-9 hours after fertilization, after which time the effect seemed to wear off.

Of the 18 observations with Caffein, only 11% were inhibitory, none stimulative, and 89% showed no effect in the concentrations used.

From the above 2 types of experiment, it appears that Pilocarpine may be either stimulative or inhibitory, depending on the concentrations used. Caffein was practically without effect, except in the highest concentrations, which were inhibitory. Atropine is more strongly inhibitory than either Pilocarpine or Caffein, but may produce a slightly stimulative effect which wears off readily.

By the time 12 hours have elapsed since fertilization, the effect of all 3 alkaloids seems to be negligible, except in higher concentrations. In order to test the further effect on development of these embryos, studies were made, using the same concentrations, and developmental rates of various body regions were observed over longer periods of time. The results of such studies will be briefly summarized in the following paper.