Some Effects of High and Low Incubating Temperatures on Chick Behavior[1]

W. C. Gunther, R. K. Jones, and Paul Manske, Valparaiso University

Introduction

This report is concerned with the investigation of the post-hatching behavior of Leghorn chicks which were incubated at abnormal temperatures during the first stages of their embryonic life.

Several investigators have concerned themselves with the effects of abnormal incubation temperatures upon the development of the chick embryo (1-7). Gunther (3) appears to have been the first investigator to make a systematic study of the behavior of chicks hatched from eggs subjected to abnormal temperatures.

The project reported herein is the initial stage of a much larger research understaking on the part of the senior author. In carrying out this larger undertaking, the investigators will consider a greater range of abnormal incubation temperatures and will investigate the development of the neural tube of embryos when it has been established that there is abnormal behavior in hatched birds which were incubated under similar conditions. Consequently, the prime objective of this initial project was to detect and make note of any significant behavioral differences between the groups being investigated. The projected implication of positive results in this and successive projects is the possibility that the aberrant behavior of mentally retarded human beings might conceivably be the result of similar temperature abnormalities during the gestation period.

Methods

In Part I of this project the authors compared the behavior of chicks hatched from eggs incubated at temperatures above the normal incubation temperature with the behavior of a control group hatched from eggs incubated at normal temperatures. Part II of the project was concerned with the behavior of chicks hatched from eggs incubated at temperatures below normal.

In both groups simple observational tests were devised to study differences in behavior. These are described below. Movies were made of the animals as they were put through the various tests. In addition, some of the animals mentioned in Part II were tested in a T-maze and in a discrimination apparatus, the details of which are presented in the appropriate section.

Materials, Results, and Discussion

Part I

Twenty-four Leghorn chicken eggs were incubated at the normal temperature, 37.5° (all temperatures herein are reported as centigrade), in a Montgomery Ward Standard Forced Air 416-egg incubator. Twenty-

1. This investigation was supported in part by research grant B-2128 from the Neurological Diseases and Blindness Council, National Institutes of Health, Public Health Service, United States Department of Health, Education and Welfare.

one chicks hatched within 22 days. One of the chicks hatched with a poorly developed leg which caused it to walk with a limp.

Twelve chicken eggs were incubated at 41° for 5 days in a round portable incubator and were then placed in the Montgomery Ward incubator at 37.5° for the remainder of the incubation period. Five chicks hatched within 23 days. One of these chicks had a poorly developed leg and walked with a limp. (Hereafter, the chicks in this group will be referred to as the "abnormals." This designation is used in reference to the abnormal incubation temperature.)

The chicks in both groups were observed frequently for 5 weeks in a 3′ x 3′ demonstration box for periods of 15 to 30 minutes. Motion pictures were taken when the chicks were 2, 22, and 32 days old, and many behavioral differences between the groups are evident in these films.

The most notable differences were the greater social aggressiveness and activeness of the normals and the apparently heightened "fear" response shown by the abnormals. This statement is based on the following observations:

1. The normals pecked at the floor, at scraps of newspaper, at knotholes in the wooden demonstration box, etc. While the abnormals carried on similar activities, they did so much less frequently.

2. The normal birds sauntered around the demonstration area quite freely without demonstrating any hesitancy. The abnormals, on the other hand, tended to move with short, jerky movements—almost reluctant to proceed without first looking around. The abnormals were quite hesitant about investigating strange objects, such as an aluminum water pan, while the normals were curious about such objects and did not seem to be afraid of them.

3. Frequently the chicks were intentionally startled by clapping hands, scraping a metal chair across the concrete floor, exploding firecrackers, and by shouting. The animals in the two groups responded quite differently to these types of stimulation. The normals tended to scatter around the demonstration box in response to the stimuli. When the abnormals were exposed to the same stimuli they huddled together or congregated in a corner. Even when one of the investigators attempted to scatter them by waving a hand at them, the chicks continued to cower in the corner. This difference was observed consistently.

4. The normals were highly competitive. They often contested for the same piece of food or the same scrap of paper. The abnormals seemed quite satisfied with the food in front of them and did not concern themselves with the activities of the other animals in their group.

5. The normals not only pecked frequently at inanimate objects, but also pecked at, and fought with, each other. The abnormals never fought among themselves.

These observations suggested to the investigators that the normal chicks tended to be more aggressive, more active, and less fearful than the abnormal birds.

There were also certain physical differences between the two groups. The feather coat of the abnormals was noticeably poorer than that of the normals and took longer to develop, patches of exposed skin being clearly

evident even after 7 days. The crippled abnormal chick died when it was 8 days old. An autopsy revealed a very poorly developed digestive tract. There were no apparent differences in the motor responses of the two groups.

Part II

Twelve chicken eggs were incubated at normal temperatures in the same manner as that described in Part I. Eight chicks hatched within 22 days.

Twenty-four eggs were incubated at 34° for 10 days and then placed into the 37.5° incubator. Three chicks hatched within 22 days, one of which had a crippled leg. The term "abnormals" will now be used to refer to this group of chicks.

These animals were observed in the same manner as those in Part I. There were no apparent physical differences and, once again, the motor responses of the two groups were not noticeably different. However, the abnormals seemed to be more hostile and more sensitive and responsive to environmental stimulation than were the normals. The abnormals also appeared restless, hypertense, and hyperexcitable. These inferences are based on the following observations:

1. The abnormals seemed to tear newspaper and peck at the floor more viciously than did the normals.

2. Six chicks were handled every day for psychological testing (to be described in detail below). Not once did the normals peck at the hands of the investigator who was working with them; nor, for that matter, did any of the normals described in Part I above. The abnormals, on the other hand, frequently pecked the investigator's hands when they were handled. Such behavior might be understandable if the chicks had been handled infrequently. Even normal chicks could be expected to peck under such circumstances. Since they were handled every day, the pecking would appear to suggest restlessness, hypersensitivity, and excitability on the part of the abnormal animals.

3. It appears to be normal behavior for chicks to peck at crippled birds in the community. This behavior was observed in both groups of chicks in Part I, as well as in the abnormals in Part II. Neither of the crippled chicks in Part I attempted to fight back or retaliate, but each resigned itself to its low position in the pecking order. However, when the cripple from the Part II group of abnormals was pecked by other birds, it was quick to fight back in retaliation. It seemed to refuse to assume its low position in the pecking order simply because of its physical deformity.

4. Instead of retreating from a hand fluttered in front of them—as was the case with all other groups tested—these abnormals occasionally "stood up" to the investigator's hand and did not cower.

In addition to these differences which are thought to be evidences of hypersensitivity, hyperexcitability, greater restlessness and hostility among the abnormals, another deviation from normal behavior was noted. Pecking at knotholes appears to be an innate response of chickens. Although the abnormals pecked at the floor and at each other with regularity, they rarely pecked at the knotholes in the walls of the wooden demonstration box; certainly they did not do so as often as the normals did.

In an attempt to identify possible differences between the groups in terms of learning ability, the animals were run in a simple T-maze in which they learned a position response. Figure 1 is a diagram of the maze employed. The walls are 10⅞" high, the leg is 23" long, the arms

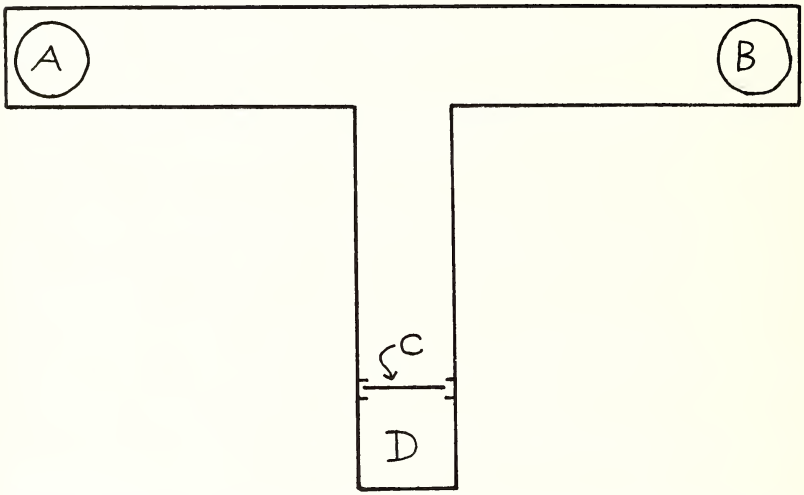

Figure 1. T-maze. A, false food well; B, food well; C, sliding panel door; D, starting box. See text for detailed description.

are each 24" long, and the alleys are 6" wide throughout. The interior of the maze is black, and during the tests it was illuminated by a 40-watt fluorescent bulb situated approximately one inch above the top of the maze and extending the length of the arms. The leg and arms of the maze are covered with a removable wire screen.

The animals proceeded from a 6" x 6" starting box and learned to execute a right turn at the choice-point in order to obtain food placed in the right arm of the maze. The left arm contained a false food receptacle; that is, the food, placed in one Petri dish covered by another Petri dish, could be seen but not obtained by the animals.

Four normal animals and two abnormal animals were each given one trial per day in the maze, from the 4th through the 33rd day after hatching. On the 21st through the 33rd days after hatching the animals were first run in a discrimination apparatus (to be described below) and subsequently in the T-maze. The animals were deprived of food but not water for approximately 12 hours prior to each testing period. The response measure obtained was running time in seconds on each trial.

Mean running times per trial were calculated for both groups, the times based upon the data for days 4 through 33 (all trials) and the data for days 21 through 33 (trials completed after the animals were introduced to the discrimination apparatus). The running times used in the computation of the means were for those days on which complete data were available for all animals. In Table 1 are given the group means per trial for days 4 through 33 and days 21 through 33.

Table 1. Mean running times per trial of 4 normal and 2 abnormal chicks (times are expressed in seconds)—differences between means are not significant.

Group	Days 4 through 33 (All trials)	Days 21 through 33 (Trials completed after animals were introduced to discrimination apparatus)
Normal	17.95	20.08
Abnormal	18.63	38.77

Although the means for the abnormal group are greater than those for the normal group, the differences failed to attain significance at the 5% level of confidence when tested by means of Student's "t-test." The larger difference between the group means for days 21 through 33 suggests that the T-maze performance of the abnormal animals may have been depressed by some aspect of the experience of performing in the discrimination apparatus. This point is discussed in detail later on.

In an effort to identify stimulus preferences which might subsequently be incorporated into tests of learning, a discrimination enclosure (Figure 2) 42″ long, 18″ wide, and 18″ high was designed. A 6″ x 6″ starting

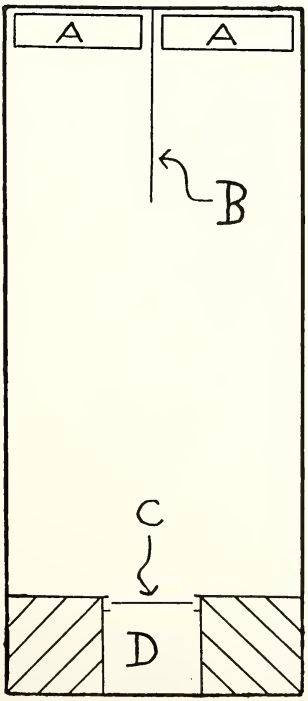

Figure 2. Discrimination apparatus. A, food trays; B, partition; C, sliding panel door; D, starting box. See text for detailed description.

box, with sliding panel door, is located at one end and a 12″ partition extends into the enclosure at the other end, separating this end into two presentation areas. The interior of the enclosure is painted a neutral gray. The stimuli employed were three removable food trays, each a different color. The food receptacles are 8¾″ x 2¼″ x 1½″ and have backs 12″ high. The three food trays are painted with Devoe Mirrolac Enamel, and the colors used are Chinese Red, Holland Blue, and Jade Green.

In the series of tests to be described the food trays were presented in pairs, both trays containing food on each presentation. The positions of the differently colored receptacles were randomized left and right in each series of trials. The 4 normal and 2 abnormal animals were each given 10 trials per day in the discrimination apparatus, from the 21st through the 33rd day after hatching. The animals were deprived of food but not water for approximately 12 hours prior to each test period. The following 3 color combinations were used in order of mention: red-green; red-blue; green-blue. The trials for each color combination were run on successive days, all animals receiving 30 trials on the red-green combination, 30 trials on the red-blue, and 40 on green-blue. Table 2 is a summary of the results of this series of tests.

Table 2. Stimulus preferences shown by 4 normal and 2 abnormal animals. Values of chi-square are based upon one degree of freedom. The double asterisk denotes significance beyond the 1% level of confidence.

Group	Stimulus combination	Preferred stimulus	Value of chi-square
Normal	red-green	red	20.28**
	red-blue	red	12.22**
	blue-green	none	1.70
Abnormal	red-green	red	9.12**
	red-blue	red	24.70**
	blue-green	none	0.15

Both the normal and the abnormal animals demonstrated significant preferences for the red stimulus over the green and for the red stimulus over the blue. Neither group showed a significant preference when presented with the blue-green combination.

No attempt was made to determine the extent to which the preferences manifested by the animals were functions of hue differences or of differences in reflection between the stimulus pairs.

Several qualitative observations of behavior of individual chicks may prove to be significant:

1. One of the abnormals consistently turned to the left in both the T-maze and the discrimination apparatus. When in the T-maze, it always investigated the false food well on the left before proceeding to the right food well. When on one occasion it did go initially to the correct food well, it first turned to the left and then had to twist itself to the right. It

seemed to know where the food was located but could not make the proper turn. When placed in the discrimination apparatus, the chick went to the food well to the left, regardless of the color choice involved. The animal was examined and no apparent physical abnormalities were found which might explain this favoring of a turn to the left over a turn to the right. It seems possible that the abnormal incubating temperature somehow affected the area of the brain which controls the learning of a response to the right.

2. The second abnormal chick was a so-called "master of the T-maze." It made very few incorrect turns and would proceed immediately and directly to the proper food well when placed in the T-maze. When introduced into the discrimination apparatus, it showed an immediate preference for the red stimulus over both the green and the blue. It was the only chick which maintained an exclusive preference for the red stimulus throughout all presentations of the red-green and red-blue combinations. Each day when placed in the T-maze—after going to the red food well in the discrimination apparatus for 10 consecutive trials—this chick seemed to lose its ability to comprehend the T-maze situation quickly as it had once been able to do. Instead of proceeding directly to the right food well as it had done prior to the beginning of the preference tests, it wandered about the maze and took 20 times longer to find the food than it did before it was run in the discrimination apparatus. Its initial concern appeared no longer to be the desire to satisfy hunger, but rather the desire to escape. This happened 7 consecutive days. When the red food tray was removed from the discrimination apparatus on the 8th, 9th, and 10th days and the blue and green trays were presented, the chick returned to its previously learned adaptive behavior in the T-maze and found the food quickly. In order to test the obvious hypothesis that the red food box was confusing the animal, the investigator again exposed the chick to the red food tray prior to putting it into the T-maze. Again the chick appeared to become completely confused and did not find the food until it had wandered aimlessly through the maze for some time.

This chick seemed to be able to learn one thing and one thing only. When the learning situation became complex, it appeared to forget everything but the most recently learned responses.

Behavior patterns similar to those just described were not observed among the normal animals. Since environmental influences were presumably homogeneous for both groups of chicks, it seems likely that the deviant behavior may have represented manifestations of the effect of the abnormal incubation temperature on the nervous system of the animals.

Summary

The behavior of chicks incubated at abnormal temperatures during the early stages of their embryonic life was compared with the behavior of chicks incubated at normal temperatures. The chicks which had been incubated for 5 days at 41° were less aggressive, more fearful, and, generally speaking, less active than the normally-incubated animals.

The chicks which had been incubated for 10 days at 34° were restless, hypertense, and hyperexcitable compared with the normal group of chicks. Although mean running times in the T-maze for the chicks incubated at

this subnormal temperature were higher than those for normal chicks, the mean differences failed to attain significance at the 5% level of confidence. On the other hand, for this same group, both normal and abnormal chicks showed highly significant preferences in a discrimination apparatus for the colors red over green and red over blue. None of the chicks tested showed a significant preference when presented with blue-green color combinations. The individual chicks of this abnormally incubated group demonstrated certain interesting behavioral patterns, which could possibly be interpreted as evidence of limited mental ability resulting in reduced ability to function adaptively.

The investigators realize that it is difficult to arrive at any definite conclusions on the basis of the above results. More temperatures must be considered, different behavioral situations must be presented, and refined techniques must be devised for testing. However, we do feel that the evidence from this particular project is indicative of a possible relationship between the temperatures at which chick embryos are incubated and the behavior of the hatched chickens.

Literature Cited

1. ALSOP, F. M. 1919. The effect of abnormal temperatures upon the developing nervous system in the chick embryo. Anat. Rec. 15 : 307-331.
2. FERE, CH. 1894. Note sur l'influence de la température sur l'incubation de l'oeuf de poule. Journ. de l'anatomie et de la physiologie, Paris, T. XXX : 155-161.
3. GUNTHER, W. C. 1958. Effect of abnormal incubating temperature on chick behavior. Proc. Ind. Acad. Sci. 66 : 363-366.
4. HARRISON, JOHN R. 1957. Morphogenesis of chick embryo in vitro after exposure to lowered temperatures in ovo. Physiol. Zool. 30(3) : 187-197.
5. HARRISON, JOHN R., and IRVING KLEIN. 1954. Effect of lowered incubation temperature on the growth and differentiation of the chick embryo. Biol. Bull. 106(1) : 48-59.
6. MORENG, ROBERT E., and REECE L. BRYANT. 1956. The resistance of the chick embryo to low temperature exposure. Poultry Sci. 35 : 753-757.
7. TAYLOR, LEWIS W. 1949. Fertility and hatchability of chicken and turkey eggs. John Wiley & Sons, Inc., New York.