CRITICAL PERIODS AND CAUSES OF DEATH IN AVIAN EMBRYONIC DEVELOPMENT

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AVIAN embryonic mortality has long been a subject of biological interest. It is also a problem of obvious economic importance. The total embryonic mortality of domestic fowl, *Gallus gallus*, has been progressively increasing for some time. Less than 30 years ago, when flocks were small and kept at large, the embryonic death rate rarely exceeded 10 per cent. As simple and primitive methods of poultry management have been replaced by such efficient modern practices as intensive feeding, breeding in confinement, and large-scale artificial incubation, embryonic mortality has frequently risen to 25 per cent or more.

Numerous investigations have attempted to determine the causes of death during the incubation period, and to explain the rising embryonic death rate. Studies have been made not only of chickens, but also of a number of other domestic birds and some game birds raised in captivity.

DISTRIBUTION OF MORTALITY

In 1919, Payne pointed out a fundamental fact regarding embryonic mortality in the fowl when he published his data on the distribution of deaths throughout the period of incubation. His observations of 2,142 dead embryos indicated that mortality is especially high between the third and fifth days, and again on approximately the nineteenth day. He also found that artificially incubated embryos were much more likely to die during the later period of susceptibility than embryos incubated under the hen. This discovery immediately suggested that environmental factors were at fault, an inference that has subsequently been borne out by the results of many studies.

Later, Riddle (1930) extended Payne's findings on embryonic mortality to include doves, *Streptopelia risoria*, and pigeons, *Columba livia*. Although the incubation periods of doves, pigeons, and chickens are of different lengths, the curves of embryonic mortality are nevertheless similar for all three species. Two peaks are seen at essentially equivalent points, one early in embryonic life, the other shortly before the hatching date. In fact, avian development in general is characterized by these two periods of increased mortality. Data on turkeys, *Meleagris gallopavo*, ducks, *Anas platyrhynchos*, pheasants, *Phasianus torquatus*, grouse, *Bonasa umbellus*, and quail, *Colinus virginianus* (Romanoff, 1934 and unpublished ms.; Romanoff, Bump, and Holm,

1938) substantiate this statement. In pheasants and quail, the embryonic death rate is probably lower under natural conditions than it is when these birds are incubated artificially. It should be noted that quail, when raised in captivity, are subject to a third period of increased embryonic mortality approximately at the midpoint of incubation.

General Causes of Embryonic Mortality

From the above observations on the distribution of embryonic mortality, two questions logically follow. Why do avian embryos tend to die at certain ages, rather than at others? What are the principal causes of death?

At the outset, it may be stated that gross structural abnormalities and teratism account for a relatively small percentage of embryonic deaths. Various studies have demonstrated this fact. Byerly (1930) examined approximately 2,000 dead chicken embryos and found that only about eight per cent of them were terata. Hutt (1930) detected only 559 malformed embryos (3.1 per cent) in 17,700 eggs that failed to hatch. Of the various types of anomalies that he identified, hyperencephaly, microphthalmia and exencephaly were most frequent.

Some monstrosities evidently arise from genetic causes, but it is probable that a much greater number are induced by the conditions of incubation. Dareste (1891), Stockard (1921), and others have shown that various abnormalities may result when the rate of embryonic development is retarded or accelerated, especially if interference occurs in the early stages of incubation. The developmental rate has been altered experimentally by subjecting the embryo to the influence of ether, alcohol fumes, narcotics, various gases, abnormal temperatures, restricted or excessive amounts of oxygen, and excessive quantities of carbon dioxide.

Only eight types of monstrosities in birds are known to be the result of genetic lethal characters. Death in the first half of the incubation period is the result of three of these characters: dominant creeper (Cutler, 1925), recessive white (Dunn, 1923a), and the "talpid lethal," which produces malformed extremities (Cole, 1942). Development continues until later in the incubation period in the presence of any of the remaining characters—recessive abnormal upper mandible (Asmundson, 1936), homozygous short legs (Landauer, 1935), diplopodia (Taylor and Gunns, 1947), simple recessive "stickiness" (Byerly and Jull, 1932), and incompletely dominant "crested" in ducks, *Anas platyrhynchos* (Rust, 1932). Ordinarily, lethal characters account for a very insignificant proportion of embryonic deaths and

are likely to be present only in inbred flocks. Byerly, Knox, and Jull (1934), as well as others, have shown that inbreeding increases embryonic mortality at both critical periods, but to a greater extent late in incubation.

In addition to structural anomalies leading to the death of the embryo, there are also malpositions that may contribute to mortality. Malpositions become evident at or near the end of incubation and are of several types. Hutt (1929) observed that 56 per cent of all chick embryos dead at 18 to 20 days were in abnormal positions. There are at least four malpositions that make hatching extremely difficult or impossible, either because the chick's head is turned so that the air in the air cell is inaccessible, or because movement is so restricted that the chick is incapable of striking the shell, or because of a combination of both reasons. It is possible that a very strong chick is occasionally able to overcome the respiratory handicap. However, no chick can hatch if its head is buried between its thighs, a malposition which has been found in nine per cent (Hutt, 1929) to 13 per cent (Sanctuary, 1925) of all embryos dead in the final stages of incubation. It is interesting to note that the incidence of malpositions may be increased considerably by various environmental factors, such as abnormal temperature (Romanoff, Smith, and Sullivan, 1938) and an excess of atmospheric carbon dioxide (Romanoff and Romanoff, 1933). These findings indicate that, as Dove (1935) suggested, malpositions are not always the primary cause of death, but may be secondary to unfavorable environmental conditions or other lethal factors. Heredity probably plays but a small part as a cause of malpositions.

CAUSES OF DEATH AT CRITICAL PERIODS

A number of explanations have been advanced to account for the peaks in the mortality curve which are present early and late in incubation. Riddle (1930) suggested that the first critical period was caused by respiratory maladjustments before the establishment of the special respiratory surfaces (area vasculosa, chorio-allantois) that appear during the first three or four days. Carbohydrates are the chief source of energy for the embryo at this time, as the high respiratory quotient indicates (Bohr and Hasselbalch, 1903), and carbon dioxide perhaps accumulates in sufficient quantity to prove fatal. It has also been pointed out (Tomita, 1921) that there is a maximum concentration of lactic acid at a time when the enzyme decomposing lactic acid is present in very small amount. Needham (1926) noted that nitrogen, early in the developmental period, is excreted largely in the form of ammonia which is very toxic to the embryo (Dareste, 1891).

According to Boyden (1927), hydronephrosis possibly results from mechanical obstruction of the mesonephros as this organ begins to function.

In the late critical period, death may perhaps be caused by failure to make a proper transition from allantoic to pulmonary respiration, as Brody (1927) suggested. Pohlman (1919) and Dunn (1923b) noted poor development of the hatching muscle (musculus complexus) as a contributing factor. In artificial incubation, deficient oxygenation may result if the oxygen tension of the air becomes too low (Arbuckle, 1918). If the eggshell is highly permeable and the relative humidity is too low, excessive evaporation from the egg may lead to water-starvation (Riddle, 1921). It should also be pointed out that, at the end of the developmental period, the cumulative effect of all unfavorable conditions may be felt, with the result that the viability of the embryo is lowered. Abnormal changes in the physicochemical state of the embryonic fluids are especially important.

The middle critical period, previously mentioned as characterizing the embryonic development of quail in captivity, ordinarily does not appear unless birds are fed a diet deficient in animal proteins (certain amino acids), minerals (especially calcium), or vitamins. Byerly, Titus, and Ellis (1933) noted a pronounced increase in embryonic mortality halfway through the incubation period in flocks fed diets of vegetable origin exclusively. Smith (1930) found that the middle critical period became evident in winter, if birds received insufficient amounts of sunshine or vitamin D, and disappeared if they were given cod liver oil or were irradiated with ultraviolet light. According to Adamstone (1931), a lack of vitamin E in the egg, although increasing mortality most frequently on the fourth day of incubation, is sometimes responsible for death at about the eleventh day. A deficiency of riboflavin in the hen's diet is particularly likely to lead to embryonic deaths in the midperiod of incubation and increases their incidence at the other critical periods as well (Lepkovsky, Taylor, Jukes and Almquist, 1938). The distribution of deaths caused by riboflavin deprivation, however, depends upon the relative lack of the vitamin in the egg. The major peak in the mortality curve falls at a progressively earlier date as the degree of vitamin deficiency increases (Romanoff and Bauernfeind, 1942).

Some abnormalities of growth in riboflavin-deficient embryos may be mentioned. In general, body weight in such embryos is consistently below normal. Embryos that die in the middle critical period may weigh from 20 to 90 per cent of normal. Those that survive until the final stages of incubation tend to fall into two distinct groups,

those that weigh less than 40 per cent of normal, and those that weigh about 90 per cent of normal. It appears that some embryos, before dying, pass through a prolonged period of morbidity, during which their growth is greatly retarded. Other embryos evidently develop at more nearly normal rates and die very suddenly.

It may be of interest to add that the oxygen consumption of the moribund embryo, whatever the cause of eventual death, often provides a clue to the embryo's condition (Romanoff, 1940). Although abnormally large amounts of oxygen may be used by some embryos (usually those that die in the last critical period, probably when they go through a death struggle), the oxygen consumption of the dying embryo is frequently less than normal for several days before death (Romanoff, 1941). In addition, there is a linear relationship between the percentage reduction in oxygen consumption and the percentage retardation in growth.

CONCLUSION

Embryonic deaths in birds occur principally at three critical periods which, in the chicken, fall on the third to fifth, twelfth to fourteenth, and eighteenth to twentieth days of incubation, respectively. The appearance of three peaks in the embryonic mortality curve is, so far as is known, specific to birds. The causes of death at each critical period are more or less distinctive, and many of them are specific for Aves.

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