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Embryology, Incubation, and Hatching

Proper incubation and hatching is vital for aviculture success. Veterinarians must have an understanding of embryology and incubation procedures to assist the aviculturist with minimizing embryonic mortality and providing neonatal care. We work with a broad range of species but most of what is known about incubation is derived from poultry. For other groups, such as waterfowl and psittacines, more information is becoming available. For rare and endangered species, or species not commonly found in captivity, little is known, and information must be gleaned from successes and problems with other species.[1]

EMBRYOLOGY

The ovum of an avian egg includes the yolk, which is produced by the ovary. The protoplasm containing the nucleus floats above the yolk and under the plasma membrane. Upon ovulation the ovum moves through the fimbria and, shortly after ovulation, into the oviduct. Fertilization takes place in the upper infundibulum and the first cleavage occurs 4 to 5 hours after ovulation. Rapid cell division follows as the yolk passes down the oviduct. Albumin, the inner and outer shell membranes, and finally the shell are applied.[2,3]

Embryonic development begins prior to laying. When the egg is laid, an embryo already consists of 2000 to 60,000 cells in the form of a circular disc called a "blastula," which is in the process of gastrulation. Gastrulation is the stage in embryonic development in which the blastula folds into itself to form the embryonic gut. The resultant embryo is visible on the surface of the yolk shortly after laying.[4] A fertile unincubated egg can be distinguished from an infertile egg by selective staining of the blastula (embryo) at the time of laying. The trained eye may also be able to recognize an embryo, especially if death occurs after a few days of incubation.[2–4]

The egg membranes, the amnion, chorion, and allantois, are sheets of living tissue that develop from the embryo itself. A pouch-like outgrowth of the digestive system encircles the yolk to form the yolk sac. In the last one-third of the incubation period the embryo produces enzymes to digest the yolk and transport nutrients through its blood vessels. The amnion and chorion develop as folds of the body wall and surround the embryo.[2] The amnion develops into a fluid-filled sac in which the embryo grows, allowing movement. The allantois, an extention of the digestive tract, fuses with the chorion to form a compound membrane, the chorioallantoic membrane (CAM). The CAM is filled with many blood vessels, necessary for exchange of oxygen and carbon dioxide through the shell. All of these critical developmental stages occur within the first one-half of the incubation period. The embryo is most susceptible to adverse conditions or trauma during this time.[2]

INCUBATION TECHNIQUES

The success of incubation and hatching techniques is indicated by fertility and hatchability rates. Fertility is the percentage of eggs found to be fertile, as indicated by evidence of embryonic development. Hatchability is the percentage of fertile eggs that hatch.

Candling is the oldest and most frequently used method to monitor embryonic development. The term "candling" dates this technique to the days when a candle was the light source used in the procedure. A candling lamp may consist of an electric light source within a box or a small bright flashlight. Light passes through a raised circular opening within a rim of rubber or flexible plastic. The egg is held tightly against the rim. With the large end (air cell) against the rim, the egg is rotated to identify different structures inside. The light source must be adequate for viewing but not so strong or hot that it will damage the egg. It must be used in a dark room. A 40-watt bulb at 4 cm (1.5 inches) from the egg will work well with chicken-sized eggs. Abnormal shell development may affect visibility as well as hatchability (Fig. 5–1). For smaller eggs, a lower wattage bulb is recommended. Colored or spotted eggs are difficult to candle. In a newly laid egg the air cell is very small, the albumin clear, and the yolk appears yellow (see Color Fig. 5–2). Embryonic development appears as a darker area. In early development, the vascular ring is often visible as a red circle, and the embryo as a small dark or red spot within the blood ring (see Color Fig. 5–3). The beating heart is usually visible by the end of the first third of incubation.

The air cell is the lightest colored part of the egg, usually found in the blunt end of the egg. With practice, infertile, dead, or damaged eggs can be identified (see Color Figs. 5–4 and 5–5). Cracks are more visible during candling. Seal cracks with a small amount of paraffin, beeswax, or white glue. (Avoid products containing acetone because it is toxic to the embryo.) Eggs should be candled once or twice weekly and damaged or dead eggs removed from the nest or incubator.[4, 6]

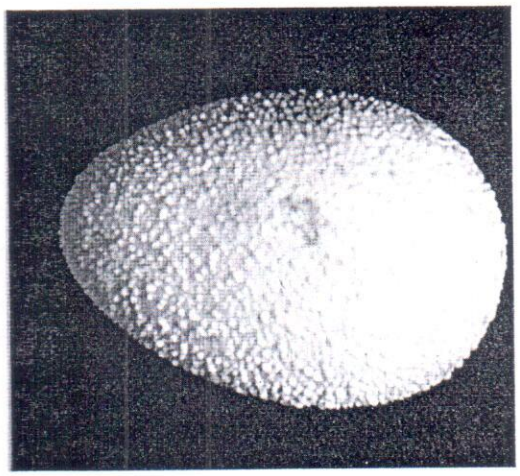

Figure 5–1

Egg with abnormal calcium deposition laid by a scarlet macaw with salpingitis.

Egg Hygiene

Improper egg handling contributes to decreased hatchability and can foster certain types of disease. An incubation temperature of 99.5°F (37°C) is ideal for bacterial growth.[6, 7] Eggs should always be handled with clean or gloved hands.

The egg is laid at the body temperature of the hen and begins cooling immediately. As the egg cools, its contents contract and a negative partial pressure forms, drawing air into the egg, forming the air cell. During the cooling process, contaminants on the shell (such as bacteria, fungi, and viruses) can be drawn into the egg.[2, 4, 6]

Egg contamination can be reduced in the nest and during egg collection. Offer only fresh, suitable nesting material to the parent birds. Keep the nest clean and dry because wet, rotting bedding can be a source of *Aspergillus* spores. Keep the cage clean; parent birds can track fecal contamination back into the nest on their feet and feathers. Eggs to be artificially incubated should be collected as soon as possible after laying to help reduce the chances of contamination. In some species, and in aviaries using some incubation equipment, artificial incubation from day 1 may not be recommended.[8] Both the collection basket and the aviculturist's hands should be clean. Take care to ensure that eggs are not chilled when they are moved from the nest to

the incubator, especially if the hen has already started to set.

Washing Eggs

Washing eggs, if done properly, can reduce contamination; however, improper washing may result in contamination. For species such as ostriches and psittacines, routine washing is not recommended.[5, 7] Washing may remove the protective cuticle layer on the outer shell. Bacteria enter the pores of a wet egg more readily than those of a dry egg. Washing can be beneficial if the egg has visible contamination, such as adherent fecal material.[4]

To avoid causing further contraction of the egg contents and movement of contaminated water into the egg, always use water that is warmer than the egg (110°F, 43°C). Higher temperatures (43–60°C) have been used successfully on eggs for short periods (3–5 minutes) but can be dangerous for small eggs. Detergents or disinfectants are added to the wash water (e.g., 10% povidone-iodine added to warm water to make a 1% solution, or chlorhexidine, sodium hypochlorite, quaternary ammonia, and phenolic disinfectants have all been used).[1] The use of detergents and some disinfectants can be harmful to passerines and other species with small porous shells. Cleaning by gently scraping with a bristle brush or sanding is also effective in removing gross organic debris clinging to the shell.[4]

Fumigation

Fumigating both eggs and incubator is an effective method for disinfecting both. Egg fumigation is most effective shortly after laying. Fumigation of eggs after incubation has begun can result in toxicity unless levels of fumigants are reduced. Place eggs in an incubator and allow them to warm. Then, inside the incubator, mix 0.33 ml of 40% formalin and 0.175 g of potassium permanganate (per cubic foot of incubator space) in a clean glass jar. Allow the gaseous fumigant cloud to remain in contact with the eggs and inner incubator surfaces for 20 to 30 minutes before exhausting the fumigant to the outside.

Use caution! Do not inhale the fumigant mix It is carcinogenic and may cause severe irrit to the skin, eyes, and respiratory tract. Pe fumigation only in a well-ventilated room leave the room while fumigation is in pro Safety badges should be worn to detect dang levels of formalin that may be present in th The U.S. Department of Occupational Safety Health Assessment (OSHA) may prohibit the u fumigants in the workplace.

Egg Storage

Storage of eggs before incubation may be us synchronize incubation and hatching, and to duce even-age chicks. Hatch synchronization portant for neonatal care of precocial specie may not be of importance for altricial species. eggs at 12.8 to 18.3°C (55–65°F)[2, 3] and 75% re humidity. Chicken egg hatchability is reduc about 2% per day when stored. Storage may in unacceptable losses in nondomestic specie

Egg storage for more than one week is no ommended. Warming the eggs to 27°C (80°F minutes and turning each egg 90 degrees m prove hatchability, because this reportedly r the behavior of some wild birds that lay clutches that hatch simultaneously.[4] Other a argue that egg turning when eggs are stored f than a week is not required.[5]

ARTIFICIAL INCUBATION

As desirable as parental incubation may be, in cases this is not possible and the aviculturis rely on artificial incubation (Table 5–1). A incubation may be used to increase product encouraging multiple clutching. It may be nec when incubating birds are lost, when rem eggs from wild nests or nests in large flight and when inappropriate incubation behavior served in parent birds. Artificial incubation r needed until suitable parents are available.

A variety of commercial incubators are ava Small, tabletop units are practical and cost-ef for the small aviculture collection. Incubators

Table 5–1

INCUBATION AND HATCHING TIMES FOR COMMON PSITTACINES (Forced Air Incubation)

Species	Length of Incubation (days)	Pip to Hatch (hours)
Macaws		
(*Ara* spp.)	26	24–48
Hyacinth macaw (*Anodorhynchus hyacinthinus*)	26–28	24–72
Conures		
(*Pyrrhura* spp. and *Aratinga* spp.)	23–24	24–48
Grey parrots		
(*Psitticus* spp.)	26–28	24–72
Senegal parrots		
(*Poicephalus senegalus*)	24–25	24–48
Eclectus parrots		
(*Eclectus roratus*)	28	24–72
Monk parakeets		
(*Myiopsitta monachus*)	23	24–48
Budgerigars		
(*Melopsittacus undulatus*)	18	12–36
Cockatiels		
(*Nymphicus hollandicus*)	21	24–48
Yellow Amazons		
(*Amazona ochrocephalia*)	28–29	24–48
Other Amazons		
(*Amazona* spp.)	24–26	24–48
Cockatoos		
(*Cacatua* spp.)		
Large cockatoos	26–29	24–48
Small cockatoos	23–25	24–48
Palm cockatoos (*Probosciger aterrimus*)	28–30	48–96
Lories		
(*Eos* spp., *Lorius* spp., *Chalcopsitta* spp.)	26–27	24–36
Lovebirds		
(*Agapornis* spp.)	22	24–48
Caiques		
(*Pionites* spp.)	25	24–48
Pionus parrots		
(*Pionus* spp.)	25–26	24–48

Data from Jordan,[6] Joyner,[7] and Clubb and Phillips.[8]

in size from these tabletop units to large walk-in units that hold thousands of eggs (Fig. 5–6).

Incubation Temperature

Temperature and humidity requirements differ with the species of bird and may vary with the microenvironment within the incubator and the room environment in which the incubator is housed. If the species is commonly hatched in captivity, incubation requirements may be published. If incubation information is unavailable, the following guidelines may be useful: small eggs are incubated at 37°C (99.5°F), whereas larger eggs require lower temperatures, usually 0.25 to 0.50°C less.[1] Ostriches are incubated at 36.0 to 36.4°C (96.8–97.5°F).[5] One author feels that cockatoos and macaws do well at slightly below 37°C, while galahs (rosebreasted cockatoos) and Australian parakeets do best at (98.7°F).[9]

Temperature requirements are critical, especially during the first one-third of the incubation period. A temperature elevation of as little as 1.0°C can kill an embryo in the first one-third of incubation. A marginally higher temperature during incubation increases the incidence of late-dead embryos. Embryos that survive to hatch are small and weak, and many have unhealed navels or exposed yolk sacs. Elevated incubation temperature also increases the incidence of crossed or scissor bills, curled toes, and wry necks. Temperature is less critical in the last one-third, as the embryo gains some ability for thermoregulation. During this period a slight elevation of incubation temperature may produce only a hatch earlier than expected.[2–4]

Temperatures lower than recommended during incubation will result in developmental problems as well that vary in severity based on the margin of error. A slight lowering of temperature each day has little effect on the embryo other than a possible

Figure 5–6

Table-top incubator with automatic turning device. A halogen light source with fiberoptic light cable is ideal for candling eggs in the incubator without handling them.

slight delay in hatching. However, a low temperature throughout incubation increases the incidence of late-dead embryos. Chicks surviving to hatch are weak, with large, soft bodies resulting from a large yolk sac, and have weak legs and a poor sense of balance. Hatching is delayed and may take several days.[2, 4]

Humidity

Relative humidity requirements for proper incubation vary with species but, generally, they are not as critical as proper incubation temperature. An embryo has some ability to compensate for differences in humidity, particularly during the last one-third of the incubation period. During this period the embryo actually swallows amniotic fluid and remaining albumin to compensate for water loss resulting from low incubation humidity. Low relative humidity, especially in the first one-third of the incubation period, interferes with proper mobilization of eggshell calcium required for bone development and may result in a stunted embryo. In late incubation, low relative humidity results in dehydration of the embryo. Albumin appears glue-like in consistency, and the kidneys fail because of insufficient water. This often results in the death of the embryo just before entering the air cell. Increased humidity levels during incubation result in eggs with small air cells and soft, edematous chicks with increased incidence of poor umbilical seal and exposed yolk sacs. Embryonic mortality can result from inadequate space in the air cell for pipping.

Egg Weights

Eggs naturally lose weight during incubation because of metabolism and evaporation of water. The rate of weight loss is dependent upon environmental humidity but also varies as a result of shell porosity, air flow, and temperature, so that even in a single species individual variability in weight loss occurs. A useful technique to monitor the health of eggs is to weigh them periodically and chart the weight loss. A mathematical relationship exists between shell porosity, incubation time, and relative humidity. During the entire incubation period, acceptable total weight loss averages 13% (11–16% range). Weight loss varies with the incubation stage, generally being greater during both early and late incubation. However, even during these periods, weight loss should not vary by more than 3% from expected. Air cell size, determined through candling, is also proportional to weight loss and can be used by experienced persons to monitor water loss. If humidity is too low, the air cell is larger than normal; conversely, high humidity results in a smaller than expected air cell.

Turning

Eggs require turning for proper incubation and hatching. Parent birds on average turn an egg every 35 minutes. Turning eggs is required to prevent adhesions of the embryo to the shell membranes. Inadequate turning results in early-dead or malpositioned, late-dead embryos. During artificial incubation, eggs should be rotated at least five times daily. The better quality commercial incubators, including some of the smaller tabletop models, have mechanical turners and timers to automatically rotate eggs.

EMBRYONIC MORTALITY

The egg and developing embryo are very susceptible to unfavorable environmental conditions. Considering the complexity of the developmental process it is surprising that so few defects occur during incubation. Research using fruit flies, frogs, and mice has shown that radiation (x-rays), ultraviolet rays, temperature changes, and a variety of chemical substances induce alterations in development of the embryo. The type of defect that occurs depends more upon the stage of development of the embryo at the time of exposure to the teratogenic agent than on the agent used. Such observations have led to the concept of critical periods in development, when particular organs or systems are developing rapidly and are most susceptible to interference.[2, 4]

In poultry, 33% of embryonic mortality occurs during the first critical period (first week of incubation), and approximately 60% occurs during the

hatching period, with little mortality during other periods. In one study of psittacine embryonic mortality, similar patterns of mortality were observed in psittacine eggs.[2, 4, 9]

Many factors are responsible for mortality during the first week of incubation, including rough egg handling, improper incubation parameters when artificial incubation is used, failure of the parents to properly incubate eggs, high or low temperatures in the nest, inbreeding or genetic abnormalities, eggborne infection, and contamination. Artificial incubation parameters that may result in early embryonic mortality include improper temperature or humidity levels, excessive vibration, improper egg turning, and poor ventilation, leading to buildup of carbon dioxide.

A blood ring, which is extravasation of blood into a ring surrounding the remnants of the embryo and embryonic circle of blood vessels, indicates early embryonic death.

The period of least mortality and risk to the embryo is midincubation. Improper incubation parameters, especially overheating, are the most common risks. Nutritional deficiencies in breeding stock, eggborne infections, and toxins such as chemical fumes around the incubation area may also result in mortality.

HATCHING

Hatching is a complex process. Problems during incubation may be reflected in mortality at hatching. As an egg nears hatching, the head of the embryo shifts within the egg. From its position in the narrow end of the egg the head moves up under the right wing, with the tip of the beak pointed toward the air cell. Carbon dioxide levels in the embryo begin to rise because the allantoic circulation no longer has the capacity to meet embryonic needs. The rising carbon dioxide levels produce spasms or twitching in the embryo's neck muscles, which forces the egg tooth on the tip of the beak to puncture the air cell membrane. Internal pip occurs when the chick breaks through the chorioallantoic membrane and its head enters the air cell. At that time, the embryo has direct access to air and begins gulping it. As this happens, the lungs begin to function in air exchange, and a right-to-left ventricular shunt closes. In species that vocalize, a gentle peeping sound may be emitted by the embryo. The elevated carbon dioxide levels that produce contractions of the neck muscles, and the struggle associated with hatching, also cause abdominal contractions that slowly pull the yolk sac into the abdominal cavity.

Pipping

As an embryo breathes from the air cell, the gaseous exchange through the shell is unable to meet the demand. Gradually, the carbon dioxide levels rise as high as 10%, inducing even stronger muscle contractions. These contractions eventually force the beak through the shell, creating the pip hole and increasing the air circulation to the embryo. Elapsed time between entering the air cell and pipping ranges from as little as 3 hours to as long as 3 days, depending on the species.[1] For most psittacine species this interval is 24 to 48 hours (see Table 5–1). Knowledge of hatching sequence and time intervals for a species is important to diagnose hatching problems and to determine if intervention is appropriate to help produce a viable chick.[11] Opening an air cell too soon interferes with other aspects of the hatching process that require elevated carbon dioxide levels (Fig. 5–7).

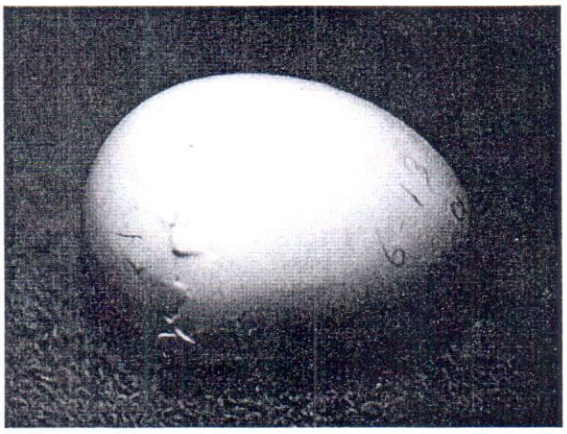

Figure 5–7

Embryo is cutting out of the shell, moving in a typical counterclockwise fashion.

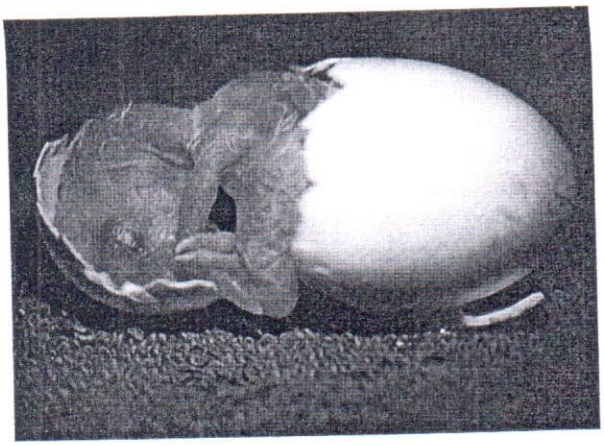

Figure 5–8
Blue and gold macaw chick emerging from the egg.

The Hatch

When the chick's head is in the air cell, activity alternates between jerking head movements and prolonged contractions of the head and back. The head movement further chips the shell, while the contractions of the neck and back help draw remaining exteriorized yolk sac into the body. Contractions force the body to rotate slightly in a counterclockwise direction. This rotation positions the head over a new section of the shell. As the chick rotates and further pips at the shell, it makes a circular pattern of connected holes in the cap or wide end of the egg. This chipping away the shell is referred to as "cutting out." Finally, during one of the contractions, the head is pushed against the weakened shell and forces open the top of the egg (Figs. 5–8 and 5–9). The chick then proceeds to kick free. The time between pipping and kicking free is species-dependent and ranges from 0.5 hours to 3 days. The normal interval for most psittacines is less than 24 hours. After hatching, the exhausted chick will rest and dry. As the chick dries, the sheaths around the down feathers are shed, and this is sometimes referred to as "incubator fluff."

Critical Stages of Development

In the absence of disease that is transmitted through the ovary or oviduct infection, the egg is essentially

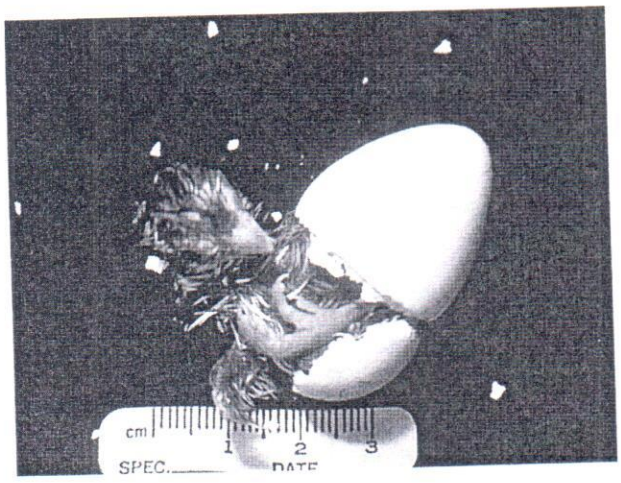

Figure 5–9
Masked bobwhite quail chick kicking free in normal hatch.

Figure 5–10

Malposition with head in the small end of the egg. (malposition 2.)

a sterile package. After laying, however, the egg is exposed to environmental and handling factors that can result in mortality from contamination or trauma.[4]

Hatching is another critical developmental stage and is the time of highest mortality. Mortality at this time may be related to improper hatchery environment, such as low humidity or temperature fluctuations (possibly caused by a concerned aviculturist who opens the incubator or hatcher door too frequently). Chicks that pip a blood vessel, rupturing it during pipping, can die from the resulting hemorrhage. They are usually malpositioned and typically are found in poultry embryo malposition 4 (see Table 5–2), with the head rotated away from the air cell. In this position the chick is unable to pip into the air cell.[9]

Some embryos fail to hatch when malpositioned (Table 5–2). There are several causes for malpositioned embryos, some of which are correctable. If the head is in the small end of the egg (malposition 2) the egg may have been set small end up, or it may have been incubated horizontally (Figs. 5–10 and 5–11). With a small embryo or round egg, the embryo may be located crosswise in the egg and may try to hatch on the side of the egg. Other causes of malpositioned embryos include failure to turn the egg sufficiently, excess carbon dioxide levels, low oxygen levels, and delays in development.[7] Described malpositions of psittacine birds are similar to those of poultry (Figs. 5–12 and 5–13).

FACTORS REDUCING HATCHABILITY

Embryonic mortality can occur during any stage of development, from the time of egg formation in the

Table 5–2

CLASSIFICATIONS OF MALPOSITIONED POULTRY EMBRYOS

Number	Posture of Embryo	Outcome
1	Head between the thighs	Early normal position for embryo
2	Head in small end of egg	Will result in death if development is delayed
3	Head to left (under left wing)	Only lethal about 50% of time; good success if hatching is assisted
4	Body rotated along long axis of egg with head away from the side of the egg, not in the air cell	Lethal Because the beak is pointed away from the air cell, position is often fatal
5	Feet over the head	Embryo cannot kick to rotate the body when cutting out; therefore often fatal unless assisted
6	Head over right wing instead of under	Usually has live hatch without serious complications
7	Embryo lying crosswise in egg	Seen with small embryos or spherical eggs; often have other defects, fatal

Data from Brown,[4] Joyner,[7] Clubb and Phillips,[8] and Olsen and Duvall.[11]

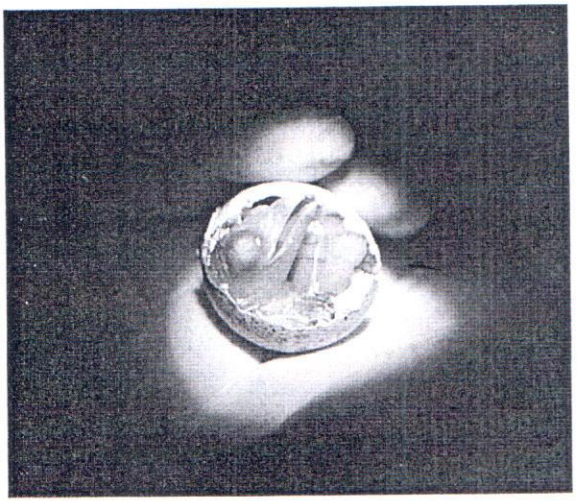

Figure 5–11

Head over right wing (malposition 6) in a Hawaiian crow embryo.

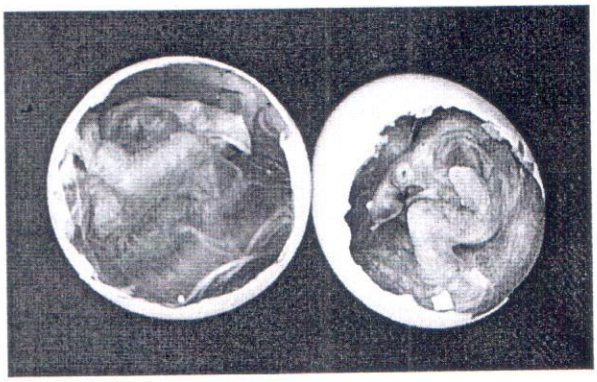

Figure 5–12

Embryo on right (yellow-crowned Amazon) shows normal position. Embryo on left (umbrella cockatoo) is malpositioned with the head under the left wing. (malposition 3.)

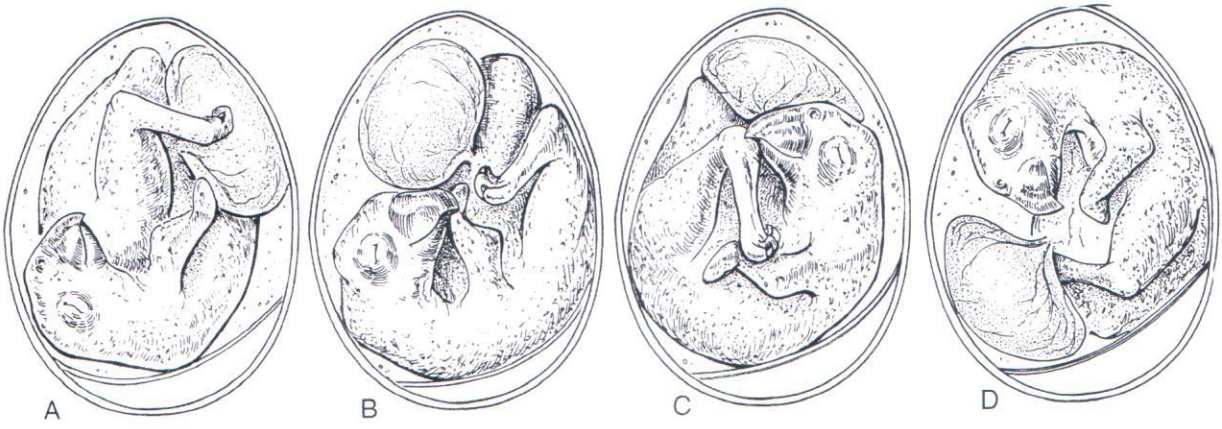

Figure 5–13

Malpositions of psittacine birds. (*A*) Normal position. (*B*) Beak rotated away from air cell. (*C*) Back of chick is toward air cell with head near large end of egg. (*D*) Head at small end of egg. (From Schubot RM, Clubb KJ, Clubb SL: Psittacine Aviculture Perspectives, Techniques, and Research. Loxahatchee, FL: Aviculture Breeding and Research Center.)

oviduct until hatching. Detailed records of incubation parameters, parental health, previous production by the pair, and egg handling techniques complement the necropsy determination. Reduced hatchability may be due to parental age, nutrition, or genetics.

Parental Age

Both fertility and hatchability are known to change with the age of the parent birds. Young and old parent birds often have reduced hatchability. For young parents, this may be the result of an immature reproductive tract, inexperience, or behavioral problems. In older birds, underlying health problems, nutrient exhaustion, poor shell quality, or deficiencies related to chronic conditions may contribute to reduced hatchability.

However, age is a species-relative factor. In commercial chicken, duck, pheasant, and quail flocks, reproductively active birds are not maintained beyond two breeding seasons. Certainly this is not true in longer-lived birds such as cranes, psittacines, and raptors. At the Patuxent Environmental Science Center, an Andean condor pair were still reproductively active when the male was 44 and the female 31 years old. A whooping crane pair is still reproductively active and producing viable eggs at 31 years for the male and 24 years for the female. Macaws have been reported to breed successfully at 35 years of age.[10] Generally, the longer the species takes to reach sexual maturity, the longer its active breeding life.

Inherited Factors

The genetic material an embryo receives from its parents' egg or sperm is sometimes defective. Genetic defects may be expressed at some point in the embryo's development. The most obvious defects are malformations seen at hatching. An especially high rate of embryonic malformation is seen in ostrich chicks, which may be related to genetics or incorrect incubation. But in other cases, embryonic development may proceed to a certain point and then cease. Determining the cause of death in some of these cases can be difficult and is based on eliminating other causes of mortality. Using genetic epidemiology to correlate the ratio of similarly dead embryos with hereditary factors is also helpful.

Genetically lethal traits are usually recessive genes. The incidence of genetic defects can increase when inbreeding birds for desirable traits or when breeding endangered species in which low founder flock numbers and genetic bottlenecks are frequent problems.

Embryonic mortality or deformity may also be the result of teratogenic factors such as pesticide exposure. An apparently normal-looking embryo may have died from lethal traits that prevented it from metabolizing certain nutrients or synthesizing compounds required for normal development.

Dietary Factors

A number of dietary deficiencies can contribute to poor hatchability. Minor deficiencies in the female bird can be magnified over the course of a breeding season, especially if multiple clutching techniques are used to maximize the pair's reproductive potential. The most obvious deficiency is a general lack of nutrients or a debilitated state in a laying bird (or, to a lesser extent, a male bird). However, such cases are not common because ovulation is usually inhibited to conserve nutrients for the adult bird.[11]

Some vitamins are extremely important for proper embryonic development. Dietary levels of vitamins sufficient for maintenance of an adult bird for a period of time without clinical signs of disease may be insufficient for the laying female and may result in deficiencies in her eggs.[11, 12]

Marginal deficiencies in vitamin A can lead to poor hatchability. However, deficiencies of vitamin A in a laying bird on a balanced diet or one in which synthetic vitamin A is added are rare.

Of the B vitamin group, deficiencies in thiamine (B_1), niacin, biotin, and pantothenic acid are rare. Riboflavin (B_2) is extremely important for incubation, and large quantities are found in egg white. Deficiencies reduce hatchability, causing curly toe paralysis and clubbed down feathers in the embryos that survive to hatch. The quality of the diet may be adequate to prevent clinical signs in the laying bird but insufficient for the embryo.[11, 12]

Pyridoxine (B_6) is also important for hatchability and early chick growth. Pyridoxine is needed for the breakdown and synthesis of proteins. Laying

birds on high protein diets have increased demand for B_{6}, which may result in egg deficiencies. Simple deficiencies of B_6 may result in embryonic death with no definitive signs. However, a more common appearance of B_6 and manganese deficiency in poultry is perosis, with soft developing bones and slipped gastrocnemius tendons. Vitamin B_6 is commonly found in nature, and marginal deficiencies are more common than absolute deficiencies.[12]

Folic acid is required for proper synthesis of red blood cells. Deficiencies result in early embryonic death because of failure of blood to form properly. Folic acid is synthesized by bacteria in the intestines; therefore, natural deficiencies are uncommon. However, laying birds on antibiotic therapy can rapidly develop deficiencies in eggs being laid at the time of therapy. Cyanocobalamine (B_{12}) is also required for proper blood formation in embryos, with reduced hatchability even with minor deficiencies. This vitamin is produced by both bacteria and molds, and synthetic B_{12} is added to bird rations.

Vitamin D is required for proper calcium and phosphorus metabolism. Marginal deficiencies in an egg interfere with the embryonic mobilization of eggshell calcium and lead to embryonic deaths. In the laying female bird, insufficiencies in vitamin D can lead to abnormal calcium metabolism for normal eggshell formation. Most birds exposed to natural sunlight or ultraviolet light can manufacture their own vitamin D (as D_3).[11, 12]

Deficiencies in vitamins C, E, and K are rare in pet birds.

Infectious Agents

Investigating possible microbiologic agents as causative factors in episodes of reduced hatchability is important. A number of bacterial agents in addition to *Chlamydia* and some viruses are pathogenic to avian embryos. When signs seen in association with embryonic mortality are consistent with one of these agents, every effort should be made to isolate and identify the pathogen. Direct ovarian (transovarian) transmission as well as transmission through shell penetration by pathogens can result in disease. Transovarian transmission of many poultry pathogens is well documented, including that of *Salmonella* (*S. pullorum, S. enteritidis, S. gallisepti cum*), *Mycoplasma*, viruses of the leukosis/sarcom group, adenoviruses (group I and egg drop syn drome), and avian encephalomyelitis virus.[13] Nota bly, investigators of transmission of some disease important to nondomestic birds have failed to dem onstrate transovarian transmission of chlamydiosis *Mycobacteria* infection, pox, and Newcastle dis ease.

Salmonellosis is a common cause of embryoni mortality and loss of chicks shortly after hatching Postmortem lesions include an enlarged congeste liver with the normal yellow-orange colored live streaked with areas of hemorrhage, coagulated yol material, a congested and enlarged spleen, and con gested kidneys. Pinpoint necrotic foci may be foun in the liver. Pericarditis is also seen. Bacterial cu ture is necessary for a positive identification.[1, 12]

Staphylococcus species are common bacteri pathogens. The avian embryo can be highly suscep tible to some strains of staphylococci but resista to other strains. Infected wounds on parent birds on the hands of the aviculturist can lead to infecte eggs. The organism proliferates readily in the env ronment of a mechanical incubator. Infection ca result in death within 48 hours or less, especial with some strains of *S. aureus*. Embryonic mortali is known to decrease with increasing age of th embryo at first exposure.[12, 13]

Staphylococcus bacteria have been isolated fro the brain, kidney, liver, and heart of dead embryo Postmortem lesions include hemorrhagic encepl losis plus hemorrhage, and necrosis of nervous t sues throughout the body. Frequently, hemorrha is seen in the kidney and liver. In addition, son structural distortion occurs in liver tissue. Hemo rhage and pericarditis characterize cardiac lesion especially with *S. epidermis* infections.[12, 13]

Prevention of *Staphylococcus* infections in pare birds requires that people handling eggs minim embryonic exposure to this pathogenic agent. Fee ing low levels of antibiotics, especially such pro ucts as penicillin or chlortetracycline, increases t possibility of developing resistant *Staphylococ* strains. Therefore, this practice should be avoid unless indicated by a more pressing disease pro lem (such as chlamydiosis requiring a treatm regimen of tetracyclines).

Streptococcus faecalis, commonly found as n

mal flora in the intestinal tract of many bird species, can be a cause of embryonic mortality. A laying bird's ovary can become infected, resulting in the *S. faecalis* organism entering the forming egg. Contamination of the egg in this manner may lead to a 20 to 50% mortality rate. Culturing eggs is important to identify the causative agent and carrier hens.

Escherichia coli can enter the egg through the shell, if there is contamination of the shell with feces containing the bacteria. Dirty nests and contaminated pens or cages are sources of contamination. Parent birds in dirty pens can track feces onto eggs from feet or feathers.

E. coli may also enter the egg from infections in the reproductive tract of the female bird. Aviary dust can be a source of contamination. Dust from poultry houses has been found to have 10^5 to 10^9 bacteria per gram. Low brooding temperature also contributes to higher incidence of *E. coli*–infected eggs (Fig. 5–14).[12]

The most common site of *E. coli* infection is the yolk sac. Yolk sac contents may appear yellow-green or yellow-brown and watery in consistency. There may be an associated omphalitis, with the yolk sac wall appearing edematous. Histologically, the outer connective tissue layer is followed by a layer of inflammatory cells characterized by heterophils and macrophages, then a layer of giant cells, and finally a layer of necrotic heterophils and bacteria next to the yolk materials.[13]

Many infected embryos die late in incubation or shortly after hatching. The incidence of infection causing death is greatly reduced by 6 days after hatching in both cranes and chickens. Post-hatching yolk sac infections (omphalitis) and poor weight gain in young nestlings are associated with *E. coli* infections acquired during incubation. Mushy duck disease, in which the duckling appears edematous, can be the result of several bacterial agents, but *E. coli* is most common and is isolated in 70% of such cases.

Reducing fecal contamination and dust in the aviary and incubator rooms is important for control. There is no recommended treatment for infected eggs. However, fumigating or disinfecting eggs shortly after laying is commonly used for poultry and reduces the incidence of *E. coli* infections.[14] Cracked eggs are more likely to become infected and serve as a source of infection for other eggs in the nest or incubator. Discarding cracked eggs or sealing the crack as soon as possible helps prevent contamination and the spread of disease.

Mycoplasma infections are transmitted with the egg and reduce hatchability. The role of *Mycoplasma* infections in cage birds is not as well documented as it is for poultry. *Mycoplasma* can spread to the egg from an infected oviduct or from the semen of infected male birds. Transmission from contaminated facilities or equipment is not well documented.[15]

The infection is commonly seen in upper respiratory passages and clavicular and thoracic air sacs. Catarrhal or caseous exudates are characteristic gross lesions. By day 13 post infection, 37 to 100% of turkey eggs will show air sac involvement. Other gross lesions include dwarfing, generalized edema, liver necrosis, enlarged spleens, and joint abscesses. Joint involvement occurs as subcutaneous periarticular granulomas with necrotic centers, bordered by epithelioid cells and some giant cells.

Treatment of eggs for *Mycoplasma* infections is possible. Tylosin (0.5–1.0 mg/dose) is injected into the air cell at the start of incubation. A small hole is drilled into the egg using a fine surgical burr. This is often best done by rotating the drill bit or burr by hand to avoid breaking delicate eggs. The hole is sealed afterward with a drop of beeswax. A combination of lincomycin and spectinomycin is also effective for egg injection.[16] Dipping eggs in antibiotic solutions is effective in reducing the incidence of disease. Eggs are first warmed to 35 to

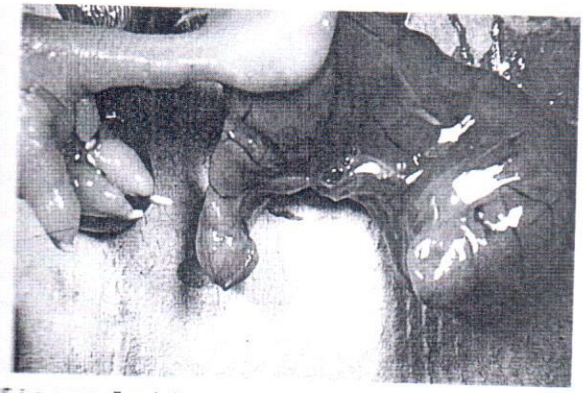

Figure 5–14

Consolidation of yolk material due to *E. coli* infection in a full-term dead-in-shell crane embryo.

37°C then dipped into an antibiotic solution maintained at 2 to 8°C for 5 to 20 minutes. Antibiotics of choice include tylosin (1000–3000 ppm or 1–3 mg/ml) or gentamicin (400–1000 ppm or 0.4–1.0 mg/ml), plus a disinfectant (such as quaternary ammonium at 250 ppm). Dilute in sterile water.

A third treatment technique that has proved effective to break the transmission cycle of *M. gallisepticum* and *M. synoviae* is to take eggs from a room temperature environment (26.5°C) and place them in a forced-air incubator. Then the temperature is elevated to 46°C for 12 to 14 hours before returning to room temperature or normal incubation temperature. This technique inactivates the *Mycoplasma* organisms but has the disadvantage of reducing hatchability by 8 to 12%.

In 1934 *Chlamydia psittaci* was found to be an egg-borne pathogen in parakeets. The organism can be isolated from the ovary of the female bird and from eggs. Chlamydiosis results in embryo death in 5 to 12 days. Characteristic pathologic findings include congested or grossly hemorrhagic yolk sac membranes.[1, 4]

Several viral diseases have been documented as adversely affecting avian embryos. Budgerigar herpes is transmitted within the egg and causes reduced hatchability. Avian paramyxovirus (Newcastle disease) will enter eggs contaminated with infected fecal material. Transmission from an infected female bird is possible, but usually viremic female birds cease laying. Embryos infected with paramyxovirus are retarded in growth and show defects in the neural tube, eye lenses, auditory vesicles, visceral arches, limb buds, and olfactory pits.[1] Embryo mortality approaches 100% with all Newcastle disease strains.

Aspergillus species is the most important fungal pathogen of eggs. Spores originate from an outside source or another infected egg. The disease is especially a problem in forced-air incubators. Embryos either die before hatching or are weak, gasping, and dyspneic at hatching. Nervous system symptoms and diarrhea are less common occurrences in embryos that survive to hatching. Typical lesions found at necropsy include small yellow foci in the lungs,[17] bronchial plugs,[18] and air cell plaques.[19] The fungus grows readily on the air cell membrane. Cultures should be taken from lesions in the lung or air sac and from the air cell membrane (Fig. 5–15).

Figure 5–15

Aspergillus infection in the membranes between the embryo and the air cell resulted in death of this Canada goose embryo.

Treatment of eggs infected with *Aspergillus* is not currently feasible. However, prevention of infection is possible. Incubators should be cleaned and fumigated between groups of eggs. Individual eggs should be candled frequently to monitor viability and dead eggs removed quickly to minimize potential for the spread of aspergillosis as well as other diseases.

Parasitism

The primary effect of parasites on egg development is indirect. Parasitic infestations, if severe, reduce the nutritional status of the adult bird and lead to deficiencies in eggs laid by these birds.

Several species of parasites have been documented to occur within eggs. Adult ascarids are occasionally found within eggs. These worms enter the forming egg by reverse peristalsis, moving from the cloaca up the oviduct.[20] The fluke *Prosthogonimus ovatus* is found in the oviduct of Galliformes and Anseriformes. The fluke may be found within the egg but, more often, fluke infestation leads to abnormal shell formation (failure to form a shell or soft-shelled eggs).[21]

Other parasitic infections have been documented only experimentally. Eggs inoculated with *Coccidia* show 100% mortality at day 3, 80% mortality at day 12. Lesions seen in coccidial infections include

nicrocephalus (64% of eggs examined), anomalies f the central nervous system (60%), microphthalnia (53%), atrophy of the lower body (47%), abnornal limb development (46%), and lordosis (34%).[13] Certainly, cracked eggs present the potential for *Coccidia* to enter the egg. Therefore, as with bacterial infections, care must be taken to avoid fecal ontamination, clean eggs that are contaminated vith feces, and seal cracked eggs.

Plasmodium has been experimentally inoculated nto eggs, resulting in death within 9 days. Necropsy ndings included green-colored liver, spleen, and mbryonic membranes.[13] *Plasmodium* theoretically ould be inoculated into an egg in the oviduct as a esult of hemorrhage from the female bird in the ormation of a blood spot. The fact that this apparntly does not occur may be the result of some natural immune mechanism.

oxic Substances

With the plethora of oil spills around the world, eterinarians are frequently called upon to save iled birds and advise others on the impact of oil n wildlife. In addition, occasionally caged birds, specially those in outside aviaries, are exposed to etroleum products. The initial reports of reduced atchability associated with petroleum products on eathers was with terns and gulls.[22, 23] Originally, oil oxicity in eggs was believed to result from oil oating the eggshell and preventing proper gaseous xchange between the embryo and the environnent. Work in the last 15 years has shown that even mall amounts of oil (1–10 μl), when deposited on he eggshell, can penetrate the egg and be emryotoxic or teratogenic.[24–29] In one study, as little s 0.3 μl of a commercial grade oil used on roadvays killed 50% of developing mallard embryos Fig. 5–16).[30] Typical lesions seen in eggs exposed o crude oil include extensive edema, enlarged eart and spleen, and hepatic necrosis.[31–33] Polyhlorinated biphenyls (PCBs) and dioxins can also e toxic to embryos.[42]

Mineral oil, often used in the preparation of melicinal ointments, can be transferred from an inubating female bird to her eggs, reducing hatchbility.[34] The primary mechanism appears to be blockage of shell pores by the oily preparations. Therefore, caution should be exercised when prescribing oil-based preparations to actively laying or nesting birds or in egg-bound birds.

Some insecticides have long been known to be toxic to birds and their eggs. Organophosphate insecticides cause malformed embryos with defects including scoliosis, lordosis, and possible reduction in total length of the entire vertebral column (Fig. 5–17, *A* and *B*).[35] With exposure to parathion, these defects are most pronounced in the cervical region. Diazinon exposure results in incomplete caudal ossification and stunting of embryonic growth. Of the common insecticides, carbaryl, malathion, permethrin, and phosmat are relatively nonembryotoxic if used properly in small amounts.[35]

Herbicides, once thought to be nontoxic to vertebrates, have proved to be equally or more toxic than insecticides when applied to eggs (Fig. 5–18). Paraquat and trifluralin are two highly embryotoxic herbicides in studies using mallard eggs.[35] Trifluralin exposure causes beak defects, whereas paraquat produces extensive edema, exencephaly, or anencephaly.[35]

Components of automotive exhaust are harmful to avian embryos. Carbon monoxide at 100 ppm is known to decrease hatchability by 21%, and at 200 ppm a reduction in hatchability of 83% was seen.[36] Carbon monoxide decreases embryonic growth while producing hypertrophy of the liver, spleen, and heart. Serum albumen is increased, globulin decreased, hematocrit increased, serum alanine transfer increased, and lactic dehydrogenase increased.[37, 38] Other components of automotive exhaust are considered hepatotoxic. Exposure of avian embryos to exhaust gases causes increased heart and liver-to-body weight ratios. Catalytic converter–treated exhaust has smaller effects on these organs and on embryo hatchability as compared with untreated exhaust.[37]

Dietary selenium in excess of 4 ppm reduces hatchability and is teratogenic. The naturally occurring organic form of selenium, selenomethionine, when present in excess in the diet of a laying female, accumulates in eggs but does not always produce clinical disease in the female bird. Malformations seen in embryos include ectodactylia, hydrocephaly, microphthalmia or anophthalmia, and beak defects (Figs. 5–19 and 5–20).[39, 40]

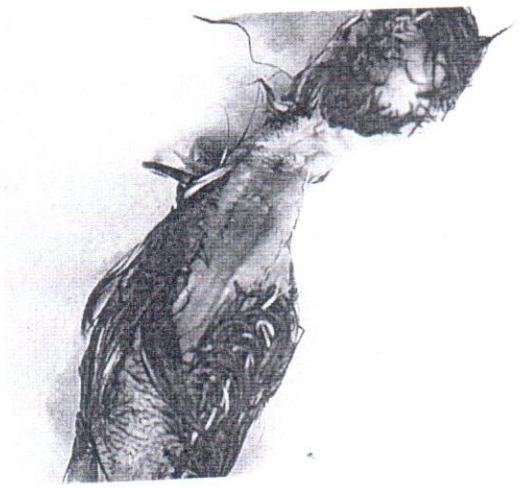

Figure 5–16

Rachischisis and encephalitis in a mallard duck embryo experimentally exposed to crude oil. (Courtesy of David Hoffman.)

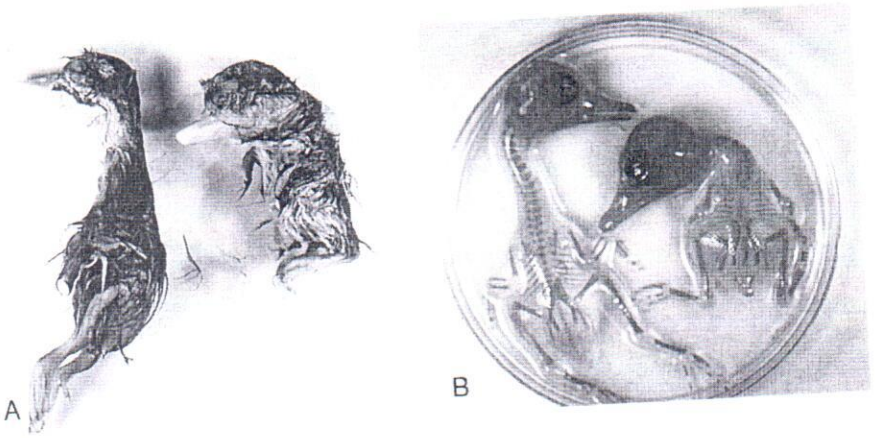

Figure 5–17

A. Normal (*left*) and abnormal (*right*) 18-day-old mallard embryos. Abnormal chick shows shortened axial skeleton, cervical lordosis, and subcutaneous edema associated with experimental application of low-level organophosphate insecticide to the shell. (*B*) The same embryo cleared to illustrate skeletal deformities. (Courtesy of David Hoffman.)

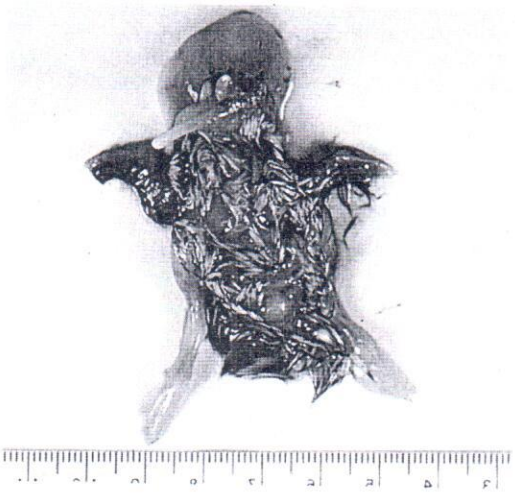

Figure 5–18

Extensive edema and an encephaly in a mallard duck embryo associated with herbicide application to the shell. (Courtesy of David Hoffman.)

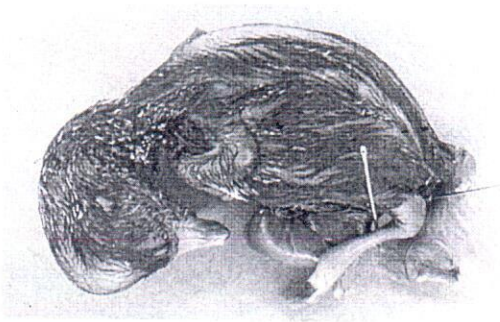

Figure 5–19

Selenium toxicity is evident by deformed toes and microphthalmia in a coot embryo. (Courtesy of David Hoffman.)

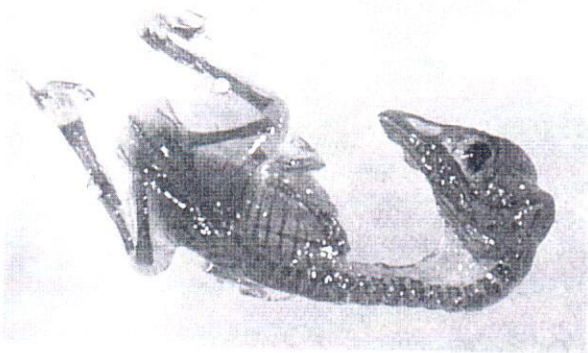

Figure 5–20

Mallard duck embryo with soft tissue cleared and bones stained, showing malformation of the feet caused by selenium toxicity. (Courtesy of David Hoffman.)

Some antibiotics are known to affect avian embryonic development. Penicillin causes hemorrhage and edema of the limbs and head.[11] Exposure to tetracycline-type antibiotics produces stunted embryos through the inhibition of skeletal mineralization and erosion of long-bone cartilage. Chloramphenicol also inhibits embryonic growth, but does not produce malformed embryos. Sulfa drugs lead to multiple embryo problems including granular degeneration of urinary tubules, enlarged head, micro- or macrophthalmia, beak hypoplasia, knee and toe joint amylosis, and regressive changes in liver cells.[13] Most of the research has been performed with the older antibiotics. Any embryotoxic or teratogenic effects of the newer antibiotics have not been discovered. The best advice is to use caution when giving any antibiotic to a laying female bird or one about to lay and to advise the client of a possible risk in such therapy.

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