Further Observations on Effect of Nonoptimally High Incubation Temperature on Frequency of Pecking And Color Preference in the Chick[1]

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Introduction

The results of our previous investigations have indicated that the critical period for embryonic nervous system development in chicks is during the early part of the incubation period; this suggests that during this time the embryo should be as free from stress as possible. Gunther (2, 3) and Gunther and Jones (4-8) have described various types of structural, behavioral, and physiological abnormalities in the subsequently hatched animals when nonoptimally high temperature was the stressor during the first several days of incubation of chick eggs, that is, during the early period of the development of the nervous system. Romanoff (9) summarizes some of the effects of higher temperature, including those on the brain and spinal cord.

Gunther and Jones (6, 7), appear to be among the first investigators to suggest, and to provide experimental evidence (at least in the terminal stages), that heat stress at any time during the embryonic period is damaging to the nervous system and that such damage will produce defects in behavior, possibly as much during subsequent phases as during the initial phases of incubation.

It will be interesting to follow up this research with application of heat to normal-temperature chicks immediately after hatching, since the thermo-regulatory mechanism is said to be inoperative until about the seventh post-hatching day (10, 11; Gunther, unpublished data). Also unexplored is the application of heat stress during the middle of the incubation period, from incubation days 5 through 15. Work on these aspects is scheduled in the laboratory.

In a previous work on pecking frequency and on color preferences (5) it was found that the heat-stressed birds (incubated initially for 3 days at 41° C.) pecked at the targets less frequently than did the control animals, and that the stressed chicks preferred the blue-green region of the spectrum whereas the control birds preferred the yellow-orange region. The research reported herein is designed to compare the pecking frequency and the orange-black preference of chicks hatched from eggs incubated initially and terminally for 36 hours at 41° C. with the pecking frequency and color preferences of chicks hatched from eggs incubated at the optimal temperature of 37.5° C. for the entire period of incubation.

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Materials and Methods

Two-hundred forty White Rock eggs (called experimentals) were incubated initially at 41° C. for 36 hours, transferred to normal temperature (37.5° C.) incubators for 18 days, then returned to the 41° C. incubator until hatched. An additional 240 eggs (called controls) were incubated for the entire incubation period of 37.5° C. Incubators, conditions of incubation and hatching, etc., have been described in previous publications.

One-hundred ninety controls and 115 experimentals hatched. The experimentals hatched somewhat precociously, all in about 20 hours after being returned to the 41° C. incubator on day 19½. The controls hatched on day 21 of the incubation period, from 10 to 20 hours after the experimentals.

Forty-three controls and 43 experimentals were run in the pecking apparatus. These chicks were all experimentally naive and were handled only when transferred to and from the pecking apparatus, and when switched from side to side in the pecking apparatus. Since effects of handling are as yet incompletely understood, it was decided to handle the chicks as little as possible. Work in the laboratory subsequent to this research indicates that handled chicks perform significantly faster in learning situations.

The pecking apparatus has been described (5). The targets on the cylinders consisted of a ¼" black or orange dot, painted on the inside of a clear plastic disc on the end of the cylinder. The black paint was a Smith-Alsop product, Saco Sheen Black; the orange paint was a special mix, judged by 3 independent observers to approximate most closely the color of the dull side of Ostwald chip #3 from the Ostwald color harmony manual (1). The cylinders were attached to the arms of microswitches. Each time the cylinder was moved—that is, when the chick pecked at it—the circuit was closed and an electric counter connected to the microswitch was moved ahead one integer. Since each cylinder, target, microswitch, and counter functioned as a unit, it was possible to record automatically and accurately the number of pecks delivered to each target. Pecking frequency was determined by using the black dots only on all 16 cylinders. As is customary, the experimentals and controls were tested simultaneously, separated from each other in the pecking apparatus by a wooden partition. The orange-black preference task was run by placing orange and black dots on alternating microswitches. Thus, during any testing period there would be 8 alternating orange and black targets on each side of the wooden partition. The birds were interchanged from one side to the other at 1½ hour intervals, or once during each 3-hour testing session. Each test was run for two weeks, one 3-hour session each day. The chicks were deprived of food, but not water, approximately 12 hours prior to each testing session. When the chicks were one week old they were first run for 2 weeks on the black dot targets in the pecking frequency test, and after three weeks of rest they were tested for another two-week period on the orange-black combination.

Results and Discussion

Table 1 summarizes the results of the pecking frequency test. The chi-square value indicates a very highly significant difference in the number of pecks delivered, the controls pecking more frequently than the experimentals. There appeared to be no diminution of or other change in pecking frequency with increased age of the chicks. This was mentioned as a possibility in a previous report (5).

The result of the orange-black preference test is shown in Table 2. It is seen that both groups showed a significant preference for orange over black. The strength of this preference was about the same in the 2 groups.

TABLE 1

Pecking frequency of control and experimental groups
Targets: Black dots

Group	Total responses	n	Chi-square (1 df)
Control	6,836	43	731.62*
Experimental	4,018	43	731.62*

* Highly significant difference; P = < .001

TABLE 2

Orange-black preference test in the pecking apparatus
Targets: Orange and black dots, alternated

Group	Total Responses	Stimulus Combination	Preferred Stimulus	n	Chi-square (1 df)
Control	5,655 3,589	Orange- Black	Orange	43	461.74*
Experimental	3,635 2,016	Orange- Black	Orange	43	463.92*

*Highly significant difference; P = < .001

The difference in pecking frequency is very similar to that seen in a former experiment (5). The controls peck much more frequently than do the experimentals. One might conclude that the experimentals are less active and that the heat stress in general depresses motor activity. Observations of the chicks, however, do not support this theory. The experimental chicks eat as much as the controls, and are every bit as active in other situations; they may not be aware of their surroundings as the controls. The disturbance causing this depressing action on pecking activity is probably one involving several brain levels, among which are learning, innate response to pecking and perhaps to color, and black-white recognition or discrimination. These results do not provide any clue as to the nature of the damage (to the brain or other locus).

Proportionately the orange-black preference experimentals delivered far more pecks to the targets than did the color preference experimentals from a previous experiment (5) in which the chicks were hatched from eggs placed in the nonoptimally high temperature initially for 3 days. One might conclude that the depressing effect of such stress is lessened when spread over 2 widely-separated incubation periods, that is, 1½ days initially and 1½ days terminally. However, since the experimental chicks in this experiment received on the average about 16 hours less exposure to the heat stress, it would be equally valid to state that this is the reason for the difference. A third possibility is that the number of different colors (16 spectral hues) in the previous experiment mentioned above tended to confuse the experimental chicks, depressing their pecking activity considerably. The relatively simple choice of orange or black left little room for hesitancy of choice, which might tend to lower the pecking frequency of the experimentals, other than the usual depressed pecking frequency of heat-stressed birds. This is suggested by the comparison of the total number of pecks delivered to the targets by both groups in both of these situations. On the black dots the experimentals delivered 37% of the total pecks registered, and on the orange-black targets the experimentals also delivered 37% of the total pecks registered. In other words, there was no difference in the percentage of pecks registered in the two situations. This seems all the more convincing when one takes into consideration the fact that the two experiments were separated by a time interval of three weeks.

The preference of orange over black for both groups is interesting in the light of previous research (5). It was mentioned as a possibility then that perhaps some kind of imprinting had occurred, promoting the preference of the controls for orange and the experimentals for blue. This nebulous preferential imprinting, though interesting as a theory, hardly seemed to bear up under close scrutiny. It was true, however, that the chicks in that experiment had been exposed to all the different color stimuli several days before testing had been begun. Nevertheless, for various reasons it appeared doubtful that imprinting played any role in that experiment. In the research reported here the birds had first been exposed to the black discs for frequency of pecking. After a three-week rest they had been run in the pecking apparatus with alternate orange and black targets. If any imprinting had occurred it should have been on the black. Actually, both controls and experimentals preferred orange to about the same extent (61% and 64% respectively). This would tend to discredit a differential imprinting theory. The lack of any difference in the orange preference of either group indicates that either the duration of the heat stress was too short to alter the normal orange preference, or the stress imposed during the final days of incubation produced little or no effect on the as yet unsolved neural bases of color preference behavior.

Summary

Chick eggs were exposed to the nonoptimally high temperature of 41° C. for the first 1½ days of incubation, transferred to normal temperature incubators until day 19½, and then transferred to the 41° C.

incubator until hatching. The hatched chicks were compared with control chicks incubated at the optimal temperature of 37.5° C. for the entire incubation period. Both groups were tested simultaneously in a pecking apparatus over a period of 6 weeks.

Pecking frequency in the controls was significantly higher than in the experimental chicks.

Orange over black was preferred by both controls and experimentals to about the same degree. This preference was highly significant.

Discussion centered on comparison with previous work in which it was found that controls preferred the orange-yellow and experimentals the blue-green region of the spectrum (the latter chicks being incubated under somewhat different conditions, however).

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