THE INCUBATION PATCH OF PASSERINE BIRDS

By ROBERT E. BAILEY

Shortly before incubation in most groups of birds the down feathers of one or more areas of the ventral surface are molted and the skin of these areas becomes thickened and richly supplied with blood vessels. This adaptation facilitates the transfer of heat from the body of the incubating bird to the eggs; thus these areas are called incubation patches.

Incubation patches were first described at length by Friedrich Faber (1826) in his book on water birds of northern Europe. He named them "Brutflecke" and rightly ascribed to them the function of permitting a more intimate contact between the incubating birds and their eggs. The absence of feathers was considered by him to be the main criterion of an incubation patch and he thought that the feathers were either pulled out by the birds or were lost partly because of the abrasive action of the nest and eggs and partly because of the "greater animal heat that is concentrated in the abdomen during incubation" [translated]. Barkow (1830) was the first to examine the microscopic structure of the incubation patch and compare it with normal skin. His work was done on the European Coot, grebes of the genus *Podiceps* [=*Colymbus*], and chickens. A lack of feathers and greatly increased vascularity were described by him as the main features of the structure.

Little work on the incubation patch was done, except for occasional references to its occurrence, until the first of the twentieth century. Interest in the evolution of the mammary glands led to a study of the incubation patch by Lange (1928), and his work is a considerable contribution to our knowledge of the microscopic and macroscopic structure of the patches in the several groups that he investigated. The work of Freund (1926) and of Koutnik (1927) concerned mostly the histological changes that occur in the formation of the incubation patch, but their work was restricted almost entirely to domestic fowl.

Incubation patches are found in almost all orders of neognathous birds. They are similar in their function and basic structure but differ in size, location, and number. The chief and most obvious characteristic of all incubation patches is the absence of feathers so that there exists at the time of incubation a naked place on the skin which can come into direct contact with the eggs. Such a loss of feathers seems necessary if the higher body temperature of birds is to be utilized to its fullest capacity in providing a constant, warm environment for the eggs, since feathers are poor conductors of heat. Usually only down feathers are lost, but in some groups contour feathers are also molted so that the patches are enlarged or two patches may be united to form a single large patch. In some orders no down feathers are found in the apteria and thus naked spaces are always present. These, however, should not be considered as incubation patches unless the other characteristics are also observed.

Since incubation patches occur almost exclusively in the apteria, it is to be expected that feather arrangement will have a strong effect on the position and number of the patches. By far the most common condition is a single patch located in the ventral

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apterium. It is referred to as the median patch and is usually limited in size by the extent of the ventral apterium, although in some groups it is enlarged by a loss of contour feathers. A single median patch is found in the Colymbiformes (grebes), Procellariiformes (petrels, fulmars, albatrosses), Falconiformes (hawks), Columbiformes (pigeons and doves), Strigiformes (owls), and in all the so-called higher orders of birds such as the Passeriformes (song birds) and Piciformes (woodpeckers). Lateral patches occur in some of the groups which have a very narrow ventral apterium and lateral apteria of considerable size. These patches are always paired and lie in the region lateral to the main ventral feather tract. Lateral patches occur in the Charadriiformes (gulls, shorebirds, auks), Galliformes (pheasants, quail, and grouse), and Gruiformes (rails, cranes). They may or may not be accompanied by a median patch. In the Galliformes both lateral and median patches are present and originate separately but soon unite into a single large patch by a loss of contour feathers in between. No patches have been found in the ratite birds, the Pelecaniformes (pelicans), or the Anseriformes (ducks, geese, swans).

All incubation patches show increased dermal and subdermal vascularity. There is not only an increase in the size and number of capillaries and smaller veins and arteries but also an increase in the size of the larger vessels supplying the incubation patch region. In this way a capillary bed forms just under the epidermis and the radiation and conduction of heat from the incubating bird to the eggs is enhanced. Dermal edema accompanies and perhaps results from the increased vascularity. It is difficult to ascribe any functional significance to the edema, and detailed investigation aimed at this particular response will probably be necessary to determine its relationship to the incubation patch as a whole. In addition to the responses already mentioned there is usually a disappearance of feather papillae, dermal muscles, and subdermal fat.

Incubation patches are found in both males and females according to their participation in incubating the eggs. Insufficient data are available to state definitively the situation in all of the living orders. Certain trends, however, can be noted with reservations. In most orders both sexes incubate and both sexes have incubation patches. This is true for the Colymbiformes, Procellariiformes, Columbiformes, Piciformes, most Charadriiformes, and most Gruiformes. Only females incubate and have patches in the Strigiformes, Falconiformes, Trochilidae, and most, if not all Passeriformes. Only males incubate and have patches in the Phalaropidae, Jacanidae, and some Scolopacidae.

The theory that the birds pull out their feathers to form the incubation patch was attacked by Lange. He believed that the loss of feathers was due to a special molt and cited as evidence the presence of patches in an immature gull (*Larus ridibundus*) that did not breed. These patches were identical in size and location with those of the breeding gulls and he attributed their occurrence to a hormonal influence. The fact that the patches in any one species are always found in the same location and are of the same size is further evidence that the defeatherization is not due to a haphazard plucking of the feathers by the birds.

The purpose of this investigation has been to study through various attacks the nature of the incubation patch in the order Passeriformes. Both field and laboratory work has been carried on and much has been added by the combination of the disciplines of natural history and physiology.

FIELD STUDY

MATERIALS AND METHODS

For the study of the microscopic and macroscopic structure of the incubation patch in passerine birds 125 specimens representing 12 families were examined. All these were collected in California or Nevada. In addition, data for six more families were obtained from the literature and from field notes deposited in the Museum of Vertebrate Zoology. Whenever possible both members of a nesting pair were collected and every effort was made to obtain complete information about the reproductive activities of the birds. In many instances it was possible to locate the nest and to determine the age of the young or eggs. On other occasions it was necessary to estimate the stage of the reproductive cycle by examining the gonads.

Several pairs of nesting birds were marked with colored bands for closer observations. These were trapped and examined daily and the changes in the structure of the incubation patch were correlated with the events of the nesting cycle. Only a few pairs were studied in this manner, but satisfactory information about the duration of the various stages of the incubation patch was obtained for the White-crowned Sparrow (*Zonotrichia leucophrys*), Oregon Junco (*Junco oreganus*), and Song Sparrow (*Melospiza melodia*).

A complete series of the various incubation patch stages was obtained and studied microscopically. Pieces of the skin of the incubation patch were removed from freshly killed birds, fixed in Bouin's fluid or 10 per cent formalin, sectioned at three to 10 microns, and stained with Delafield's hematoxylin and eosin or Mallory's stain.

MACROSCOPIC STRUCTURE

The incubation patch of passerine birds presents no exception to the general rule that the most important factor regulating the size, number, and location of the incubation patches is the arrangement of the feathers on the ventral surface. In passerine birds the ventral feather tract divides in the neck region into two symmetrical branches that continue posteriorly to the cloaca. These branches diverge rapidly and on the breast and abdomen are widely separated. They are broad on the breast but narrow sharply at the anterior edge of the abdomen. The apterium that they enclose medially is broad and proportionately much larger than that found in most orders of birds. It is covered sparsely with down feathers, especially on the abdomen (fig. 1).

The incubation patch is located within the large ventral apterium and is in fact coincident with it. It extends from the anterior edge of the breast to the cloaca and laterally to the median edge of the ventral feather tracts. All the down feathers in the apterium are molted in the first stage of the development of the patch. During incubation it is vascular and edematous; the skin appears reddish in color and is considerably thickened. No contour feathers are lost in the formation of the incubation patch of passerine birds.

The blood supply of the patch of passerines shows no marked deviations from that found in other groups. It was described adequately by Lange (1928) and I have examined only a few birds in this regard. Some minor variations exist, but for the most part only three main sources are important in relation to the incubation patch.

The breast region is supplied by two branches of the external thoracic artery. One becomes subcutaneous in the shoulder and continues posteriorly along the median edge of the ventral feather tract. It sends branches into the feather tract and into the ventral apterium. The second becomes subcutaneous under the ventral feather tract about midway along the breast. It also sends branches to the ventral feather tract and to the breast region of the ventral apterium. The arteria incubatoria, which is an important vessel
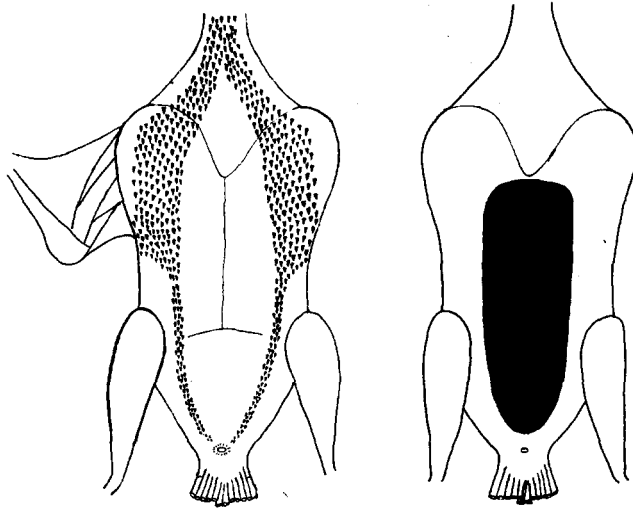

Fig. 1. Ventral surface of White-crowned Sparrow showing relationships of the ventral feather tracts and ventral apterium (left) to incubation patch (right; black area).

in the blood supply of the incubation patch in many groups of birds, runs lateral to the ventral tract and sends no significant branches medially. It therefore plays no important role as a vessel supplying the incubation patch of passerine birds.

The abdominal part of the ventral apterium is supplied by one or more branches of the prepubic artery. This artery is a branch of the internal pelvic and is so named because it runs ventral to the pubis and then enters the abdominal musculature. From the abdominal wall several branches become subcutaneous and these provide the chief supply for the skin of this region.

MICROSCOPIC STRUCTURE AND CORRELATION OF THE INCUBATION PATCH STAGES WITH THE REPRODUCTIVE CYCLE

The nesting cycle of all passerine birds that incubate their own eggs can be divided into several stages representing various behavioristic and physiological activities. The pattern is fairly uniform and consists of (1) courtship and territory formation (if such occurs), (2) nest building (during which time copulation usually occurs several times), (3.) egg laying, (4) incubation, (5) brooding, (6) fledging, and (7) either a return to the non-reproductive condition or resumption of the cycle at the nest building or egg laying stage. Variations exist in the duration of each stage in the different species but all of them usually occur in all passerine birds in the order named with the exception of those showing brood parasitism.

The incubation patch of passerine birds also undergoes a series of changes that can be divided into several stages. These stages can be related to the stages of the nesting cycle and in fact both probably owe their appearance to the same physiological activi-

ties. The incubation patch stages and their correlation with the nesting cycle stages are diagrammed in figure 2. The diagram is based on the White-crowned Sparrow, but since the variations that exist are due to the differences in the length of the stages, it applies, except for time, to all the passerine birds observed.

During the non-breeding season the skin of the abdomen and breast is similar to the skin of other parts of the body. It is thin and for the most part only loosely fastened to the body wall. The stratum germinativum of the epidermis is made up of 1 to 3 layers of rather flattened cells and is covered externally with a thin stratum corneum. The dermis, as in mammals, is divided into a compact and a spongy layer. The compact layer consists of a closely packed mass of connective tissue fibers lying parallel to the surface. Wherever down feathers occur the compact layer follows the papilla down into the spongy layer. The spongy layer is a looser connective tissue that contains the larger arteries and veins of the skin. It is difficult to distinguish the boundary between the spongy layer of the dermis and the subdermal tissue. In passerine birds the subdermis
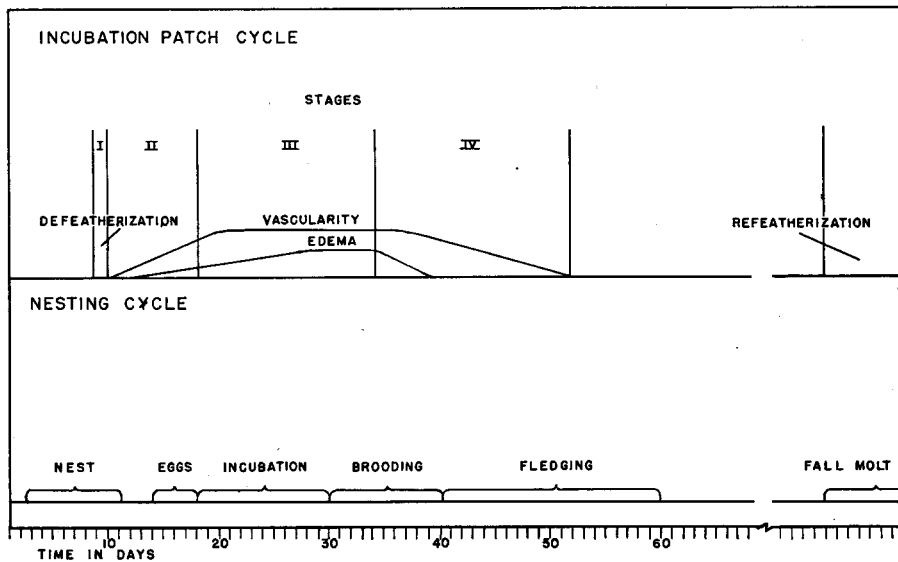

Fig. 2. The stages of the incubation patch cycle of the White-crowned Sparrow.

does not contain much adipose tissue, although usually some fat is concentrated under the feather tracts and under the skin of the abdomen, neck, and rump in preparation for migration. Figure 3 (a-f) shows some photomicrographs of the abdominal skin at various stages of the incubation patch cycle.

Stage I: Defeatherization.—Several days before the first egg is laid the down feathers of the ventral apterium are molted. This is the first change detectable in the formation of the incubation patch and the process requires about 24 hours for completion. Histological studies at this time show no changes in the structure of the skin. In the White-crowned Sparrow and Oregon Junco defeatherization occurs four to five days before the first egg. Nice (1937) reports the appearance of the incubation patch in the Song Sparrow four to six days before the first egg, and I assume from my studies that she was referring to defeatherization.

Many attempts were made to determine if the feathers were ever plucked out by the birds. Most passerines do not use feathers for the construction of their nests and thus
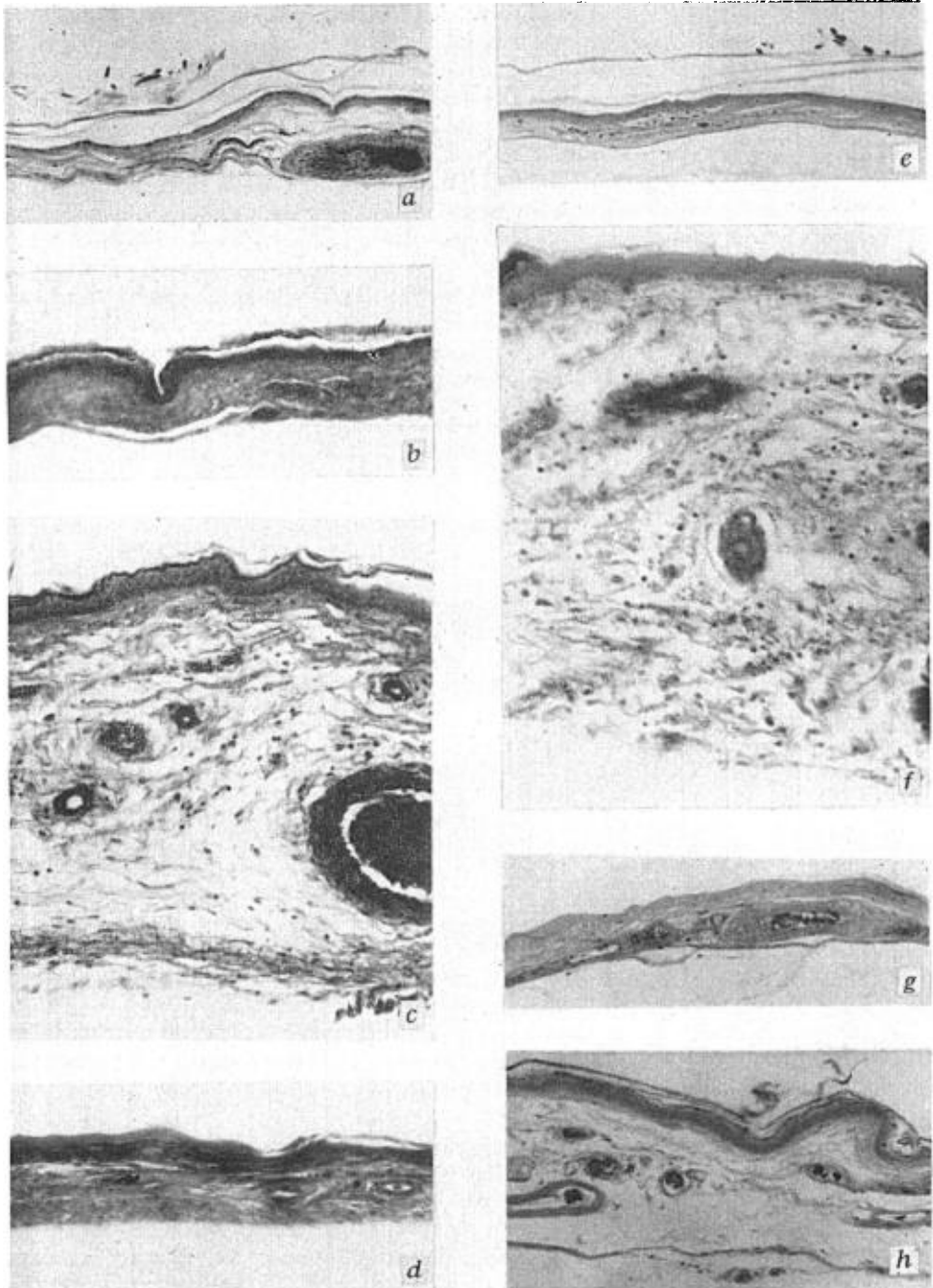
Fig. 3. Abdominal skin at various stages of the incubation patch cycle. Red-winged Blackbird: a, non-breeding (down feather papilla shows at right); b, after laying first egg; c, early incubation; d, young just out of nest. White-crowned Sparrow: e, non-breeding; f, after one week incubation; g, non-breeding hypophysectomized ♀ with estradiol for 9 days; h, non-breeding hypophysectomized ♀ with estradiol for 9 days then prolactin and estradiol for 8 more days. All × 160.

would not pull out their feathers for this reason. Never was I able to see any bird pluck feathers from its breast or abdomen nor did I see any bird with feathers in its mouth. I am convinced that the loss of down feathers in the ventral apterium during incubation is due to a special molt.

Stage II: Vascularization.—Immediately following defeatherization the blood vessels in the dermis of the ventral apterium begin to increase in size and number. By the time the last egg is laid and incubation begins, the skin is vascular and the many large blood vessels can be seen with the naked eye. In the White-crowned Sparrow this vascularization stage lasts about nine days. Toward the last of the vascularization stage a slight amount of edema occurs in the dermal tissue and a thickening of the skin results.

A section through the skin of the ventral apterium in this stage reveals a marked increase in the number of capillaries and smaller blood vessels as well as an increase in the size of the major vessels supplying the region. The edema, which will become even more marked in the next stage, is also readily noticeable microscopically. All feather papillae have already disappeared, but dermal muscle bundles can still be seen since they usually persist until the tissue is very edematous. Also, during this period, the cells of the stratum germinativum of the epidermis undergo rapid multiplication, are larger than before, and have rounder nuclei.

Stage III: Edema.—During incubation the patch continues to become more vascular and edematous, and the skin is now many times thicker than in non-breeding birds. When incubating, the female fluffs out the feathers that surround the incubation patch and the loosely folded skin is closely applied to the eggs. Thus the warmth brought to the surface of the incubation patch by the rich blood supply is efficiently transferred to the eggs. The edematous stage continues throughout the period of incubation and during the brooding of the newly hatched young. The length of incubation and brooding in the various species, therefore, is the important factor controlling the duration of this stage.

Stage IV: Recovery.—After the eggs are hatched and the young are no longer dependent on the parent for the maintenance of their body temperature as earlier, the vascularity and edema in the dermis begin to subside. This is a gradual process and usually begins when the young are about four or five days old. The edema disappears more rapidly than the vascularity. The skin, which has been somewhat stretched, is now wrinkled and scaly. The large blood vessels, no longer obscured by the edema, now stand out prominently but these also decrease in size and by the time the young are ready to leave the nest have almost returned to normal.

In many species of passerine birds a second clutch of eggs is laid after the first brood has been fledged. In such cases the incubation patch again becomes vascular and edematous as in the first cycle and continues to remain this way throughout the incubation and brooding of the second nesting. After the last clutch the skin of the abdomen and breast soon returns to normal but remains unfeathered, however, until the fall molt. During the period between nesting and the fall molt it is difficult to distinguish the site of an old incubation patch from the unfeathered ventral apterium of an immature bird.

OCCURRENCE OF THE INCUBATION PATCH

My study of the occurrence of the patch in passerine birds has been restricted almost entirely to the suborder Passeres and includes only one family (Tyrannidae) from the other suborders. An incubation patch was found in all nesting females that I examined but never have I found an incubation patch on a male passerine bird. The following is a list of the species of passerine birds of which I have personally examined males and females during the breeding season:

Tyrannidae
  Black Phoebe (*Sayornis nigricans*)
  Western Flycatcher (*Empidonax difficilis*)
Hirundinidae
  Cliff Swallow (*Petrochelidon pyrrhonota*)
Corvidae
  Scrub Jay (*Aphelocoma coerulescens*)
  Steller Jay (*Cyanocitta stelleri*)
  Clark Nutcracker (*Nucifraga columbiana*)
Paridae
  Mountain Chickadee (*Parus gambeli*)
  Plain Titmouse (*Parus inornatus*)
  Bush-tit (*Psaltriparus minimus*)
Sittidae
  Pygmy Nuthatch (*Sitta pygmaea*)
Chamaeidae
  Wren-tit (*Chamaea fasciata*) ⸱
Turdidae
  Robin (*Turdus migratorius*)
Vireonidae
  Warbling Vireo (*Vireo gilvus*)
Parulidae
  Orange-crowned Warbler (*Vermivora celata*)
  Audubon Warbler (*Dendroica auduboni*)

Ploceidae
  English Sparrow (*Passer domesticus*)
Icteridae
  Red-winged Blackbird (*Agelaius phoeniceus*)
  Brewer Blackbird (*Euphagus cyanocephalus*)
  Western Meadowlark (*Sturnella neglecta*)
Fringillidae
  Black-headed Grosbeak (*Pheucticus melanocephalus*)
  Purple Finch (*Carpodacus purpureus*)
  House Finch (*Carpodacus mexicanus*)
  Green-backed Goldfinch (*Spinus psaltria*)
  Green-tailed Towhee (*Chlorura chlorura*)
  Spotted Towhee (*Pipilo maculatus*)
  Brown Towhee (*Pipilo fuscus*)
  Oregon Junco (*Junco oreganus*)
  Chipping Sparrow (*Spizella passerina*)
  White-crowned Sparrow (*Zonotrichia leucophrys*)
  Fox Sparrow (*Passerella iliaca*)
  Song Sparrow (*Melospiza melodia*)

The incubation patch of all birds represents a series of structural and physiological changes that are closely correlated with the events of the nesting cycle. There is little doubt that these changes are functionally significant and that the incubation patch is important for the warming of the eggs. There is a high correlation in most orders between the presence of the patch and the share of the sexes in incubation. One would expect that in the passerine birds there would also be this correlation. The situation in this order, however, is complicated by the fact that, while in most instances the female alone incubates, the male may occasionally sit on the eggs and in some species does so regularly. From this it would appear that an incubation patch is not essential for the incubation of the eggs, but a closer examination of the facts does not bear out this assumption.

In the Wren-tit (*Chamaea fasciata*) and the Black-headed Grosbeak (*Pheucticus melanocephalus*) both sexes sit on the eggs in turns during the day, but the careful studies of the Wren-tit by Erickson (1938) and of the Black-headed Grosbeak by Weston (1947) show that it is always the female at night. It is, of course, during the night that the eggs are most likely to drop to such a low temperature that the development of the embryos may be impaired. At this time the adaptations which make the incubation patch especially efficient for the transferring of heat to the eggs are most needed. In the species in which only the female incubates, she may leave the nest frequently for feeding and drinking during the day; yet the eggs are not harmed. The activities of the male, therefore, in sitting on the eggs, is probably not absolutely essential. These questions can only be answered by a study of the temperature relationships involved.

LABORATORY STUDY

For reasons mentioned at the beginning of this paper it seemed obvious that the incubation patch develops in response to some internal stimulus and not to the mechanical action of the eggs against the feathers and skin. The loss of feathers is also certainly due to a special molt since they are not, except in the Anseriformes, plucked

out by the incubating birds. It occurred to me, therefore, that it might be possible to analyze the physiological mechanisms that control the development of the patch and through the manipulation of non-breeding birds to produce an incubation patch experimentally.

Since the incubation patch is directly concerned with reproduction, it was natural to turn first to the reproduction hormones as a promising approach to this study. Endocrines had already been suggested by Koutnik (1927) and Tucker (1943), but so far as I know no experiments had been made to determine which hormones are involved.

Several facts were useful in choosing hormones that might most profitably be studied. In 1935, Riddle, Bates, and Lahr found that prolactin, an anterior pituitary hormone, would induce broodiness in fowls. Prolactin is also associated with other "care of the young" responses such as lacation in mammals, crop sac secretion in pigeons and doves, maternal behavior in rats, and nest building in some fishes (see Riddle, 1935; Beach, 1948). It seemed likely, therefore, that prolactin might be able to regulate the development of the incubation patch as well as to cause broodiness. Ability, or lack of ability, to secrete this hormone could then account for the occurrence of the patch in various species and in one sex or the other. It could also explain the high degree of correlation between the occurrence of the patch and the urge to incubate.

The secretion of the gonads, that is, estrogens and androgens, seemed likely alternate factors since from studies of the nesting birds it was found that the first indications of the patch were observable in the latter part of the nest-building activities or shortly before ovulation. At this time the gonads are most highly developed and the estrogen and androgen titers would probably be at the highest. Presence or absence of the patch could then be explained by the ability of the ventral skin to respond. Possibilities that pituitary hormone other than prolactin might be involved and that perhaps the response might be initiated by the synergistic action of two or more hormones were considered.

<div align="center">MATERIALS AND METHODS</div>

Only general materials and methods are given here. Deviations will be noted in the detailed description of the individual experiments. Three species of fringillid birds were used: White-crowned Sparrow, House Finch, and Oregon Junco. All were trapped on the University of California Campus, Berkeley, and were confined in groups of four in cages 12 by 12 by 12 inches or in groups of up to 12 in cages 2 by 2 by 3 feet. The birds were provided with a plentiful supply of water, bird seeds, and grit. Occasionally pears, apples, grapes, or lettuce were added to the diet. The smaller cages were kept indoors in a well ventilated room with a large window and were exposed to normal day lengths. The larger cages were placed out-or-doors in a sheltered area.

Estrogen Administration. Estrogen was administered as estradiol pellets (Progynon Schering). At first these were placed in the abdominal cavity but since it was impossible to watch the rate of absorption they were implanted in the neck region in the later experiments. This area was chosen because the pellets could be inserted in the incision used for hypophysectomy. It provided furthermore an access to the blood stream that did not immediately pass through the hepatic portal system and thus to possible deactivation in the liver.

Androgen Administration. Androgen was given as testosterone pellets (Oreton-F Schering) in the same manner as the estrogen.

Prolactin Administration. Luteotropin Squibb was injected subcutaneously in the rump or shoulder region. The dosage was the same in all of the experiments—30 I.U. (as 0.150 ml. of aqueous solution) daily.

Albumin Administration. Albumin was injected into a few birds serving as controls

for those treated with prolactin. This was administered as 0.150 ml. of 2 per cent aqueous solution injected subcutaneously in the rump or shoulder.

Hypophysectomy. The pituitary, both anterior and posterior lobes, were removed from some of the birds by a method modified from that of Schooley (1939). The birds were anesthetized with nembutal and the feathers plucked from the ventral aspect of the neck. An incision about one and one-half inches long was made starting between the mandibles and running posteriorly along the ventral feather tract. With small hooks the trachea and esophagus were retracted to the left and right, respectively. In this way the muscles and blood vessels lying ventral to the floor of the skull were exposed. The branching of the common carotid provided a good land mark and just anterior to this, posterior to the anastomosis of the jugular veins, the thin flattened muscles were separated with forceps and cotton swabs (a small amount of cotton rolled on a toothpick). The basisphenoid was in this way exposed and by working the connective tissue aside anteriorly the parasphenoid was also exposed. Using a dental drill with a small burr, the parts of the basisphenoid and parasphenoid lying ventral to the sella turcica was removed. Drilling was started in the midline at the suture between these two bones and the hole enlarged to about 3 by 5 mm. The thin bony capsule of the sella turcica was picked away with a sharp dissecting needle and the membranes surrounding the pituitary broken. At this time if bleeding occurred, the blood was removed with a suction cannula. No attempts were made to fill the empty sella turnica with bone wax or fibrin foam. If the bleeding was not too great, the retractors were removed, the hormone pellet placed next to the esophagus, and the incision closed.

Autopsy and Histological Technique. At the end of each experiment the birds were sacrificed and pieces of the abdominal skin were fixed in Bouin's, formal-sublimate, or 10 per cent formalin. The testes and ovaries were examined and measurements taken. All the birds were captured in the winter and the gonads were small and inactive. No significant increase or decrease in size of gonads was noted at the end of the experiments.

The pieces of skin were dehydrated in dioxane, embedded in paraffin, and sectioned at 3 to 10 microns. The sections were stained with Mallory's stain or Delafield's hematoxylin and eosin.

<div align="center">EXPERIMENTS</div>

1. *Effect of Prolactin.*—Starting December 20, 1948, a pilot experiment was made to test the assumption that prolactin alone could produce an incubation patch in nonbreeding birds. Eight White-crowned Sparrows were injected daily with prolactin for six days and then sacrificed on the seventh. No controls were used. The birds were examined daily for changes in the feather distribution or vascularity of the ventral surface.

Two birds died before the injections were completed. Three adult males, two adult females, and one immature female were alive at the end of the experiment. No loss of feathers or increase in vascularity of the incubation patch area occurred. These results indicated that prolactin alone will not stimulate the development of an incubation patch.

2. *Effect of Estradiol.*—On November 23, 1950, an experiment was started to test the effects of an estrogenic substance. Estradiol pellets were implanted in three adult female and three adult male House Finches. They were placed in one of the larger cages and examined daily.

By the fifth day the blood vessels of the abdominal skin had increased sufficiently in size to be detectable with the naked eye. On the sixth day the feathers on the ventral apterium began to be shed and two days later the apterium was completely bare. This molt started at the midline in the breast and spread laterally as it does in the develop-

ment of the patch in nesting birds. By the ninth day, at which time the birds were killed, it would have been impossible to distinguish the artificially produced patch now present from that of a breeding female.

Microscopic studies of sections through the skin of the incubation patch region showed a considerable thickening of the skin due to dermal edema. In the dermis there was also a network of smaller blood vessels such as is found in an incubating bird.

Both males and females showed this response to continuous treatment with estradiol. In House Finches only the female incubates and has a patch; therefore, lack of estrogen in the male is probably the reason for the absence of the patch in males of this species.

3. *Effect of Testosterone and Estradiol.*—To test the possibility that both androgens and estrogens could produce an incubation patch, 13 Oregon Juncos were trapped and confined in one of the large cages. On December 11, 1950, testosterone pellets were implanted in seven of the birds and estradiol pellets in six. On the next day five more juncos were trapped and added as controls.

The juncos did not survive as well in captivity as the House Finches and White-crowned Sparrows. Several died in the first few days and by December 24, 1950, only 11 of the original 18 were alive. At this time the birds were examined and the incubation patch region of all the birds treated with estrogen was found to be vascular and beginning to defeather. The controls and androgen-treated birds were not changed. On January 6, 1951, the birds were sacrificed. The three estrogen treated birds remaining were all females. The ventral apterium of these was completely defeathered and the skin was vascular and edematous. Of the four remaining androgenized birds two were females and two were males. No indication of defeatherization or vascularization of the abdominal region was observable. This was also true for the controls, three of which were females and one a male.

Further evidence was thus gained that continuous treatment with estrogen will stimulate incubation patch development and that, at least in Oregon Juncos, androgens will not.

4. *Effect of Estradiol and Prolactin.*—Although satisfactory evidence had been obtained that estradiol could produce an incubation patch, there still remained the possibility that estradiol was stimulating the pituitary to secrete some hormone which was actually responsible for all or part of the response. After a technique had been perfected for the removal of the pituitary an experiment was started using hypophysectomized as well as intact (non-hypophysectomized) birds. White-crowned Sparrows were chosen for the experiment since they were best suited for the surgical procedures. Trapping was started on January 3, 1951, and the birds were confined indoors in the small cages. Since it was impossible to accumulate a large number of hypophysectomized birds due to a high mortality, the experiment was spread over a period of several weeks. The results of this experiment are shown in figure 4. Although this experiment was designed to test the effect of estrogen and prolactin on hypophysectomized birds, several other groups of birds were treated at this time with testosterone and prolactin to substantiate further the earlier experiments.

Non-hypophysectomized—Estrogen and Prolactin Treated. Starting January 23, 1951, four birds were treated with estradiol. By the seventh or eighth day the incubation patch area had become vascular and by the fifteenth day all down feathers on the ventral apterium had been lost and the skin was edematous as well as vascular. On January 26, four more birds were treated with estradiol and on the eighth day, after the apterium was vascular, two were injected daily with prolactin and two with albumin. The birds given prolactin lost the down feathers sooner than those that were injected with albumin. The birds receiving prolactin also became edematous sooner. This experi-

ment indicated that the vascular response and the defeatherization-edema response are probably initiated by separate hormones. It also suggests that the hormone responsible for the defeatherization and edema is probably prolactin.

Hypophysectomized—Estrogen and Prolactin Treated. Twenty-one birds were successfully hypophysectomized between January 4, 1951, and February 8, 1951, and were treated with estradiol pellets. A number of these died within a few days and only nine lived long enough to yield significant results. In all these the abdominal skin became vascular within six to 11 days (average seven days). One was killed on the ninth day and a cross section through the skin is shown in figure 3g. None of the hypophysectomized birds showed any defeatherization or edema as long as they received only estradiol,
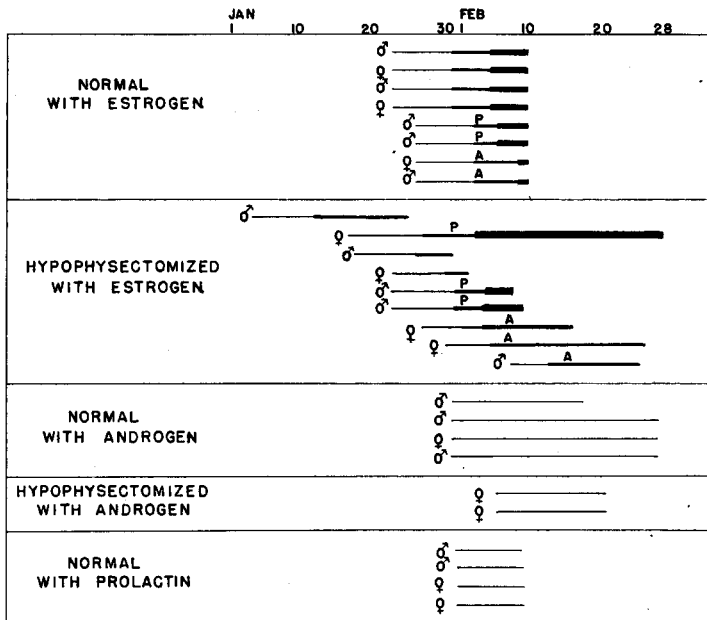

Fig. 4. Results of experiment 4. Narrow lines indicate normal condition of skin in non-breeding birds; medium lines indicate vascularity; heavy lines indicate vascularity, edema, and defeatherization. The dates on which daily administration of prolactin and albumin was started are indicated by the letters P and A. Androgen and estrogen were administered as pellets implanted at the time indicated by the beginning of the lines.

but when prolactin was given to three of the birds after they had become vascular, defeatherization and edema appeared within three or four days. Microscopic sections show that the skin (fig. 3h) is almost identical to that of an incubating female although not as thick. Three of the birds were treated daily with albumin, after they had become vascular, until they died, seven, nine, and 18 days later, respectively. One bird remained on estrogen alone. Both of these latter groups continued to be vascular but in neither did the apterium show the defeatherization response.

Non-hypophysectomized—Androgen Treated. Four birds treated with testosterone pellets for 20 to 31 days showed no increased vascularity or loss of feathers of the incubation patch region.

Hypophysectomized—Androgen Treated. Four hypophysectomized birds were

treated with testosterone pellets starting February 5, 1951. Only two of the birds, both females, survived for more than a few days. No change was found in these by the sixteenth day at which time they were sacrificed.

Non-hypophysectomized—Prolactin Treated. Four birds were injected daily with prolactin starting February 1, 1951. The injections were continued for 10 days but no changes were noted by this time and the birds were sacrificed.

To summarize, estradiol, which can stimulate the production of a complete incubation patch in intact (non-hypophysectomized) birds, produces only the vascularity response in hypophysectomized birds. When prolactin is given to hypophysectomized estrogen-treated birds, a complete patch develops. Prolactin has no effect when given alone. Testosterone has no effect on hypophysectomized or intact birds. One can conclude that the incubation patch is formed by the synergistic action of estrogen and prolactin. This response can be induced in both males and females, although only the females normally incubate and have a patch.

PROLACTIN CONTENT OF INCUBATING AND NON-INCUBATING CALIFORNIA GULLS

Initially, assays of the prolactin content of the pituitary were made on small birds in the hope that they could be made on the same passerine species used in the experimental and nesting studies. It was soon discovered, however, that in small passerines as many as fifteen pituitaries from incubating birds were needed to detect the presence

Table 1

Assay of Prolactin in Adult California Gulls

Date	Locality	Pituitaries implanted	Reaction*
April 14	Oakland	1 Male	—
April 21	Oakland	1 Male, 1 Female	—
		2 Male, 2 Female	—
		3 Male, 3 Female	*
May 31	Mono Lake	1 Male	*
		1 Female	*
		1 Male, 1 Female	**
		2 Male, 2 Female	****
November 3	Oakland	1 Female	—
		1 Male, 1 Female	—
		2 Male, 2 Female	—

* Minus sign indicates no proliferation, one asterisk a minimal reaction, and four asterisks a strong reaction.

of prolactin by the pigeon crop-sac assay method. Reliable quantitative results seemed improbable with such large numbers. California Gulls (*Larus californicus*) were eventually chosen for the assays when it was found that a single pituitary from an incubating bird would give a response. That the gulls are quite far removed, phylogenetically, from the fringillids used for the greater part of this study is recognized. Data from the gulls are admitted as a necessity but also on the supposition that the incubation patch among all birds is homologous and that the regulating mechanism is very similar in different groups.

The California Gulls used for the assays were collected at Oakland, Alameda County, California, and at Mono Lake, Mono County, California. The heads were removed within an hour after the birds were shot and were frozen in dry ice or a refrigerator tray. The pituitaries were removed and implanted, one to six together, subcutaneously over the crop sac of eight-week old White King pigeons. In several of the assays pieces of liver, testes, breast muscle, and spleen were implanted as controls. The pigeons were killed on the fifth day. The crop glands were removed and examined for proliferation

of the epithelium and then were fixed in 10 per cent formalin, sectioned, and stained with Mallory's stain.

The results of the assays are shown in table 1. The gulls collected at Mono Lake were incubating and had incubation patches. Those collected at Oakland, although adult, were not in breeding condition and did not have incubation patches. The prolactin content of the pituitaries of the incubating birds was greater than that of the non-incubating birds. No differences in the prolactin content of males and females were found, which correlates with the fact that both sexes of the California Gull incubate and have incubation patches.

DISCUSSION

From the results of the foregoing experiments it appears that the incubation patch, at least in frigillids, develops in response to the synergistic action of estrogen and prolactin. The incubation patches produced by these two hormones are similar to those normally occurring in these birds in the breeding season. Estrogen is capable of exerting its influence alone. It results in increased vascularity of the dermis. Prolactin, however, requires the presence of estrogen before it is able to initiate its effect, which is, the loss of feathers and dermal edema.

Studies of the reproductive cycle of many birds show that the gonads increase in size as nesting time approaches. The oviducts become larger and singing and posturing occur more often at this time. These latter responses are probably initiated by the sex hormones. While evidence is lacking for the species studies, it is almost certain that the estrogen titers are highest at the time when the changes in the incubation patch that are attributed to this hormone occur.

The evidence for the presence of prolactin at the appropriate time is even better established than that for estrogen. Prolactin assays of California Gulls show more prolactin in the pituitary during incubation than at other times in the year. Burrows and Byerly (1936) found more prolactin in the pituitary of broody races of hens than in non-broody races. These same workers also found more prolactin in broody hens than in laying hens or males (Byerly and Burrows, 1936).

The development, following estrogen treatment, of a complete incubation patch in normal birds and not in hypophysectomized ones leads to the assumption that estrogen stimulates the production of prolactin in the bird's own pituitary. That estrogen has this effect on mammalian pituitaries has been well established (Reece and Turner, 1936). Nevertheless, Meites and Turner (1947) have shown that estrogen seems to have no effect on the prolactin content of pigeon pituitaries. Riddle (1935) has suggested that the elaboration of prolactin may occur as the results of psychic stimulation, and it is possible that estrogen brings about environmental or internal relationships in the White-crowned Sparrow that initiate the necessary psychic stimulations. In any case, it seems that the estrogen does result in increased prolactin production or release through some mechanism, either directly or indirectly.

Androgens probably play no role in incubation patch formation in passerine birds. Androgen treatment did not result in vascularity in the birds tested as did estrogen treatment. This is to be expected since no males in this order have incubation patches. In some orders of birds, however, the males do incubate and have incubation patches. Androgen may, in these cases, have the same relation to the incubation patch as does estrogen in the passeriforms.

SUMMARY

The incubation patch of passerine birds was studied in the field and laboratory. Over 125 specimens from 12 families were examined and no significant variations in structure

were observed. Passerine birds have a single large incubation patch located in, and coincident in size with, the ventral apterium.

Formation of the incubation patch of passerine birds can be divided into several stages that correlate with the stages of the nesting cycle. They are:

I. Defeatherization Stage: All the down feathers of the ventral apterium are molted several days before the first egg is laid.

II. Vascularization Stage: Immediately after defeatherization the size and number of the blood vessels in the dermis increase, the skin becomes slightly thickened due to dermal edema, the feather papillae disappear, and the epidermis undergoes rapid cell division. This stage lasts until incubation begins.

III. Edematous Stage: The patch continues to become edematous and vascular and the dermal muscles disappear. This stage lasts through incubation and the first part of brooding.

IV. Recovery Stage: The edema and vascularity subside and the epidermis returns to normal. These changes are gradual, and by the time the young are able to fly and feed themselves, the skin has returned to normal (unless another clutch is laid, whereupon the cycle is repeated). Refeatherization of the patch occurs during the fall molt.

It was found that an incubation patch can be produced in non-breeding birds by continuous treatment with estradiol. If the birds are hypophysectomized, however, estrodiol will produce only vascularity. If both estradiol and prolactin are given to hypophysectomized birds a complete patch develops. Prolactin alone or testosterone, alone or in combination with other hormones, have no effect. Assays of the prolactin content of the pituitary of California Gulls by the pigeon crop-sac method shows that gulls with incubation patches have more prolactin than those without patches.

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