Genetic and Environmental Factors Affecting the Development of Avian Embryos

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Individuals considering starting student research programs will be interested in this description of many of the factors relative to the hatching process.

Introduction

An avian embryo is amenable to a variety of manipulations at almost every stage. Its overall rate of growth as well as the development of individual structures and organs can be drastically altered by different environmental factors. Disturbances in development may be caused by factors either located within the egg or acting through the outer environment. People who are not primarily concerned with hatchability problems and incubation techniques frequently pay little attention to some of the important factors which have a definite influence on the development of the embryo. This often leads to erroneous conclusions, and makes the reproduction of data difficult.

For workers concerned with studying the norm of development or exploring the causal factors in developmental anomalies, it is of the utmost importance to standardize both material and experimental conditions in order to obtain some harmony in the development of an embryo. The purpose of this manuscript is not to review extensive literature in avian development, but to acquaint researchers and teachers of biology with the roles played by genetics and various extrinsic environmental factors in the development of early embryos. The material is discussed under the following headings:

1. History of the breeder stock.
   a. Genetic background
   b. Diet of the breeders
   c. Diseases
   d. The age of the layers
2. Preincubation storage environment.
   a. Length of preincubation storage
   b. Storage temperature
   c. Humidity level
   d. Turning of eggs
   e. Gaseous environment
3. Incubator environment.
   a. Incubator temperature
   b. Humidity level during incubation
   c. Turning of eggs during incubation
   d. Gaseous environment during incubation
4. Physical and oviposital conditions of the eggs.
   a. Shell porosity and thickness
   b. Egg weight
   c. The time of the day when the egg is laid
   d. Jarring of eggs
5. Optimal conditions for using avian eggs for experimental studies.

1. History of the Breeder Stock

a. *Genetic background*: The role of heredity in avian development has been demonstrated among various breeds and strains of domesticated birds. These observations clearly indicate that besides the physical and chemical environment of the egg, the genetic constitution of the embryo plays an important role in its capacity to develop normally. The rate of embryonic development in both chickens and turkeys is superior in eggs from genotypes selected for high hatchability than from genotypes selected for poor hatchability; the latter are also associated with poor fertility, high developmental variability among embryos, and also with high incidence of early mortality and malformations during incubation. Superior development in eggs from high hatchability genotypes may be due to the fact that they contain blastoderms in the comparatively

more advanced stages of gastrulation at oviposition than do eggs from poor hatchability genotypes. Interestingly, birds selected for large mature body size show some characteristics similar to those of low hatchability genotypes mentioned above.

Early embryonic development in chickens and turkeys is superior in crossbreeds as compared to inbreds; the latter are usually associated with low fertility and a marked delay in the initiation of development. Furthermore, the maternal effect in the rate of embryonic growth has also been observed for F_1 embryos from crosses of various inbred lines of chickens.

b. *Diet of the Breeders:* A variety of nutritional substances are essential for normal growth and development of the avian embryo. These substances should be available in the diet of the mother in sufficient quantity to be deposited in the egg and so made available to the developing embryo. If such substances are lacking in the fertilized egg or, are present in an insufficient amount, death of the embryo may occur sooner or later. Starvation or the limitation of protein supply and particularly the deficiency of essential amino acids are of a relatively more critical nature for the breeders. Although certain types of embryonic abnormalities are unique to a particular nutritional deficiency, most anomalies may have alternative causes involving more than one nutritional deficiency or nonnutritional condition of genetic or environmental origin. Syndromes produced in chickens by deficiency of several water-soluble vitamins such as riboflavin, biotin, pantothenic acid, folic acid and vitamin B_{12} have been reported in detail in the literature. Deficiency of vitamins and minerals also leads to a retardation of embryonic growth, embryonic mortality, and malformations. Various shell defects are associated with the lack of calcium salts in the diet of the hens. Fat soluble vitamins such as vitamins A, E, and K do not show a definite effect on embryonic development but have been found to be essential for the maintenance of fertility in the breeder stock. A reduction in the sperm number and an increased portion of deformed sperms were observed to be associated with the deficiency of vitamins A and E. Some embryonic mortality and malformation may be associated with fertilization by defective or stale (aged) sperm. The presence of toxic elements or compounds in the diet such as fungicides, insecticides, selenium, etc., may have adverse effect not only on the mother but also on the embryos developing in eggs laid by such hens.

c. *Diseases:* One of the most important factors affecting the development of embryos is the health of the breeder stock. Besides nutritional deficiencies, a diseased condition of the layers is very detrimental to the viability of the embryo. Excessive embryonic mortality and malformations have been reported in the chicken eggs from flocks suffering or having suffered from various respiratory diseases such as Newcastle's disease, infectious bronchitis, etc. High embryonic mortality was also reported in flocks having *Salmonella pullorum* infections where the pathogenic organisms are present in the ovarian ova and in the yolks of the eggs laid by infected hens. Certain other types of bacteria, such as the coliaerogenes group and fowl typhoid, have also been isolated from eggs containing embryos that have died early in the incubation. In general, the embryonated egg provides an ideal growth medium for a variety of common bacteria, molds, and viruses.

d. *Age of the layers:* Fertility and shell quality has been found to decrease with an advance in age of the layers. Increasing age of the mother is also a predisposing factor for the occurrence of malformations; the frequency of malformed embryos was found to increase with an advance in the maternal age. High levels of embryonic mortality during the first week of the incubation are seen in the initial eggs from pullets which have just reached sexual maturity. Accordingly, it has been suggested that chicken eggs for experimental studies should be obtained from females in egg production for at least two months and not more than 12 months. In quails, the hatching eggs should be collected from 3-8 month old birds.

2. Preincubation Storage Environment

a. *Length of preincubation storage:* It is a common practice in the hatchery industry to hold eggs for some time in a suitable environment before incubating them. The length of the period for which eggs can be kept without affecting their potential to hatch normally is different in various species. The detrimental effect of storage on the hatchability and early embryonic development in chickens and turkeys has been demonstrated repeatedly. Subjecting eggs to extended preincubation storage even at a so called optimal temperature (13°-16°C) is damaging to the embryo; this is expressed either in the inability of the affected blastoderms to initiate development after being placed in the incubator or to sustain growth after development was initiated. The net result is an increased incidence of early mortality and of malformed embryos. (These observations indicate clearly that the blastoderms are not "preserved" during storage but continue to deteriorate.)

The relative sizes as well as the weights of chicken and turkey embryos decrease progressively as preincubation storage extends beyond 7-10 days. Such embryos also exhibit disproportionate development of different embryonic structures or areas. This leads, finally, to various types of malformations. Variation in the rate of growth among embryos is also very high in eggs stored for prolonged periods.

Heredity has been found to be a factor in the survival of embryos during storage and their ability to undergo subsequent normal development. Blastoderms from high hatchability genotypes are not only more resistant to storage stress but are also superior in development following extended storage periods.

Prolonged storage may, especially under unfavorable conditions, lead to bacterial and fungal invasion and spoilage of hatching eggs.

b. *Storage Temperature*: Numerous investigations have been conducted to determine an optimal temperature for holding hatching eggs for prolonged periods without severely affecting their potential to develop and hatch normally. These studies have shown that embryonic development is not only influenced by the age of the egg but also by the temperature at which eggs are held before incubation. In practice, storage temperatures in the range of 13°-18°C are commonly utilized in hatcheries. In developmental studies, it was observed that growth of chicken embryos was superior when eggs were held at about 18°C for a period of 1-3 days than eggs held at 13°C and 7.5°C for the same period. A reverse was the case when the storage extended for 7-14 days or more. Storage at 18°C beyond 7 days induced regressive structural changes in the blastoderms. These storage effects were expressed later, not only in an increased embryonic mortality both before and after initiation of development, but also in depressed growth and abnormal embryogenesis of the affected embryos. Effects were not so severe when the eggs were held at 13°C and 7.5°C for the same periods. These observations clearly indicate that duration of storage and storage temperature have an important effect on the viability of the blastoderms.

Exposing avian eggs to prolonged periods of freezing and sub-freezing temperatures during winter and to hot weather conditions (temperature such as 38°-41°C) during summer is detrimental to the embryos: they soon lose their potential to develop normally. Under extreme weather conditions, the eggs from the nest or cages should be collected as frequently as possible and soon after each collection the eggs should be transferred to favorable preincubation storage conditions.

c. *Humidity level:* In case the eggs have to be stored for some time before incubation begins, the control of relative humidity of the atmosphere in which the hatching eggs are held is very essential. The literature does not provide convincing evidence for an optimal humidity requirement during storage, but it does suggest that the loss of water from the egg is greater at a higher holding temperature. The smaller the loss of water from the egg during the period of storage, the better it is for subsequent embryonic growth and hatchability. A relative humidity level of 70-80% has proved successful in our laboratory. Evaporation of water from eggs during storage can be prevented by keeping eggs in Cryovac bags.

d. *Turning of eggs*: Turning of eggs during the storage period is not very essential according to some workers. They found no significant difference in hatchability among eggs which were turned regularly during storage and those which were not turned. However, the literature does suggest that turning of the eggs during storage may be beneficial if eggs are held longer than 10-14 days.

e. *Gaseous environment:* Evidences have been accumulating suggesting that it may be possible to reduce the loss of blastodermal viability in stored eggs by exposing them to an altered gaseous environment; storing fertile chicken eggs in an atmosphere of nitrogen was shown to help in this direction. Undoubtedly, more research is needed along this line before the biological basis for these observations can be understood.

3. Incubator Environment

a. *Incubator temperature:* Although embryonic development may be initiated over quite a wide range of temperatures, it proceeds to normal completion only within a comparative narrow range. A temperature of 37.5°-38°C is considered optimal incubator temperature and is widely used in the poultry industry. Deviation of temperature even a few degrees from the optimum, is harmful for the development of the embryo.

Although high temperatures accelerate the rate of embryonic development during early incubation, continued incubation results in embryos which exhibit moribundity and various types of structural abnormalities. Under high incubation temperatures eggs tend to lose an excess of water; this, in turn, causes embryonic mortality and smaller size of the embryos. Lower incubation temperature, on the other hand, may cause a delay or a decrease in the rate of development. If incubation a low temperatures continues beyond a certain time, irreparable damage may ensue, and result in malformations and death of the embryos. It has been observed with chicken eggs that at 34.4°C incubation temperatures nearly all embryos died in the shell and at 30°C, no embryo survived beyond the fourth day of incubation.

Maintaining a constant temperature during the course of incubation is essential. Both decreases and increases in temperature during the incubation period is damaging to the embryos and may lead to embryonic mortality or disproportionate development of certain organs and structures. Surprisingly, a rise of 2°C above the optimal incubation temperature produces more drastic effects on the embryos than a corresponding fall of 2°C.

b. *Humidity level during incubation:* In addition

to an optimal incubation temperature, a suitable humidity level is also very essential for the harmonious development of the avian embryo. In a very low relative humidity, there is an excessive evaporation of water from the egg contents; the shell membranes and other embryonic sacs become unusually dehydrated. As a result, the normal metabolic activities and respiration of the embryo may suffer interference, resulting in the death of the individual. Embryonic mortality is also observed when the relative humidity is too high. The shell membranes and outer shell surface become unusually watery and this, in turn, may interfere with gaseous exchange through the shell.

Although the optimal level of humidity during incubation has not been determined satisfactorily, good results have been achieved with a relative humidity between 70-80%. In ordinary laboratory incubators, water kept in an open pan at all times during the incubation period is satisfactory. Pheasant eggs require a higher level of humidity at the beginning rather than towards the end of incubation period, whereas the reverse appears to be true for chickens and quail eggs.

c. *Turning of eggs during incubation:* Turning of eggs during the course of incubation is essential, particularly after the first two days of incubation. Lack of turning results in the abnormal adhesion of embryonic and extra-embryonic structures to the shell membranes and ultimately leads to retardation in the growth of the embryo, developmental anomalies and death. Turning eggs 6-8 times a day through an angle of 90° is beneficial. It is better if the incubator is fitted with an automatic turning device.

d. *Gaseous environment during incubation:* An adequate supply of oxygen is imperative for successful development of the embryo. Early chick embryos are very susceptible to reduced as well as high oxygen tension. Oxygen levels below 18% tends to reduce hatchability quite proportionally to the decrease in the oxygen concentration. Very low oxygen tension during early stages of incubation is teratogenic. The best hatchability results have been obtained with 21% oxygen and 0.05 to 0.12% carbon dioxide in chickens. A carbon dioxide concentration above 1% results in slow embryonic growth malformations, and early death of the embryos.

Toxic fumes from substances such as formaldehyde, turpentine, ammonia, etc., are harmful to the embryo.

4. Physical and Oviposital Conditions of the Eggs

a. *Shell porosity and thickness:* The shell not only protects the embryo against various unfavorable external agencies but also acts as a good medium for the exchange of gases between the developing embryo and the environment. Any structural changes in the shell will affect the biological quality of the egg. Excessive porosity and thinness of the shell permits the loss of water by evaporation from the egg, which is definitely harmful to the embryo during incubation. Under such conditions an increased frequency of embryonic deaths and malformations have been observed. Shell porosity is greater during the second laying year of hens. The egg occupying the first position in the clutch is relatively less porous than those occupying subsequent egg positions. Poor shell quality is often associated with a lack of calcium in the diet of layers.

Eggs used in experimental studies should be devoid of cracks. When the shell of an egg is cracked, the developmental potential of the embryo is destroyed. Quail eggs are extremely sensitive to breakage and shaking and should be collected as soon as possible after they have been laid. Cracks in the shell are generally produced by rough and frequent handling of eggs.

Soiled eggs should be discarded since the closing of the pores of the shell prevents necessary gaseous exchange between the embryo and the environment. Eggs intended for experimental studies should never be washed.

b. *Egg weight:* Although there are some conflicting reports on record with regard to the relationship between egg weights and the rate of early embryonic development, it is well established that embryos from larger eggs are somewhat heavier than those from smaller eggs during the latter part of the incubation period. It was observed in this laboratory that embryos in both large and very small eggs, at 38 hours of incubation, exhibited a higher developmental variation and increased frequency of malformations as compared to embryos in medium sized eggs.

c. *Time of the day when the egg is laid:* Some degree of relationship between hatchability and the time of the day when eggs are laid has been reported by some workers. Eggs laid between 9:00 a.m. and 2:00 p.m. had better hatching potential than those laid either earlier or later. The incidence of malformed embryos was higher in eggs laid during the afternoon than in eggs laid in the forenoon. Conflicting reports are also on record with regard to such a relationship.

Eggs retained in the oviduct for unusually long intervals of time exhibit a high frequency of early embryonic mortality and malformations. At the same time such eggs are expected to contain blastoderms at a relatively more advanced stage of development at oviposition.

d. *Jarring of eggs:* Prolonged and violent vibrations or the mechanical shaking of eggs during the course of incubation results in increased early mortality, hemorrhages, rupturing of the yolk sac and various other developmental malformations. The frequency of malformations was also higher in eggs shaken prior

to incubation. Shaking eggs with the large ends up seems to be relatively less damaging to the embryo than shaking with the small end up. Jarred eggs develop a tremulous air sac, which in turn, is believed to cause the death of the embryo.

5. Optimal Conditions for Using Avian Eggs for Experimental Studies

Breeders should be healthy and free from any disease. They should be fed a diet adequately supplied with all nutrients eventually required for the normal development of the embryos. Layers should be in production for at least two months with well established high hatchability records. Eggs should be collected from the nests or cages at least 2-3 times daily or more frequently during extreme weather conditions. Soon after collection, the eggs should be transferred to a storage room maintained at 13°-16°C and 70-80% relative humidity. Eggs for experimental purposes should not be stored for more than 7 days. Under no circumstances, should the eggs held for varying durations of time and from different breeds and strains of birds, be utilized in the same experiment. Only clean eggs of uniform size and shape with good shell quality should be utilized in the experiment. Eggs retained longer than usual in the oviduct should not be utilized. A minimum handling of hatching eggs should be practiced.

Incubation should be carried out in an incubator housed in a well ventilated environment and equipped to maintain optimal conditions of temperature (37.5°-38°C), and relative humidity (70-80°). Preferably, the incubator should also be equipped with an automatic device for turning the eggs at least 6-8 times daily. The incubator should, at all times, be clean and free of any putrifying and decomposing material. A constant temperature should be maintained during the course of incubation.

When using stored eggs, it is suggested that the eggs be held at room temperature (about 23°C) for about 12 hours after removing from the storage room; during which time, the blastodermal cells will gradually return to the metabolic level which must exist before they can participate in normal development.

The study of avian embryological development and associated influences of environmental factors offer innumerable possibilities of experimentation in order to increase the basic understanding of developmental processes. The subject matter covered in this manuscript will not only add to knowledge about the material but also offers opportunity to beginning biologists and advanced students in biology for further experimentation in this area both inside and outside of the classroom.

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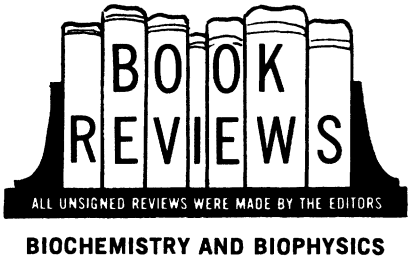

BIOCHEMISTRY AND BIOPHYSICS

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These are paperback un-books, attempting to provide subject matter in a unique format, supposedly to fill a gap in what the author believes to be the almost solely verbal treatment of the subject in textbooks and the strictly visual metabolic maps and similar aids now available. That this thesis may be specious is suggested by the many excellently illustrated books on biochemistry currently available, such as that by R. J. Light. The volumes are essentially a series of odd shaped cards (two-and-three-quarters by four inches), printed eight to the page on perforated stock and capable of being removed *ad libitum*. The cards are printed on both sides and contain formulae and reactions of biochemical compounds, tables of pertinent data, and flow diagrams and schematics of biochemical processes and concepts. The student is enjoined to take pertinent cards to appropriate lectures and, instead of taking notes, simply jot down the applicable card numbers for study