FUNDAMENTAL ANATOMICAL AND PHYSIOLOGICAL STUDIES ON THE FEATHER RELEASE MECHANISM IN THE DOMESTIC FOWL

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Orville William Ostmann
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Orville William Ostmann

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Robert K. Singer
Major professor

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FUNDAMENTAL ANATOMICAL AND PHYSIOLOGICAL STUDIES ON THE FEATHER RELEASE MECHANISM IN THE DOMESTIC FOWL

By

Orville William Ostmann

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ABSTRACT

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In the anatomical investigation, gross and microscopic studies were conducted. For the purpose of making the gross study, skin from the dorsal feather tract of a cooked turkey was used. The microscopic studies were, for the most part, made on tissues taken from the back region of chickens, although tissues from the leg, neck and breast were also

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In the anatomical investigation, gross and microscopic studies were conducted. For the purpose of making the gross study, skin from the dorsal feather tract of a cooked turkey was used. The microscopic studies were, for the most part, made on tissues taken from the back region of chickens, although tissues from the leg, neck and breast were also

studied. Tissues were taken from chickens in the anesthetized, unanesthetized and molting state. Various methods of processing and staining were used.

The feather follicle is a tubular structure that is dilated into an onion shaped area called the bulb. Attached to the bulb area of the follicle are various bundles of smooth muscle fibers. These fibers radiate from the follicle in many directions, but generally course from the area of the dermal papilla to the apex of an adjacent follicle. Thus, a unique criss-cross pattern is formed. The muscles attach to the follicle by tendons which are replete with elastic fibers. These fibers continue, in conjunction with connective tissue, around the follicle and in so doing, form the outermost layer of the follicular wall.

The muscles of the feather follicle are richly supplied with nerve fibers which terminate in free nerve endings. These fibers apparently contain both motor and sensory fibers and, based on physiological studies, are both sympathetic and parasympathetic in origin. Numerous sensory end-organs, Vater Pacini corpuscles, were observed in close association with the feather follicle.

No apparent histological differences were observed in

skin taken from anesthetized and unanesthetized birds. This was attributed to the fact that skin removed from an anesthetized bird underwent changes which allowed the feathers to return to their tightened state before fixation occurred. Skin taken from birds subjected to scalding was too distorted for any detailed histological analysis to be made.

In the physiological study, a series of experiments were conducted to evaluate the effect of 1) brain sticking; 2) various classes of drugs; 3) spinal transection; and 4) skeletal muscles on feather release in chickens. Feather pulling force, as measured with a spring scale, was used as the criterion.

In the brain sticking studies feather loosening occurred when the stick was made into the diencephalon-cerebellum or the medulla-cerebellum. Due to the procedure used in making the stick, none of these parts were pierced alone; consequently, the exact part of the brain involved could not be determined. When the cerebrum or optic lobes were pierced, feather loosening failed to occur.

Drugs which were effective in reducing feather pulling force were sodium pentobarbital, chloroform, ether, procaine (anesthetics); chlorpromazine, promazine (tranquilizers);

atropine (parasympatholytic); and yohimbine (sympatholytic). All of the neuromimetic drugs, acetylcholine, pilocarpine, carbachol (parasympathomimetic); epinephrine and ephedrine (sympathomimetic), failed to significantly alter the force required to pull an individual feather from its follicle.

All levels of spinal transection significantly reduced feather pulling force of feathers pulled from the dorsal feather tract posterior to the level of the transection. Feather pulling force of feathers pulled anterior to the level of the transection remained relatively unchanged.

Skeletal muscles apparently do not function in the release of the feather from its follicle. This was indicated when curare, a skeletal muscle depressant, failed to reduce the force required for feather removal.

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Special gratitude is extended to Mrs. Marjorie Tetzlaff for the preparation of the histological sections and to Ronald Peterson for cooperation and assistance in collecting the data.

Above all, I am unable to express adequately my gratefulness to my wife, Harriet, for her interest, patience, understanding and unending encouragement during this strenuous

period of graduate study and research. The sacrifices and understanding of my children, Belinda and Julie, are also recognized and appreciated.

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INTRODUCTION

The method by which feathers are removed during processing is one of the factors that affects final appearance, tenderness and keeping quality of the carcass, as well as processing efficiency. As it is well known, there are several methods by which the feathers may be removed. Commercially, however, the method that is unanimously employed is the "scald-pick" method. This method is based upon the fact that heat from the water denatures proteins which are present in the muscle of the feather follicle, thus allowing muscular relaxation and feather release.

During the past fifteen years, optimum scald water temperature has been the subject of considerable research. Although considerable progress has been made, the most advanced method of feather removal still falls short of the goal of producing, with minimum labor, a clean, completely feather-free carcass having a stable, attractive skin surface.

Development of new, more efficient methods of removing feathers, however, is seriously handicapped by a lack of knowledge of the forces that hold the feather in its follicle, as well as the physiological mechanisms by which these forces can be controlled.

The general objective of this investigation has been to ascertain a basic understanding of the physiological mechanisms involved in the release of the feather from its follicle.

REVIEW OF LITERATURE

A. The anatomy of the feather follicle and its immediate surroundings.

A search of the literature reveals very little information on the anatomy of the feather follicle and its immediate surroundings. Most of the information available on this subject is in regard to the muscles which move the feather and was reported in the nineteenth century. The muscles of the feather follicle were first described by Nitsch (1840), who reported that four separate muscles were usually attached to the follicle of each contour feather. He further reported that sometimes six and more rarely five were present.

Seuffert (1862) according to Langely (1904) observed that contour feather follicles of the trunk region usually had two to four separate muscles. He stated that the muscles were unstriated and attached to the follicle by elastic tendons. These muscles were observed to course from the lower part of one follicle to the upper part of an adjacent follicle. Similar observations were made by Helm (1884) as cited by Langley (1904).

Langley (1902b) noted that each feather follicle in the neck region had two sets of muscles, which he named erector

and depressor. He also noted that the number and arrangement of these muscles varied considerably and as many as sixteen muscular attachments to a single follicle were observed.

In a subsequent report, Langley (1904) presented a very detailed description of the muscles which move the feathers. It was his observation that each feather usually had four small unstriated muscles which arise within the dermis. Many times, however, one or two more were present. The muscles were of three general types: (1) erector muscles coursing from the neck of one follicle to the end (bulb) of the follicle anterior to it; (2) depressor muscles coursing from the neck of one follicle to the follicle posterior to it; and (3) retractor muscles coursing from the neck of one follicle to the neck of an adjacent follicle. He reported that these muscles were situated anterolaterally, posterolaterally and longitudinally from the mid-dorsal line. According to Langley their function was to erect, depress or retract the feather, respectively.

During the course of the many studies of Lillie and coworkers on experimental feather development, many photomicrographs and seni-diagrammatic drawings of the developing

feather were presented. From a semi-diagrammatic drawing which was presented by Lillie (1940) it could be seen that the follicle was enclosed by a circular muscle. This, in turn, was bordered by the levator muscle which moves the feather.

According to Langley (1904) the first information on the innervation of these muscles was secured by Jegorow in 1890. Using the turkey as the experimental bird, Jegorow found that depression of the head and neck feathers occurred upon stimulation of the cervical sympathetic nerve. On the other hand, Langley (1902a) reported that when the cervical sympathetic nerve was severed, feather ruffling occurred in the peripheral area of the skin which was innervated by the nerve. In addition, the latter worker reported that following transection of the cervical spinal cord, and stimulation of the lower end, the contour feathers over the entire body were depressed and drawn closely to the skin. Later, Langley (1902b) reported that feather ruffling was also under the control of the sympathetic nervous system.

Further evidence that the sympathetic system is involved in feather movement was provided by Langley (1904). Using the chicken and pigeon, he observed that if any part of the

sympathetic system was stimulated, the ordinary effect was a depression of the feathers. At times, however, a strong erection of the feathers occurred. This he attributed to stimuli affecting the spinal cord, as the same effect was observed when the cord itself was sectioned. Further evidence suggesting that the cord was involved was brought forth when strychnine, a spinal cord stimulant, caused irregular movement of the feathers.

B. Factors influencing the release of the feather from its follicle.

Influence of scalding on feather release.--The fact that scalding, submerging the bird into water at a controlled temperature for a given period of time, facilitates feather removal is well known. However, much of the information obtained has been on an empirical basis. According to Benjamin et al. (1960) the first method of scalding to be widely used in commercial processing plants was the hard-scald. Birds subjected to this method of scalding are submerged for 30-60 seconds in water at a temperature of 160-180^{o}F. The ease with which feathers may be removed from birds processed by this method is well known. It has the disadvantage of removing the epidermal layer of the skin.

Thus, bacteria may enter and seriously impair the shelf-life of the bird. Because of this adverse effect, this method was discarded in favor of the semi- or slack method of scalding. In this method, the temperature of the water is 123 to 130°F; the exact temperature depending on the kind and age of the bird and the length of immersion time. The temperature of the scald water that is generally used for chickens, and which was shown by Pearse and Lavers (1949) to result in an optimum quality carcass, is 128°F. These workers further reported the optimum immersion time to be 40 seconds. Under these conditions, feather removal by mechanical pickers was found to be incomplete; thus increasing the labor required for pinning and finishing.

The present trend in poultry processing has been toward the use of higher scald water temperatures of 138-140°F. for 30-75 seconds. This method, commonly called sub-scalding, was evaluated by Gwin (1950, 1951a, 1951b). He reported that feather removal was nearly complete by mechanical pickers, therefore, markedly increasing processing efficiency. He further reported that part of the epidermal layer of skin was removed and unless the carcass was kept moist, the flesh darkened. In a subsequent report, Gwin

(1952) pointed out that the amount of labor required for pinning and processing following sub-scalding could be reduced as much as 80 percent over lower scald water temperatures (semi-scalding). Similar results were reported by Pool et al. (1954) and Stadelman and Ziegler (1955).

Using an objective measure, Pearse and Lavers (1949) related feather pulling force to scald water temperature and length of scalding time. They reported that birds scalded in water at 125°F. for 30 seconds required 15 ounces of pulling force to remove an individual feather from its follicle. In contrast, birds subjected to scald water temperature of 136°F. for 15 seconds required only 5 ounces.

Pool et al. (1954) conducted an experiment to evaluate scalding temperatures and time of immersion on feather removal of turkeys. Individual feather pulling force was used as the criterion. They found that as the temperature of the scald water decreased, feather pulling force increased. Moreover, the increase was in a consistent manner with the decreasing scalding temperatures. When scalding time was varied from 30 to 120 seconds at a constant scald water temperature of 125°F., no significant difference in feather pulling force was noted. Thus, these workers concluded

that scalding time is a much less critical factor than scald water temperature in feather removal. Similar results were reported by Knapp and Newell (1961) in the chicken. In addition, the latter workers presented data which demonstrated that scalding time had a greater effect at scald water temperature of 128°F. than at any other temperature which they tested. The latter researchers further reported that at scald water temperature of 142°F., scalding time of 45 seconds gave optimum results.

Recently, reports have appeared in the literature in which the effects of various factors in combination with scalding were studied. Knapp and Newell (1961) studied the combined effects of scald water temperature, scalding time and length of fasting period (without feed and water) on feather removal of White Leghorn hens. The birds were fasted for either 6 or 8 hours, then killed and scalded for 45 or 90 seconds at a temperature of 128, 135 or 142°F. These researchers reported that fasting did not have any beneficial effects on feather removal. In live birds, however, fasting resulted in a slight but insignificant reduction in feather pulling force.

Klose et al. (1961) reported on the combined effects of

anesthesia and scalding. They observed that chickens which were anesthetized with sodium pentobarbital, then scalded in water at a temperature of 122, 128 or 140° F. for 60 seconds, had an individual feather pulling force lower than those birds subjected to either scalding or anesthesia alone. Furthermore, this effect was observed for all three scald water temperatures. They suggested, "that the lower (122° F.) scalding temperature might be acting through a nerve-muscle relaxing mechanism."

Although scald water temperature has been the subject of considerable research, the actual physiological process involved in the release of the feather from its follicle by this method remains unknown. The present theory is that heat, from the water, denatures proteins which are present in the muscles of the feather follicle, thus allowing muscular relaxation and feather release (Winters and Funk, 1960; Benjamin et al., 1960). This theory is supported, indirectly, by the results of Pearse and Lavers (1949), Pool et al. (1954) and Knapp and Newell (1961).

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Apparently, the first factual information on the essential area of the brain involved in feather release was provided by King (1920). Working with broilers and pigeons, he reported that none of the brain above the level of the cerebellum and medulla was required for release of the feather. In an attempt to isolate which specific part of the brain was involved, he subjected birds to ether anesthesia, which was shown to have no effect upon the feather muscles; he also removed the skull and stimulated or destroyed various parts of the brain. In eight out of ten birds tested, stimulation or damage to the cerebellum failed to result in feather loosening. On the other hand, all birds in which only the medulla was involved, exhibited loose feathers. In view of these results, he concluded that the center involved in feather release was located in the medulla.

Weaver (1936), however, indicated that the center involved in feather release was not located in the medulla, but rather in the anterior portion of the cerebellum. His conclusion was based on results obtained from studies conducted on exposed brains of birds under chloroform anesthesia. It was his contention that feathers loosened by

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Recent data, presented by Klose et al. (1962) further confirm the theory of denaturation of muscle protein. These workers reported that feather pulling force was reduced by

50-70 percent in birds subjected to radiant heat which was supplied by either a photo flood lamp or an infra-red lamp. They further demonstrated that feather pulling force was markedly influenced by the type of scalding medium used. Water was found to be the most effective in loosening the feathers, followed in decreasing order by propylene glycol and mineral oil. They suggested that these differences in effectiveness "could arise from the appreciable difference in viscosity, wetting ability and specific heat represented by the three liquids." It was also noted that under-skin temperature for birds scalded in these three liquids at 126°F. was considerably different. They reported that within one minute under-skin temperatures increased from 104°F. to 117°F. for birds scalded in water. In contrast, birds scalded in mineral oil showed an increase only to 111°F.

Influence of the nervous system on feather release.-- Another factor which is known to influence feather release is the nervous system, as indicated by feather loosening when a successful brain stick is performed. Just what portion of the brain is involved, however, is controversial, although the medulla is regarded to be the essential part.

Apparently, the first factual information on the essential area of the brain involved in feather release was provided by King (1920). Working with broilers and pigeons, he reported that none of the brain above the level of the cerebellum and medulla was required for release of the feather. In an attempt to isolate which specific part of the brain was involved, he subjected birds to ether anesthesia, which was shown to have no effect upon the feather muscles; he also removed the skull and stimulated or destroyed various parts of the brain. In eight out of ten birds tested, stimulation or damage to the cerebellum failed to result in feather loosening. On the other hand, all birds in which only the medulla was involved, exhibited loose feathers. In view of these results, he concluded that the center involved in feather release was located in the medulla.

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piercing the center in the cerebellum could be made to tighten by subsequent piercing of the medulla. In addition, Weaver stated that two separate and distinct nerves were associated with the feather muscles; one being responsible for relaxation of the feathers, the other responsible for tightening the feathers.

Recently, Klose et al. (1962) presented data which also indicate that the nervous system is involved in feather release. They reported that when the brain was pierced by a blade inserted through the eye socket, feather loosening occurred within 15-30 seconds. However, the effect lasted for less than one minute. The area of the brain in which these sticks were made was not reported.

Electric shock has also been shown to cause feather release. Rose (1939) reported that satisfactory feather release could be obtained when the birds were subjected to 450-500 volts of electricity for a period of 10 seconds. The results were inconsistent and were not quite as good as those obtained by a successful brain stick. A subjective measure was used in this study. Satisfactory results were said to be obtained when the feathers could be plucked with reasonable ease and without tearing the skin. A metal

clamp attached to the head of the bird was used to provide electrical contact.

The influence of drugs on feather release.--Until recently, the effect of drugs on the muscles of the feather follicle received comparatively little attention. Langley (1904) used atropine, curari, apocodeine, and supra-renal extracts and observed no effects upon feather movement. In contrast, nicotine, injected intravenously, resulted in a strong depression of the feathers over the entire body. A subsequent dose, however, had no further effect upon feather movement. He attributed the depression of feathers to a stimulatory effect of the drug upon the sympathetic ganglia. He further reported that strychnine, following morphia and curari, caused an irregular rhythmic movement of the feathers for one to two minutes, the movement being an erection and depression. It was his conclusion that the action of strychnine appeared to be on the central nervous system as feather movement of the head and neck failed to occur when both the cervical sympathetic nerves were cut.

King (1920) studied the effects of several classes of drugs on the muscles of the feather follicle and/or the nerve center involved in feather loosening. His results

indicated that chloroform, atropine, scopolamine, apomorphine, strychnine and amyl nitrite loosened the feathers as much as did the successful brain stick. Drugs which were tried but found to have no effect on feather loosening were eserine, adrenalin, morphine, curare, chloral hydrate, heroin, emetin and camphor.

It has recently been reported that oral administration of sodium pentobarbital was effective in loosening the feathers. Huston and May (1961) reported that feather removal was 90 to 95 percent complete when the anesthetized birds were plucked dry by commercial machines after death by bleeding. Klose et al. (1962) also studied the effect of oral administration of sodium pentobarbital on feather loosening. They, too, reported feather loosening following anesthetization. However, their data revealed that this effect could not be carried over into the post mortem state. Thus, their results are in disagreement with those of Huston and May (1961).

Intravenous injection of sodium pentobarbital has also been shown to be effective in causing feather loosening (Klose et al., 1961, 1962). In the latter report, data were presented which indicated that anesthesia reduced feather

pulling force by approximately 70 percent of the value observed in the conscious bird. These workers also presented data in the latter report on feather pulling force of fowl which were subjected to a small or massive lethal dose of sodium pentobarbital. From this study, these workers reported that feather pulling forces of birds subjected to the small lethal dose returned to 80-100 percent of the pre-injection level within 1-2 minutes after death. Massive doses, however, were found to prolong this time.

There is also evidence which indicates that tranquilizing drugs may influence further relaxation. Sturkie et al. (1958) reported that tranquilizing doses of reserpine, given to capons, apparently caused relaxation of the feather follicle, as indicated by the dropping of feathers when handled. Observations by Klose et al. (1961) revealed that reserpine, fed at 50 or 110 ppm for 1, 7 or 14 days, reduced feather pulling force in the live birds. The effect failed to persist after slaughter. Similar results were reported when the tranquilizer was administered intravenously. Knapp and Newell (1961) also observed that oral administration of reserpine was effective in reducing the force required for feather removal in the pre-slaughter state. They further

reported that practically a straight line relationship existed between the level of the drug and feather pulling force. In addition, the latter workers also observed that trifluoperazine was effective in releasing the feather from its follicle.

OBJECTIVES

The objectives of this investigation were:

1. To characterize the anatomical features of the feather follicle and its immediate surroundings.
2. To characterize, by histological techniques, the essential changes involved in feather loosening.
3. To determine the part of the brain which is involved in the release of the feather from its follicle.
4. To investigate the effect of spinal transection on the release of the feather from its follicle.
5. To investigate the effects of various synthetic drugs on the release of the feather from its follicle.
6. To investigate the role played by skeletal muscle, if any, in the release of the feather from its follicle.

EXPERIMENTAL PROCEDURE

A. General.

1. Anatomical studies.--Gross and microscopic observations on the anatomy of the feather follicle and its immediate surroundings were studied. The macroscopic anatomy was studied in skin taken from the dorsal feather tract of a "cooked" turkey. Before dissection, the tissues were placed in ethyl ether to extract a portion of the fat. The dissected tissues were studied with the aid of a dissecting scope as well as a microscope. Photomicrographs of the dissected tissues were taken with a Kodak 35 mm Photomicrographic camera.

The microscopic studies were, for the most part, made on tissues taken from the back region of chickens, although tissue from the leg, neck and breast were also studied. Tissues of approximately 10 X 10 mm were taken from birds in the following physiological states: unanesthetized, anesthetized and molting. Tissues were also taken from birds which were subjected to scalding (140^{o}F for 1 1/2 minutes). After the tissues were removed from the birds, the feather quills were cut off just above the dorsal surface

of the skin to facilitate sectioning.

Except as otherwise indicated, the sections were processed in an autotechnicon (Model 2A) in the normal manner (Armed Forces Institute of Pathology, 1957). The tissues were fixed in either neutral 10 percent formalin, Zenker's fluid or Bouin's fluid, depending on the type of stain to be used.

Following processing, the tissues were embedded in paraffin and cooled rapidly. Paraffin blocks were cut, trimmed and mounted on a metal object holder. Blocks were cut on a Spencer Rotary Microtome.

At first, sections were cut at a thickness of 4-6 microns; however, it was found that for detail study of the feather follicles these sections were inadequate. Thicker sections were found to be superior; consequently, all sections, except as otherwise indicated, were cut at 7-10 microns.

The majority of the sections were cross-sections, since these appeared to give the most information regarding the relationship of the different skin structures to the feather follicle. Longitudinal sections were also made, however, and were especially useful in demonstrating the innervation

of the muscles of the feather follicles. Only follicles containing mature feathers were studied.

The sections were floated on a water bath, affixed to clean slides with Mayer's egg albumen, dried in a paraffin oven at 56°C and deparaffinized. The sections were then stained by either a general or specific stain, depending upon the various histological details being studied. For the study of general histology, the following stains were employed (Armed Forces Institute of Pathology, 1957; Conn, Darrow and Emmel, 1960):

1. Harris's hematoxylin and eosin
2. Masson's trichrome stain
3. Gomori's trichrome stain
4. Van Gieson stain for collagen fibers
5. Heidenhain's aniline blue
6. Gallego's iron fuchsin
7. Gomori's aldehyde fuchsin; metanil yellow counter-stain
8. Hematoxylin-Shorr S_3 stain

In neurological staining, the general histological method of processing tissue is of little value. This is contributed to by the fact that a large number of structural peculiarities

exist in the nervous system. These peculiarities vary greatly in their reaction to staining. Consequently, specific techniques have been developed for demonstrating a specific structure.

In this study, nerves and their endings were of particular interest. In order that these could be demonstrated, the following methods were employed:

1. Chloral Hydrate Silver Method (Conn, Darrow and Emmel, 1960). Tissues were fixed in chloral hydrate-alcohol solution for 1-3 days. Following fixation, the tissues were stained for 5-6 days in 2 percent aqueous silver nitrate. The tissues were embedded in paraffin and sectioned on a rotary microtome at 7-10 microns.
2. Silver Method for Nerve Axoplasm (Winkelmann and Schmit, 1957). This method is dependent upon the impregnation of silver into the nervous tissue. The tissue was fixed several days in 10 percent formalin. Sections were cut at 50 microns on a freezing microtome and stained with silver nitrate.
3. Histochemical Method for Cholinesterase (Gomori, 1952). This method is based upon the demonstration

of an enzyme, cholinesterase, which is found in the membrane of all cholinergic fibers but is virtually absent from the adrenergic fibers. This permits one to distinguish cholinergic from adrenergic fibers. Acetyl and butyryl-tricholine were used as substrates. Elimination of these substrates served as the control. The tissues were fixed in 10 percent formalin for 1-4 hours. Sections were cut on a freezing microtome at a thickness of 50-100 microns. The tissues were not counterstained.

2. Physiological studies

Feather pulling force.--The criterion used in this part of the investigation was feather pulling force which may be defined as the force required to pull an individual feather from its follicle. In order that this force might be recorded, it became necessary to devise some instrument which would give accurate results. Earlier workers reported that a spring scale, with a hemostat attached to hold the feather, proved to be satisfactory (Pearce and Lavers, 1949; Pool et al., 1954; Klose et al., 1961). A potentiometer was also reported to give satisfactory results (Knapp and Newell, 1961).

At first, a balance which measured in grams was used. Attached to the pan of the balance, via a string passing over a pulley, was a hemostat. As the bird was pulled from the scale, the force required to pull the feather was recorded. Although this method proved to be accurate, it was nevertheless cumbersome. Consequently, a spring scale was employed (Fig. 1). A hemostat was used to attach the scale to the feather. The scale was calibrated from 0 to 32 ounces; however, a conversion factor (1 ounce = 28.4 gms) was used so that results could be reported in grams. The spring scale was tested for accuracy against the previously used balance and was found to be accurate. During the feather pulling procedure, the scale was held in a manner such that the line of pull tended to coincide with the angle in which the feather was imbedded.

During the latter part of these studies a spring scale, calibrated in 10 gram divisions was used. In addition, this scale possessed a maximum reading pointer and was very similar to the one used by Klose et al. (1961).

In preliminary work considerable differences in feather pulling force were found when feathers were pulled from different areas of the body. The most consistent pull

Fig. 1. Application of the spring scale used to measure feather pulling force.

occurred when feathers were pulled from the back area (dorsal feather tract). Similar observations were reported by Klose et al. (1961). Consequently, all results reported, unless otherwise specified, are from feathers pulled from the dorsal feather tract. It was also noticed that considerable variation between individual feathers within a given area existed. Much of this variation was due to the state of maturity of the feathers involved. Immature feathers released from their follicles when very little pull was exerted. Therefore, in order that an accurate feather pulling force could be recorded, only mature feathers were used. A mature feather may be defined as one which has a hard, dry quill and is embedded well into the follicle. Each feather was examined for its state of maturity before and after pulling. A minimum of five feathers were pulled for each observation made, since it was previously demonstrated that there were no significant differences in feather pulling force between the first five and second five feathers pulled from individual birds (Klose et al., 1961).

Experimental birds.--The birds used in these experiments were birds which were previously used in a nutrition and/or management study, although some cull hens were also utilized.

Only birds possessing a good set of feathers (non-molting) were used. White Leghorn hens, which were in good laying condition, were used in most of the experiment. Some White Rock hens and broilers were also used, however. In addition, a few White Leghorn cockerels were used.

3. Statistical analysis.--For statistical analysis, the "t" test was employed (Snedecor, 1955). Each drug was studied individually at each of the levels and/or methods of administration. No comparisons between drug efficacy, with respect to feather release, were made. The percent change in feather pulling force at each observation was figured from the value obtained in the normal state. The data are presented as the mean value with its standard error.

B. Special techniques.

1. Anatomical studies.--One of the objectives of the histological studies was to characterize any changes that occurred between the feather follicle and its surroundings in the tightened and the relaxed state. During preliminary observations of tissues taken from birds in the anesthetized and unanesthetized state, no apparent histological differences were noted. Thus, it was conceivable that skin, containing

feathers in the relaxed state by virtue of anesthesia, underwent changes which allowed the feathers to return to their tightened state before fixation could occur. Subsequent physiological studies, which will be discussed later, substantiated the above fact. It was obvious, therefore, that in order to observe the feather follicle and its surroundings in the relaxed state induced by anesthesia, histological techniques other than those of the standard method must be used. Consequently, the techniques of freeze-drying and frozen-sections were employed.

Freeze drying technique.--A relatively inexpensive freeze-drying apparatus was used. The apparatus consisted of a vacuum pump with a hose attached to a vacuum flask which, in turn, was immersed in a beaker containing a solution of alcohol and dry ice. Insulation was then placed around the beaker containing the alcohol-dry ice solution. A thick wall Florence Flask was attached to an outlet of the vacuum flask, for the purpose of containing the tissue.

Tissues were taken from White Leghorn males in the un-anesthetized or anesthetized state and subjected to one of the following treatments: 1) placed directly in the drying flask attachment; 2) frozen immediately by placing between

two pieces of dry ice for 1 minute, then placed in the flask attachment; 3) frozen immediately by immersion into an alcohol-dry ice solution for 1 minute, then placed in the flask. Following any of these treatments, the tissues were immediately placed in the freeze-drying apparatus and dried for a period of 5-8 hours. The tissues were then taken out of the drying flask and immediately infiltrated with paraffin in one of two ways: 1) infiltration for not less than 15 hours, in two paraffin baths in the autotechnicon; 2) infiltrated under vacuum (15 lbs./sq. in.) for a period of 15, 30 or 60 minutes.

The tissues were then taken out of their respective paraffin baths, and imbedded in paraffin. Blocks were cut, mounted, sectioned and stained according to the usual procedure.

Frozen section technique.--Tissues were taken from anesthetized and unanesthetized White Leghorn hens. The tissues were sectioned on a Spencer-Freezing Microtome (Model 880) in one of the following states: 1) fresh unfixed; 2) agar imbedded (Lillie, 1954); 3) gelatin imbedded (Lillie, 1954); or 4) fixated in 10 percent formalin. The sections were cut at either 15-20 microns or 50-100 microns depending upon the method employed. The sections were stained by the routine procedure.

Celloidin technique.--Mature feathers are highly keratinized, cylindrical structures which are devoid of any material in their lumen. Due to these characteristics, it is very difficult to section this type of tissue without some shattering occurring. Thus, the section tends to become distorted during histological processing. In an attempt to eliminate some of the distorting effects, the celloidin method of processing was used.

Tissues were taken from the leg and breast area of a White Leghorn hen following death by bleeding. The bird was then scalded for 1 1/2 minutes in water at a temperature of 140°F. Following scalding, Zenker-formalin fixative was injected, subcutaneous or intradermal, into the leg and breast area. Tissues were taken at 1, 3, 5 or 11 minutes after the fixative was injected. All tissues were processed by the Hercules method for celloidin sections (Bensley and Bensley, 1938). Sectioning the blocks of tissue was carried out according to the cedarwood oil method for dry sections of celloidin (Lillie, 1954). The sections were stained using the method for staining celloidin sections (Armed Forces Institute of Pathology, 1957).

RESULTS AND DISCUSSION

A. Anatomical studies.

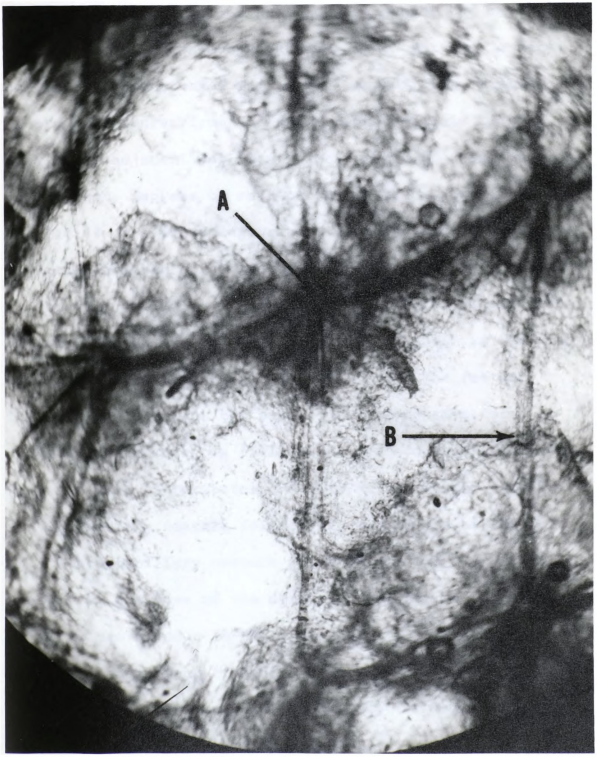
General description.--The anatomical arrangement of the feather follicles and the muscles which control their movement is essentially as noted by Langley (1904), and is described here to facilitate understanding of feather loosening. The feather follicles are arranged in rows and connected to one another by bands of smooth muscles (Fig. 2). Thus, when the dissected skin is viewed from the surface, a four-sided figure is seen between any four follicles. In the back region, the feather follicles are located further apart anterior-posterior than laterally; consequently, the typical figure usually observed is a rhomboid (Fig. 2).

The muscular system of the feather follicle is much more complex than is indicated in Fig. 2. While it is true that the muscles pass between the roots of adjoining feather follicles, they do so in a unique pattern. After attaching to the feather follicle by tendons, the muscle fibers course from the dermal papilla area of one follicle to the apex area of the follicle directly anterior to it. The muscle fibers also course from the dermal papilla area of one follicle to the apex area of the follicle directly posterior to it.

Fig. 2. Photomicrograph of skin taken from the dorsal feather tract of a turkey showing the general view of the muscular arrangement between five feather follicles. Prepared by dissection. X 9.

A. Feather follicle

B. Muscle


A
B

Thus, it is quite obvious that in this manner the smooth muscles form a criss-cross pattern. This pattern is very adequately demonstrated in Fig. 3. In addition, muscle fibers also course from the apex of one follicle to the apex of an adjacent follicle. The purpose of these muscles is to erect, depress or retract the feathers (Langley, 1904).

Fig. 4 is a photomicrograph of a dissected feather follicle from the dorsal feather tract of a turkey. Similar to the hair follicle, the feather follicle is dilated into an onion-shaped area called the bulb. The bulb is the area of the greatest diameter of the follicle. The feather follicle is hollow at the base. This depression forms an obovate cavity which is filled with loose connective tissue. This, in turn, constitutes the area of the dermal papilla.

The resting feather follicle is surrounded at the base by a loose capillary network which arises from larger blood vessels at the base of the dermis. Evidence of blood vascularity can be seen in the region of the neck of the follicle. It is apparent from Fig. 4 that these blood vessels arise from the same vessels that supply the base of the follicle. During molt, the vascularity increases remarkably and can be seen in abundance in follicles containing

Fig. 3. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken showing the criss-cross pattern of muscles as they course between adjacent feather follicles. Cholinesterase preparation. Frozen section. X 20.

A., B. Feather follicle

C. Criss-cross of muscles

B
C
A

Fig. 4. Photomicrograph of a dissected feather follicle from the dorsal feather tract of a turkey. Prepared by dissection. X 20.

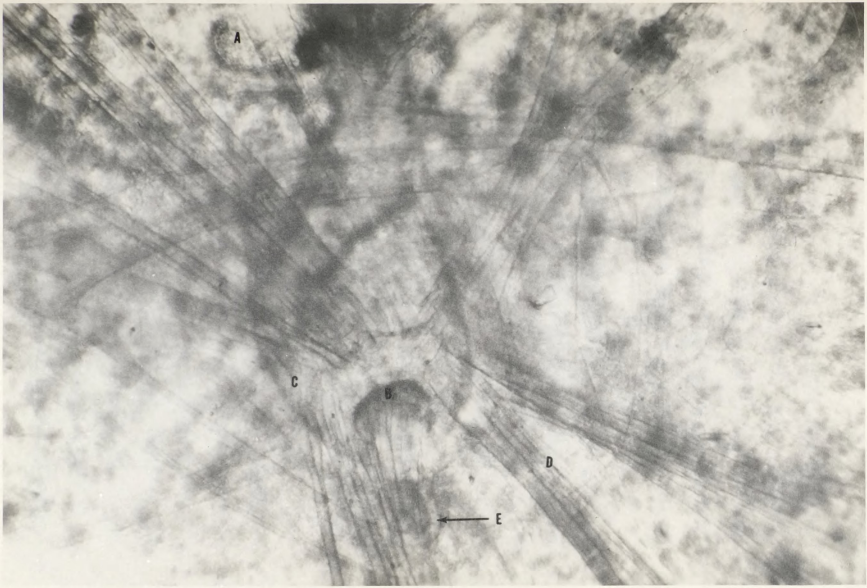
A. Apex of feather follicle

B. Base of feather follicle containing obovate cavity

C. Bulb of feather follicle

D. Smooth muscle attached to follicle at various levels and coursing in several directions.

E. Blood vessel entering cavity of feather follicle


A
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immature feathers (Fig. 5). As the developing feather matures, the pulp, along with the blood vessels, is slowly resorbed and is used to nourish the developing feather (Lillie, 1940). At maturation, the pulp is completely resorbed, hence the feather becomes a hollow keratinized structure (Fig. 5).

According to Lillie (1940) the pulp is supplied with a single axial artery which gives off many branches. The pulp is replete with pericentral sinuses which connect to form small veins. The veins, in turn, leave the follicle through the papilla.

Numerous nerves may be seen emerging from the spinal cord or from the spinal ganglia; however, as they course to the skin they become microscopic and are virtually impossible to trace macroscopically.

Histology.--In cross section, the lumen of a mature feather appears as a clear white area which is devoid of any structure (Fig. 6). In longitudinal sections of this area, keratinized membranes marking the periodic steps in the resorption of pulp can be seen. These membranes are the scala of the calamus. In contrast, the cross-section of the immature feather is characterized by the appearance of

Fig. 5. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken showing a feather follicle containing a mature feather, two feather follicles containing immature feathers and the complexity of the muscles involved. Contractile elements are also illustrated. Section stained with Heidenhain's analine blue. X 20.

A. Mature feather follicle

B.,C. Immature feather follicles, containing pulp replete with blood vessels

D. Feather bulbs

E. Muscle, longitudinal section

F. Muscle, cross section

G. Contractile elements

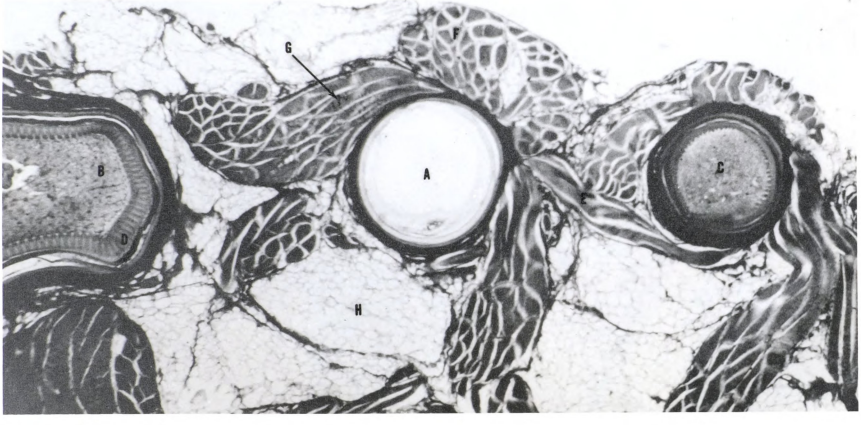
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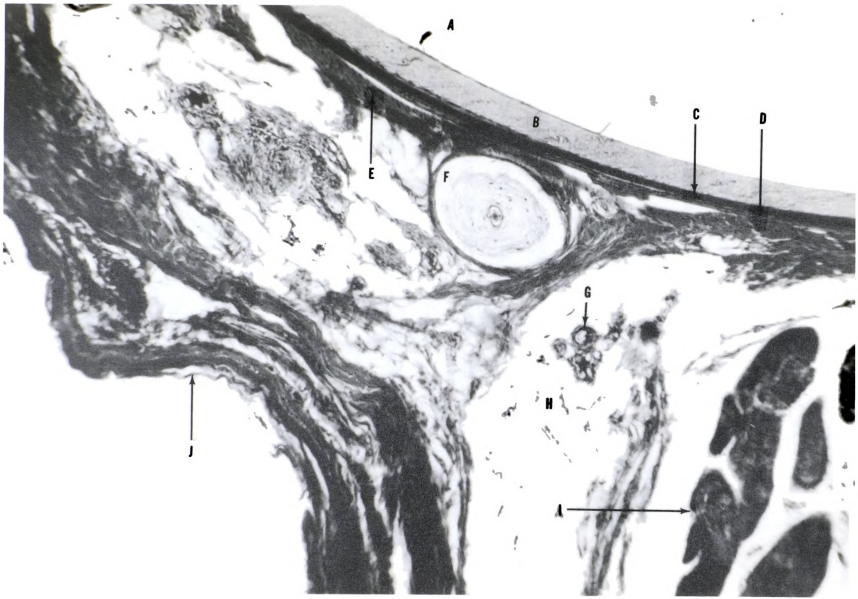
Fig. 6. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken showing a cross section of a mature feather follicle and its surroundings. Section stained with Masson's trichrome stain. X 100.

A. Lumen of the feather
B. Keratinized calamus
C. Keratinized epithelium
D. Epithelium
E. Connective tissue
F. Vater-Pacini corpuscle
G. Blood vessel
H. Adipose tissue
I. Smooth muscle
J. Epithelium


A
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J
A

the pulp with its pericentral sinuses and axillary artery together with the stratum corneun and ridges (Fig. 5). Adjacent to the keratinized calamus of the feather proper, is the keratinized layer of epithelium. In longitudinal section, this layer can be readily traced as a continuation of the epithelium of the skin (Fig. 7). The keratinized layer of the feather proper and the keratinized layer of the epithelium form a frictional bond which presumably aids in holding the feather in its follicle. When the feather becomes loose in its follicle, these two layers separate and form the follicular cavity (Fig. 8). Within the follicular cavity, numerous microscopic bridges of adherence between the two keratinized layers can be observed. When a feather is plucked from its follicle, the keratinized layer of epithelium remains attached to the follicular wall.

Lillie (1940) diagrammatically depicted the next layer adjacent to the keratinized epithelium as a circular muscle forming the follicular wall. Upon histological examination of sections stained with a specific connective tissue stain such as Masson's, Gomori's or Van Gieson's, it is very apparent that this layer is not smooth muscle but, rather, connective tissue. Moreover, the connective tissue layer

Fig. 7. Photomicrograph of a cross section of skin taken from the dorsal feather tract of a chicken showing the keratinized epithelium of the feather follicle as being continuous with the keratinized epithelium of the skin. Section stained with Harris's haematoxylin and eosin. X 35.

A. Keratinized epithelium of skin

B. Keratinized epithelium of feather follicle

C. Keratinized calamus of feather

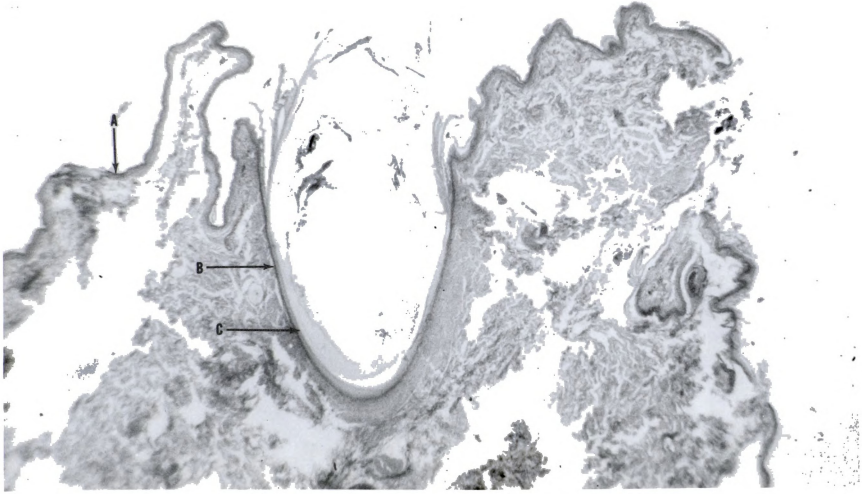

A
B
C

Fig. 8. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken showing the microscopic bridges of adherence between the keratinized calamus and the keratinized layer of epithelium. Section stained with Gallego's iron fuchsin. X 100.

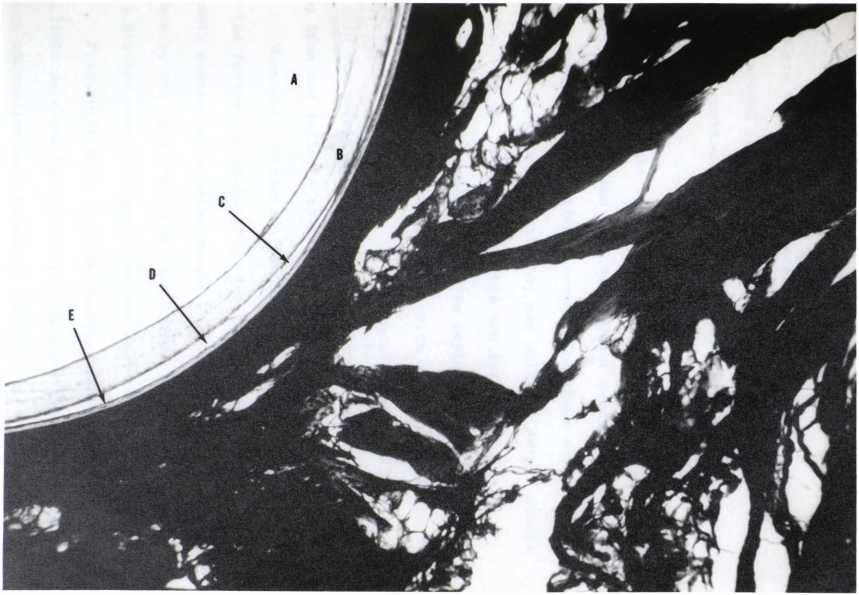
A. Lumen of the feather

B. Keratinized calamus of feather

C. Follicular cavity

D. Microscopic bridge

E. Keratinized epithelium of feather follicle


A
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D
E

is replete with elastic fibers. These elastic fibers form a network around the feather and upon close examination can be seen to be continuous with the tendon of the smooth muscle (Fig. 9).

As a rule, the muscles are found in bundles; however, in the skin they are often seen as singular muscle fibers scattered among the connective tissue. When these smooth muscle fibers parallel each other, they may unite to form a muscle bundle. As the bundle approaches the feather follicle, it abruptly ends in a tendon which is replete with elastic fibers. This elastic tissue then combines with connective tissue to form the follicular wall. These smooth muscles all arise within the dermis and, as noted previously, can be seen coursing from one follicle to another (Figs. 2, 3 and 5).

Numerous contraction bands were observed in the muscles of the feather follicle (Fig. 5). These were the result of smooth muscle cells being caught in a completely contracted state by the fixative; thus, they appear shortened, bulged and are stained more intensely than the rest of the muscle fibers.

Frequently seen in close association with the feather follicle were the Vater-Pacini corpuscles (Figs. 6 and 10). These corpuscles were numerous; usually one or two could be

Fig. 9. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken showing the attachment of smooth muscles to a follicle containing an immature feather by tendons. Section stained with Gomori's aldehyde fuchsin; counterstained with metanil yellow. X 100.

A. Smooth muscle

B. Tendon containing elastic fibers

C. Continuation of elastic fibers in the connective tissue of the follicular wall

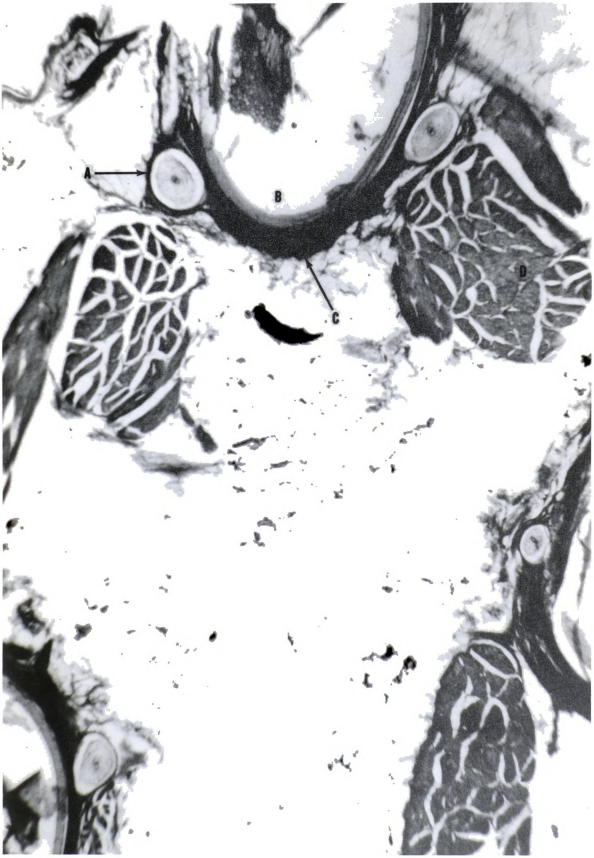
A
B
C

Fig. 10. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken showing the Vater-Pacini corpuscle in relation to the feather follicle. Section stained with Gomori's trichrome stain. X 35.

A. Vater-Pacini corpuscle

B. Connective tissue of follicular wall

C. Lumen of feather follicle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seen in relation to a given feather follicle. They were observed in tissue taken from the back, leg, breast and neck area. This corpuscle is an encapsulated nerve ending and is composed of concentric lamellae. In this study, their exact location was not determined. However, Winkelmann and Myers (1961) reported that they were located just above the insertion of the multiple smooth muscle bundle on the feather follicle.

The muscles of the feather follicle are richly supplied with nerve fibers, as demonstrated by several stains used. In Fig. 11 is shown a typical bundle of nerve fibers coursing through the connective tissue stroma of the skin. From this bundle of nerve fibers, numerous branches split off, one of which is shown in Fig. 11. These branches can be traced for some length before they penetrate the smooth muscles of the feather follicle; however, due to the thickness of the section required for nerve demonstration, this could not be reproduced photographically. As the nerve fibers penetrated the smooth muscles they were observed to do one of two things; one, they continued to course through the muscle as a small bundle; or, two, they branched repeatedly until individual nerve fibers were formed (Fig. 12). These individual nerve

Fig. 11. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken showing a nerve trunk coursing through the connective tissue stroma. Note small bundle branching from main trunk. Winkelmann's silver method. Frozen section. X 100.

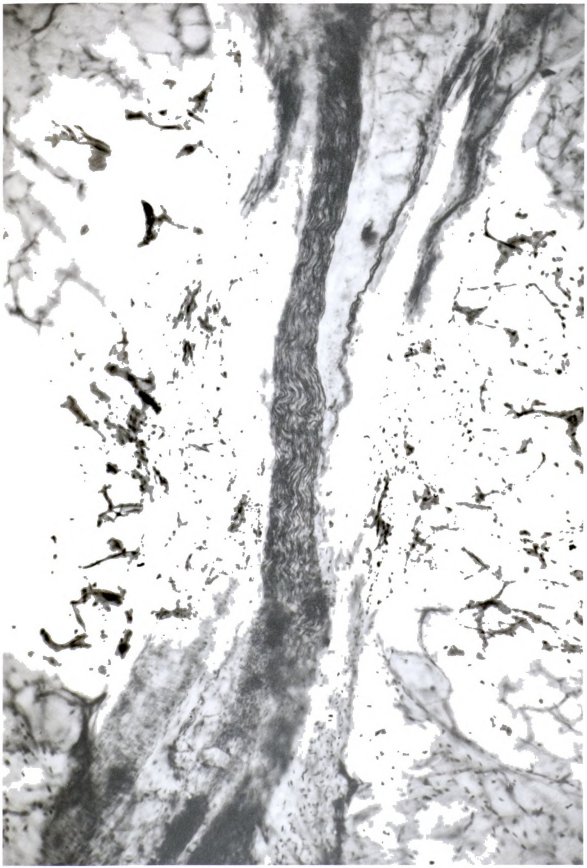
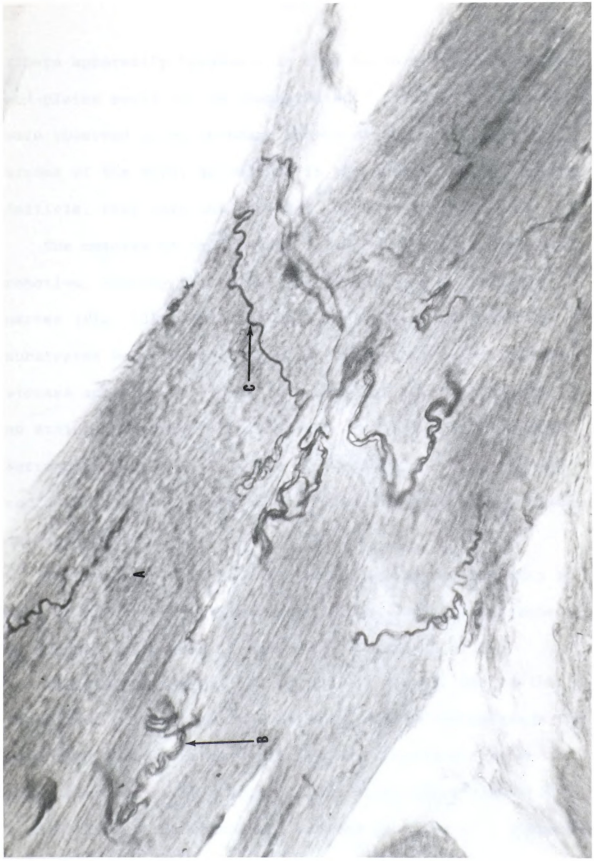


Fig. 12. Photomicrograph of a longitudinal section of muscular tissue present in skin taken from the dorsal feather tract of a chicken showing the involvement of the nervous system. Chloral hydrate silver method. X 430.

A. Smooth muscle

B. Small nerve bundle

C. Individual nerve fiber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fibers apparently terminate in free nerve endings, as motor end-plates could not be demonstrated. Although the nerves were observed to be abundant in the connective tissue stroma of the skin, as well as in the muscles of the feather follicle, they were not observed in the follicular wall.

The muscles of the feather follicle were cholinesterase reactive, thereby indicating the presence of cholinergic nerves (Fig. 13). Both the acetyl and butyrylthiocholine substrates were successful in the demonstration of cholinesterase activity. When these substrates were eliminsted, no staining reaction was observed. A nerve plexus as seen surrounding the hair follicle by Montagna and Ellis (1957) could not be shown around the feather follicle by the histochemical method.

The remainder of the microscopic anatomy of the skin is made up of the connective tissue stroma with an abundance of adipose tissue and minute blood vessels (Fig. 6).

Other observations.--As previously stated, one of the objectives of the histological study was to characterize the essential changes involved in feather loosening. In an attempt to secure this information, skin tissue was first studied from birds which were scalded in water at a temperature of 140° F for 1 1/2 minutes.

Fig. 13. Photomicrograph of a typical cholinesterase reaction of muscle present in skin taken from the dorsal feather tract of a chicken. Acetylthiocholinesterase reaction. X 100.

Observation of the scalded skin tissue in which the feathers were removed immediately before processing, reveals that much distortion of the tissue occurs during the process of scalding (Fig. 14). This, in effect, made any detailed histological analysis impossible, although it was observed that the feather separated from its follicle at the keratinized layer of epithelium. It is also evident from Fig. 14 that numerous contraction elements are present in the muscle of the feather follicle. This finding is quite interesting and suggests that muscle relaxation may not be essential for feather loosening as the present theory indicates. Sections of skin tissue taken from birds which were scalded, but the feathers left intact, were also distorted and incoherent.

In a further attempt to observe the histological changes occurring in feather loosening, skin tissue from birds under the influence of sodium pentobarbital was studied. Examination of skin tissues taken from birds in the anesthetized state revealed no apparent differences from that taken from unanesthetized birds. Thus, this suggested that skin from the anesthetized bird underwent changes which allowed the feathers to return to their tightened state before fixation

Fig. 14. Photomicrograph of a longitudinal section of skin taken from the dorsal feather tract of a chicken following scalding. Section stained with hematoxylin-Shorr S_3 stain. X 35.

A,B,C. Feather follicles

D. Muscle


A
B
C
D

could occur. Consequently, a study was undertaken to determine if similar changes occurred in skin when it was removed from an anesthetized bird.

Two White Leghorn hens were anesthetized with sodium pentobarbital. Immediately thereafter, two sections of skin, each approximately 2 X 2 inches, were taken from the back region of each bird. Feather pulling force was then measured on each of these tissues at the following times: immediately, 15, 30 and 60 minutes after the skin was removed from the bird. For a control feather pulling force, data were obtained before the birds were anesthetized.

Table 1. Feather pulling force of feathers pulled from excised skin of anesthetized White Leghorn hens[1]

Mean feather pulling force (gms)				
Before	Immed. after	15 min. after	30 min. after	60 min. after
608	40	302	453	484

[1]"Before" feather pulling force obtained on birds before anesthetization. All other values obtained from excised skin.

As can be seen from the data presented in Table 1, the mean feather pulling force of feathers pulled from the skin immediately after its removal from the body was markedly

decreased from the value obtained before the birds were anesthetized. However, this effect failed to persist. Within 15 minutes after the skin was removed from the birds, the feather pulling force returned to 50 percent of the value obtained for the birds in the conscious state. As is evident from the data, the feather pulling force continued to increase from the value obtained immediately after the skin was removed from the birds. At the 60 minute observation, the feather pulling returned to 80 percent of the control value. Thus, it is obvious that changes were occurring in the excised skin which allowed the feathers to return to their tightened state.

Histological observations of molting feathers revealed no information on the mechanism of feather release. The most obvious difference between molting and mature feathers was the presence of the pulp, together with the various layers of the developing feather, in the follicle containing the immature feather.

The previous study indicated that skin underwent some change beginning immediately after it was removed from the body. Thus, in a further attempt to observe the feather and its immediate surroundings in the relaxed state, the freeze-

drying and frozen-section techniques were employed. In addition, celloidin sections were studied.

Freeze-drying.--Much difficulty was experienced in attempting to produce microscopic sections of feather follicles processed by this method. This was due to the brittleness of the sheath of the feather proper. In addition, considerable difficulty was encountered with the process of paraffin infiltration. Extended time periods of infiltration were necessary when the autotechnicon paraffin baths were used. However, even when the tissue was infiltrated up to 15 hours, the infiltration still was far from being satisfactory. Vacuum infiltration for 60 minutes was comparable to the extended time period of infiltration in the autotechnicon. Infiltration by vacuum for periods less than 60 minutes was incomplete.

Frozen sections.--Considerable difficulty was experienced in sectioning unfixed, fresh skin tissue containing mature feather follicles. Again the difficulty appeared to be due to the brittleness of the feather sheath. Coherent sections were virtually impossible. When the tissue was fixed in 10 percent formalin, the sections were markedly improved.

Due to the fixation time involved, the feather could not be observed in the relaxed state. The gelatin and agar embedding technique (Lillie, 1954) resulted in somewhat more coherent sections. However, when the medium was dissolved for the purposes of staining, the sections failed to remain intact.

Celloidin.--The celloidin sections obtained did not appear any more coherent than the paraffin sections routinely processed. In addition, it was found that the celloidin sections stained very intense, more so than is usually desired. Due to these results, together with the lengthly procedure involved, this method was only sparingly used.

B. Physiological Studies

EXPERIMENT I

Effect of piercing various parts of the brain on feather pulling force.

No doubt there are many factors which may be involved in relaxation of the muscles of the feather follicle and, consequently, feather release. One such factor which is known to influence feather release is the nervous system, as indicated by feather loosening when a successful brain stick is performed. What general part of the brain is involved, however, is controversial. Therefore, it was the purpose of this experiment to determine the general part of the brain involved in the release of the feather from its follicle.

A total of 25 birds were used in this study. The majority of these birds were either White Leghorn or White Rock hens, although some White Rock and White Leghorn cockerels were used. After the normal feather pulling force was obtained, each bird was hung by its feet from a shackle and the external jugular vein severed. Immediately thereafter, the brain was pierced with a sharp, narrow-bladed knife inserted through the roof of the mouth. Following feather pulling

observations, the brain was dissected out of its cavity, and gross studies of the course of the knife made. The course of the knife was then plotted on a diagram of the brain for later study. The immediate area of the brain through which the knife passed was also processed for histological studies. For the histological studies, the standard method of fixation, dehydration and infiltration was used. The sections were stained with hematoxylin and eosin.

From 19 of the 25 birds studied, data on feather pulling forces were obtained immediately after the stick was made. The remaining 6 birds began struggling immediately after their brains were pierced, thereby making it impossible to obtain data until the struggling ceased. The individual data are reported in Table 2.

A study of these data indicated that only two of the 13 birds in which the stick was made into the optic lobe or cerebrum showed a significant reduction ($P < 0.05$) in feather pulling force when measured within 2 minutes after the stick was performed. In contrast, 11 of the 12 birds whose brains were pierced in the area of the diencephalon, cerebellum and medulla showed a marked reduction in feather pulling force. Neither the medulla nor the cerebellum were

pierced alone, consequently, it was impossible to accurately pinpoint the part of the brain area involved. There is some indication that the cerebellum may be the essential part. This is evident from the fact that all birds in which the stick was made in the area of the diencephalon-cerebellum showed a marked reduction in feather pulling force. Birds in which only the area of the diencephalon was pierced showed only a small reduction in feather pulling force, thus indicating that this general area has little influence on feather loosening. Further evidence suggesting that the cerebellum may be involved is found in the study of the bird pierced in the optic lobe-cerebellum. It is obvious that feather pulling force was markedly decreased in this bird. On the other hand, birds pierced only in the optic lobe showed little change in feather pulling force. When the medulla and cerebellum were pierced simultaneously, feather pulling force was markedly reduced, thus again suggesting that the cerebellum may be involved.

The increase in feather pulling force between the two observations following brain sticking was quite obvious. This increase was greatest for those birds which showed the greater reduction in feather pulling force and may be

attributed to the effects of struggling. Also attributed to the effect of struggling was the large variation between feather pulling forces of birds that were pierced in the same general area.

Histologically, the course of the knife could be seen very clearly. The most obvious indication that the knife entered a given area was the infiltration of blood. It was also noted that tissue separation occurred. Because the knife generally entered more than one part of the brain, it was again impossible for any conclusions to be made regarding the essential part involved in feather release.

Table 2. Effect of piercing various parts of the brain on feather pulling force of feathers pulled from the dorsal feather tract of chickens

Part of brain pierced		Mean feather pulling force ± standard error (gms)					
Ventral	Dorsal	Before stick	0-2 min. after	Percent change	After 2 min.	Percent change	Percent change[1]
Diencephalon	Anterior to cerebellum	780 ± 45	713 ± 106	- 8.6	818 ± 29	+ 4.9	+ 14.7
"	"	461 ± 9	-	-	363 ± 33**	-21.3	-
"	"	557 ± 35	359 ± 31**	-35.5	463 ± 43	-16.9	+ 28.9
Diencephalon	Cerebellum	737 ± 62	336 ± 26**	-50.3	566 ± 32**	-23.2	+ 68.5**
"	"	450 ± 33	218 ± 20**	-51.6	368 ± 32	-18.2	+ 68.8**
"	"	648 ± 43	151 ± 20**	-76.7	313 ± 28**	-51.7	+107.3**
"	"	605 ± 44	-	-	352 ± 26**	-41.8	-
"	"	496 ± 17	376 ± 47*	-24.2	426 ± 33	-14.1	+ 13.3
"	"	390 ± 27	113 ± 0**	-71.0	323 ± 23	-17.2	+185.8**
Diencephalon	Cerebrum	520 ± 31	571 ± 42	+ 9.8	-	-	-
Medulla	Cerebellum	458 ± 26	-	-	305 ± 16**	-33.4	-
"	"	638 ± 36	-	-	241 ± 31**	-62.2	-

* Significantly different ($P < 0.05$) from feather pulling force before stick.
** Significantly different ($P < 0.01$) from feather pulling force before stick.
1. Represents the percent change in feather pulling force between the two values obtained after brain sticking.

Table 2. (Cont'd). Effect of piercing various parts of the brain on feather pulling force of feathers pulled from the dorsal feather tract of chickens

Part of brain pierced		Mean feather pulling force ± standard error (gms)					
Ventral	Dorsal	Before stick	0-2 min. after	Percent change	After 2 min.	Percent change	Percent change[1]
Optic lobe	Cerebellum	567 ± 18	192 ± 21**	-66.1	374 ± 61*	-34.0	+94.8*
Optic lobe	Cerebrum	586 ± 32	447 ± 40*	-23.7	657 ± 73	+12.1	+46.9
"	"	632 ± 39	587 ± 47	-07.1	874 ± 61**	+38.3	+50.4*
"	"	584 ± 29	545 ± 31	-06.7	713 ± 77	+22.1	+30.8
"	"	676 ± 26	527 ± 30*	-22.0	590 ± 25*	-12.7	+11.9
"	"	492 ± 32	505 ± 12	+ 2.6	-	-	-
Optic lobe	Optic lobe	575 ± 22	-	-	579 ± 50	+ 0.7	-
"	"	606 ± 22	510 ± 57	-15.8	514 ± 17**	-15.2	+ 0.8
"	"	564 ± 31	503 ± 32	-10.8	601 ± 43	+ 6.6	+19.5
"	"	383 ± 36	-	-	361 ± 37	- 5.7	-
Cerebrum	Cerebrum	567 ± 30	463 ± 50	-18.3	438 ± 29**	-22.8	- 5.4
"	"	443 ± 24	442 ± 34	- 0.2	-	-	-
"	"	652 ± 33	581 ± 48	-10.9	-	-	-

* Significantly different ($P < 0.05$) from feather pulling force before stick.
** Significantly different ($P < 0.01$) from feather pulling force before stick.
1. Represents the percent change in feather pulling force between the two values obtained after brain sticking.

EXPERIMENT II

Effect of neuromimetic, anesthetic and tranquilizing drugs and drugs which depress the autonomic nervous system on feather pulling force.

The objective of this experiment was to study the influence of the nervous system on feather release by using four different classes of drugs, namely, anesthetics, tranquilizers, neuromimetics, and drugs which depress the autonomic nervous system. In addition, it was thought that by using drugs which act on the central nervous system, additional information could be obtained regarding the part of the brain involved in feather release.

Sixty-seven White Leghorn hens and 15 White Rock broilers were used in this experiment. Of these, 29 were given neuromimetic drugs, 12 were administered tranquilizing drugs, 30 were given anesthetics, and the remaining 11 were given blocking agents of the autonomic nervous system. The 15 White Rock broilers were used to study the effects of sodium pentobarbital and constituted a portion of the group of 30 birds used to study the effects of anesthetics. A few of the same birds were used in experiments involving different neuromimetic drugs; however, a minimum time interval of at least 5 days elapsed before any particular bird was re-used.

The neuromimetic drugs selected for use were: acetylcholine, pilocarpine and carbachol (parasympathomimetic); epinephrine and ephedrine (sympathomimetic). Atropine (parasympatholytic) and yohimbine (sympatholytic) were the blocking agents studied. Several anesthetics were also used. These included ethyl ether, chloroform, sodium pentobarbital and procaine. Tranquilizers studied were chlorpromazine (thorazine) and promazine (sparine). The pharmacology of these drugs was described by Drill (1958). All drugs, with the exception of promazine, ether and chloroform, were made up in 0.85 percent physiological saline. Injections were intravenous (i.v.), intramuscular (i.m.) or subcutaneous (s.c.). The site of injection was: brachial vein for intravenous, thigh muscle for intramuscular and the loose skin stretching medially between the thigh and the body for subcutaneous, with the exception of procaine. This drug was injected intradermally in the back region. Inhalation anesthetics (ether and chloroform) were administered by means of a cone. With the exception of epinephrine, procaine, ether and chloroform, all drugs were given on a per unit of body weight basis. Only single injections were made. The amount of the drug given may be seen in the respective tables.

Feather pulling forces were recorded before treatment, immediately after treatment and at various intervals thereafter, depending upon the route of administration of the drug involved. Various clinical symptoms were also observed and were used as an indication of drug efficacy.

The results of the effects of various anesthetics on feather pulling force are presented in Table 3. The data showed a significant reduction ($P < 0.01$) in feather pulling force for all drugs; however, ether appeared to be least effective. Due to the peculiar arrangement of the bird's respiratory system, the volatile anesthetics (ether and chloroform) were difficult to administer. The duration of anesthesia with either drug was short, consequently, the reduction in feather pulling force was of brief duration. In contrast, sodium pentobarbital was very effective in producing anesthesia. Since the route of administration was intravenous, anesthesia was produced immediately and the feather pulling force was decreased by 77 percent of the value found in the conscious bird. A similar effect was observed when procaine, a local anesthetic, was administered. This drug reduced feather pulling force by 80 percent in the area immediately around the site of injection.

Reduced feather pulling force induced with the general anesthetics was not surprising in view of the fact that the center for feather loosening undoubtedly lies within some portion of the central nervous system. The considerable differences between the results obtained with ether and chloroform are difficult to explain, especially since both of these drugs are very similar in the mechanism of action (Beckman, 1958). One possible explanation may be found in the fact that chloroform as an anesthetic is much more potent than ether. Thus, the birds which received chloroform may have been anesthetized to a greater depth than those receiving ether. It was observed that birds anesthetized with chloroform remained under anesthetization considerably longer than birds anesthetized with ether.

The effects of the tranquilizing agents, chlorpromazine and promazine, upon feather loosening are indicated in Table 4. It is obvious that both drugs resulted in a marked reduction in feather pulling force. The greatest reduction in feather pulling force was noted immediately after injection of the higher level of promazine. On the other hand, the greatest decrease caused by chlorpromazine did not occur until the 10 minute observation. Moreover, the duration of

the effect of chlorpromazine appeared to be shorter than that observed for promazine. Although no statistical analysis was applied to test the effectiveness of the differences in dosages, it is apparent that in both cases the higher levels were more effective than the lower levels.

From the studies with procaine, it becomes evident that the peripheral nervous system may also be involved in feather release. The action of this drug is local. The drug penetrates through the tissue down to the nerve endings, paralyzing the function of the nerve. Nerve impulses are, therefore, blocked from this area. As a result, relaxation of the muscles of the feather follicle occurs (feather loosening).

The results from experiments involving neuromimetic drugs are summarized in Table 5. The data show that there were no significant differences in feather pulling force as a result of these drugs. Even with large doses, which produced marked distress among most birds, feather pulling force remained approximately the same as the value observed in the normal state.

The data showing the effects of sympatholytic and parasympatholytic drugs on feather pulling force are shown in

Table 6. As is evident from the data presented, both atropine and yohimbine significantly decreased ($P < 0.01$) the feather pulling force from the value observed before drug administration. Atropine was effective immediately, even when injected intramuscularly. On the other hand, the subcutaneous injection of yohimbine required approximately 30 minutes to produce its effect. Thus, these data suggest that the muscles of the feather follicle are innervated by both sympathetic and parasympathetic fibers and that either or both may be involved in feather loosening.

Table 3. Effect of anesthetics on feather pulling force of feathers pulled from the dorsal feather tract of chickens

Anesthetic	No. of birds	Route of administration	Mean feather pulling force ± standard error (gms)		
			Before	Immed. after	Percent change
Ether	8	Inhalation	650 ± 34	428 ± 30**	-34.2
Chloroform	5	Inhalation	547 ± 35	179 ± 25**	-67.3
Sodium pentobarbital[1]	15	i.v.	398 ± 22	91 ± 12**	-77.2
Procaine[2]	2	s.c.	748 ± 28	153 ± 16**	-79.6

** Significantly different ($P < 0.01$) from feather pulling force before drug administration.

1. Dosage - 30 mg/kg body weight.
2. Total dose injected - 0.5 cc of a 2 percent solution.

Table 4. Effect of a single intravenous injection of chlorpromazine or promazine on feather pulling force of feathers pulled from the dorsal feather tract of chickens

Drug and dosage (mg/kg)	No. of birds	Mean feather pulling force ± standard error (gms)						
		Before	Immed. after	Percent change	10 min. after	Percent change	30 min. after	Percent change
Chlor-promazine								
1.0 - 2.5	4	570 ± 21	407 ± 30**	-26.6	325 ± 25**	-43.0	403 ± 46*	-29.3
3.0 - 4.0	3	587 ± 21	332 ± 53*	-43.4	276 ± 64**	-53.0	332 ± 62*	-43.4
Promazine								
1.5 - 2.0	2	743 ± 40	480 ± 74	-35.4	450 ± 63	-39.4	494 ± 2*	-33.5
2.5 - 3.5	3	714 ± 47	148 ± 36**	-79.3	226 ± 73**	-68.4	332 ± 3**	-53.5

* Significantly different ($P < 0.05$) from feather pulling force before drug administration.

** Significantly different ($P < 0.01$) from feather pulling force before drug administration.

Table 5. Effect of neuromimetic drugs on feather pulling force of feathers pulled from the dorsal feather tract of chickens

Drug and dosage (mg/kg)	No. of birds	Route of administration	Mean feather pulling force ± standard error (gms)						
			Before	Immed. after	Percent change	10 min. after	Percent change	30 min. after	Percent change
Acetylcholine 10-30	6	s.c.	542 ± 35	-	-	551 ± 43	+1.7	542 ± 34	0.0
Carbachol 0.5-1.3	4	s.c.	620 ± 34	639 ± 44	+ 3.1	693 ± 43	+11.8	-	-
Pilocarpine 10-15	3	i.v.	710 ± 46	748 ± 44	+ 5.4	775 ± 40	+9.2	-	-
200	2	i.m.	639 ± 21	587 ± 9	- 8.1	593 ± 26	-7.2	605 ± 19	-5.3
Epinephrine[1] 0.5-0.75	6	i.v.	617 ± 46	596 ± 39	- 3.4	631 ± 29	+2.3	-	-
Ephedrine 20-25	3	i.v.	557 ± 41	619 ± 46	+11.1	540 ± 41	-3.1	-	-
200-300	2	i.m.	653 ± 90	-	-	632 ± 115	-3.2	584 ± 36	-10.6
200-300	3	s.c.	638 ± 15	-	-	741 ± 54	+16.1	684 ± 64	+7.2

1. Total dose injected (cc) 1:1000 dilution.

Table 6. Effect of drugs which depress the activity of the autonomic nerves on feather pulling force of feathers pulled from the dorsal feather tract of chickens

Drug and dosage (mg/kg)	No. of birds	Route of administration	Mean feather pulling force ± standard error (gms)						
			Before	Immed. after	Percent change	10 min. after	Percent change	30 min. after	Percent change
Atropine 20	4	i.v.	678 ± 40	129 ± 2**	-81.0	169 ± 15**	-75.1	-	-
200-250	3	i.m.	696 ± 38	195 ± 62**	-72.0	187 ± 22**	-73.1	-	-
Yohimbine 33.6	4	s.c.	741 ± 22	-	-	715 ± 36	- 3.5	228 ± 32**	-69.2

** Significantly different ($P < 0.01$) from feather pulling force before drug administration.

EXPERIMENT III

Effect of spinal transection on feather pulling force.

The results from the previous experiments indicated that the nervous system was involved in the release of the feather from its follicle. Moreover, it was apparent that a depression of the nervous system was essential in this phenomenon. Since spinal transection may be regarded as suppressing the nervous activity, it seems conceivable that feather loosening might occur upon severing of the spinal cord. Therefore, the present experiment was undertaken to determine the effect of spinal transection on feather release.

General.--Spinal transections were performed in the thoracic or lumbar-sacral region of birds which were lightly anesthetized with sodium pentobarbital. Following anesthetization, the muscles over the spinal column were separated and the spinous process of the vertebra at the selected site was clipped away. A bone drill was then used to reach the cord. The cord was severed and picked out in small pieces by a small forceps. The forceps were then directed caudally and manipulated so as to disorganize the cord. The level and completeness of the transection were confirmed by autopsy.

Feather pulling forces were obtained by the routine procedure: however, in this experiment feathers were pulled from three feather tracts, namely, dorsal, femoral and pectoral. With respect to the dorsal tract, data were obtained from two different areas. One area was anterior to the level of the spinal transection, the other, posterior to the level of the spinal transection. The purpose of obtaining data anterior and posterior to the level of spinal transection was two-fold. First, the anterior area could serve as a control for the posterior area at any particular time throughout the experiment. Secondly, the anterior area could serve as a control against the effects of sodium pentobarbital which is known to reduce the feather pulling force.

Feathers were pulled before anesthetization and immediately, 25 and 60 minutes following spinal transection. Following the last feather pull, nine of the birds were sacrificed in order to determine whether feather relaxation observed in the live birds could be carried over into the post-mortem state.

Thoracic spinal transection.--Six White Leghorn hens were used in this study. Two birds were subjected to spinal

transection at the level of the fourth thoracic vertebra (T-4), one bird at the fifth thoracic vertebra (T-5), and two birds at the sixth thoracic vertebra (T-6). In addition, one other bird was subjected to a spinal transection at the level of the third thoracic vertebra (T-3); however, feathers from the pectoral tract were not pulled, thus there was only a total of five birds studied with respect to this feather tract.

The results from the effects of severing the spinal cord in the thoracic region on feather pulling force may be seen in Table 7. The data revealed that a significant reduction in feather pulling force occurred in the dorsal feather tract when the feathers were pulled posterior to the level of the transection at each observation. In contrast, feathers pulled anterior to the transection showed an increased feather pulling force. This increase was significant ($P < 0.01$) at the 25 minute observation and may be attributed, in part, to the fact that it became necessary to pull feathers anterior to the level at which they were pulled in the normal state. This, of course, was necessitated when the level of the transection was performed at the anterior part of the back (thoracic vertebra). When values anterior and

posterior to the transection were compared, it was noted that the feather pulling force posterior to the transection was reduced by approximately the same value as it was reduced from the feather pulling force in the normal state. Thus it is obvious that the effects observed are those resulting from the spinal transection and not sodium pentobarbital. This fact is further substantiated by the fact that an increase in feather pulling force occurred anterior to the level of spinal transection.

It can also be readily noted with respect to the femoral feather tract that a 74 percent reduction ($P < 0.01$) occurred in the force required to pull an individual feather from its follicle immediately after the spinal transection was performed. A similar effect was observable throughout the experimental period.

The effect of thoracic transection on feather pulling forces of feathers pulled from the pectoral tract is not as clear as it is on the dorsal and femoral tract. As can be seen from the data presented in Table 7, birds which were subjected to spinal transection at the level of the fourth or fifth thoracic vertebra showed a significant decrease in feather pulling force whereas those sectioned at the level

of the sixth thoracic vertebra showed essentially no change. The considerable differences in feather pulling force between birds subjected to spinal transection at the level of the fourth or fifth thoracic vertebra may be attributed to the fact that nerves arising from the spinal cord branch considerably as they course out to the periphery of the body. As a result, they may innervate a large number of muscles. Apparently, all of the feathers pulled from birds subjected to spinal transections at the level of the fourth thoracic vertebra were associated with muscles innervated by nerves arising from the spinal cord posterior to the level of transection. Thus, when the transection was performed, these muscles lost their direct connection with the brain. Consequently, the muscles relaxed and a reduction in feather pulling force occurred. On the other hand, feathers pulled from the pectoral tract of birds subjected to spinal transections at the level of the fifth thoracic vertebra have muscles innerved by nerves arising from the spinal cord anterior to the level of the fifth thoracic vertebra. Since these nerves branch considerably, it is suggested that some of the feathers pulled from these birds were associated with muscles which were in direct connection

with the brain, whereas other feathers may have been associated with muscles in which this connection was severed. Thus it may easily be seen that these results were subjected to considerable variation, a fact that is indicative of the large standard error which is reported. Although the numbers are small, these data suggest that the muscles of the feather follicles in the pectoral tract may be innervated by nerves arising from the spinal cord anterior to the level of the sixth thoracic vertebra.

Lumbar-sacral spinal transection.--Five White Leghorn hens were used in this study. Spinal transections were performed at the following levels: first (L-1), sixty (L-6), eighth (L-8), twelfth (L-12), or thirteenth (L-13) lumbar-sacral vertebra. Feather pulling force was not obtained from the pectoral feather tract of the bird subjected to the transection at the level of the first lumbar-sacral vertebra. Consequently, the results are reported as the mean value for five birds on the dorsal feather tract and four birds on the pectoral feather tract (Table 8). With respect to the femoral feather tract, individual data are presented (Table 9).

As can be seen from the data presented in Table 8, a

marked decrease in feather pulling force occurred only in the dorsal feather tract, posterior to the level of the spinal transection. Feather pulling force of feathers pulled from the pectoral feather tract was significantly decreased; however, it is obvious, as indicated by the percent decrease, that this reduction was minor. Thus, these data further support the earlier statement that in order to loosen feathers in the pectoral feather tract, the cord should be severed anterior to the level of the sixth thoracic vertebra.

The results from the effect of severing the spinal cord in the lumbar-sacral region on feather pulling force of feathers pulled from the femoral tract may be seen in Table 9. A significant reduction in feather pulling force was noted for each of the levels immediately after the spinal transections were performed. Similar results were noted at the 25-minute observation. At the 60-minute observation, however, the results became complicated. It is obvious that only the bird in which the spinal transection was performed at the level of the first lumbar-sacral vertebra continued to show a consistent decrease in feather pulling force. The feather pulling force in all other birds, and

especially in the bird in which the transection was at the thirteenth lumbar-sacral vertebra, increased considerably. Although there appears to be a definite trend for the feather pulling force at 60 minutes to increase above that observed at 25 minutes, the values reported are still significantly different ($P < 0.01$) from those observed in the normal state with the exception of the bird in which the cord was severed at the thirteenth lumbar-sacral vertebra. Why this increase in feather pulling force occurred is difficult to explain. One explanation may be simply that muscle tone, which undoubtedly is diminished following spinal transection, may have been regained.

Carry-over effect.--Although the main objective of this experiment was to contribute to the fundamental understanding of how the nervous system may influence feather retention and release, it was nevertheless important to obtain information on the question: "Can feather relaxation in the live bird be carried over into the post-mortem state?"

For this study nine White Leghorn hens which were subjected to spinal transection in the previous studies were used. Two methods of killing were studied, namely, a lethal dose of sodium pentobarbital and bleeding. In the case of

sodium pentobarbital, feathers were pulled from the dorsal feather tract, both anterior and posterior to the spinal transection, at the following times: before death, immediately after death, and approximately 5 minutes after death. On the other hand, for birds killed by bleeding, feathers were pulled only posterior to the spinal transection before death and after struggling.

It is obvious from the data presented in Table 10 that with both methods of killing the feather pulling force posterior to the transection increased very markedly after death. In the case of sodium pentobarbital, this increase was 94 percent of the value observed immediately before drug administration, whereas in the case of bleeding this increase was 160 percent. Thus, these data are in agreement with those of Klose et al. (1962).

Table 7. Effect of spinal transection at the thoracic level on feather pulling force of feathers pulled from the dorsal, femoral and pectoral feather tracts of White Leghorn hens

Feather tract	No. of birds	Mean feather pulling force ± standard error (gms)						
		Before	Immed. after	Percent change	25 min. after	Percent change	60 min. after	Per cent change
Dorsal:								
Anterior	6	692 ± 16	672 ± 55	- 2.9	809 ± 32**	+16.9	759 ± 51	+ 9.7
Posterior	6	735 ± 40	183 ± 17**	-75.5	189 ± 27**	-74.3	238 ± 27**	-67.6
			- 72.8[1]		- 76.6[1]		- 68.6[1]	
Femoral	6	752 ± 69	198 ± 43**	-73.6	261 ± 15**	-65.3	246 ± 42**	-67.3
Pectoral:								
T-4	2	896 ± 22	323 ± 44**	-63.9	352 ± 98*	-60.7	362 ± 14**	-59.6
T-5	1	968 ± 19	480 ± 49**	-50.4	830 ± 72	-14.3	598 ± 35**	-38.2
T-6	2	900 ± 51	663 ± 103	-26.3	740 ± 48	-17.8	904 ± 53	+ 0.4

* Significantly different ($P < 0.05$) from feather pulling force before spinal transection.

** Significantly different ($P < 0.01$) from feather pulling force before spinal transection.

1. Indicates the percent change in feather pulling force figured from the value obtained anterior to the level of transection.

Table 8. Effect of spinal transection at the lumbar-sacral level on feather pulling force of feathers pulled from the dorsal and pectoral feather tracts of White Leghorn hens

Feather tract	No. of birds	Mean feather pulling force ± standard error (gms)						
		Before	Immed. after	Percent change	25 min. after	Percent change	60 min. after	Per cent change
Dorsal:								
Anterior	5	742 ± 36	572 ± 70	-22.9	657 ± 76	-11.5	695 ± 54	- 6.3
Posterior	5	783 ± 31	205 ± 34**	-73.8	254 ± 34**	-67.6	288 ± 47**	-63.2
			- 64.2[1]		- 61.3[1]		- 58.6[1]	
Pectoral	4	906 ± 18	700 ± 81	-22.7	691 ± 98*	-23.7	730 ± 19**	-19.4

* Significantly different ($P < 0.05$) from feather pulling force before spinal transection.

** Significantly different ($P < 0.01$) from feather pulling force before spinal transection.

1. Indicates the percent change in feather pulling force figured from the value obtained anterior to the level of transection.

Table 9. Effect of spinal transection at the lumbar-sacral level on feather pulling force of feathers pulled from the femoral feather tract of White Leghorn hens

Level of transection	Mean feather pulling force ± standard error (gms)						
	Before	Immed. after	Percent change	25 min. after	Percent change	60 min. after	Percent change
L-1	628 ± 22	248 ± 19**	-60.5	270 ± 37**	-57.0	258 ± 39**	-58.9
L-6	879 ± 18	301 ± 40**	-65.8	374 ± 23**	-57.4	629 ± 68**	-28.4
L-8	703 ± 29	227 ± 31**	-67.7	255 ± 13**	-63.7	431 ± 52**	-38.7
L-12	598 ± 22	258 ± 22**	-56.9	316 ± 37**	-47.1	394 ± 37**	-34.1
L-13	845 ± 29	641 ± 82*	-24.1	425 ± 37**	-49.7	788 ± 66	- 6.7

* Significantly different ($P < 0.05$) from feather pulling force before spinal transection.

** Significantly different ($P < 0.01$) from feather pulling force before spinal transection.

Table 10. Feather pulling force of feathers pulled from the dorsal feather tract of White Leghorn hens before and after death by bleeding or sodium pentobarbital

Method of killing	Relationship to spinal transection	No. of birds	Mean feather pulling force ± standard error (gms)				
			Immed. before	Immed. after	Percent change	5 min. after	Percent change
Sodium pentobarbital	Anterior	3	641 ± 24	204 ± 17**	-68.2	669 ± 34	+ 4.3
	Posterior	3	262 ± 30	165 ± 15*	-37.0	509 ± 64*	+94.3
Bleeding	Posterior	6	272 ± 28	707 ± 43**	+159.9	-	-

* Significantly different ($P < 0.05$) from feather pulling force before spinal transection.

** Significantly different ($P < 0.01$) from feather pulling force before spinal transection.

EXPERIMENT IV

Effect of skeletal muscles on feather pulling force.

According to Langley (1904), the muscles which are responsible for feather movement are of the involuntary type. Furthermore, he indicated that only intra-dermal muscles are involved. Since it is well known that the skin adheres to a large proportion of the skeletal muscular system, it is conceivable that muscles of the skeletal system could possibly influence feather release. This experiment was undertaken to investigate the effect of skeletal muscle on feather pulling force.

Curare, a drug which paralyzes skeletal muscle but not smooth muscle, was used. The drug was injected intravenously into each of eight White Leghorn hens. Five birds received the drug at the dosage of 0.0267 mg/kg of body weight; the remaining three birds were administered the drug at the dosage of 0.05 mg/kg of body weight. Artificial respiration was provided for birds administered the drug at the higher level according to the method described by Burger and Lorenz (1960). Data on feather pulling force were obtained on feathers pulled from the dorsal feather tract in the usual manner. Data were obtained before injection, immediately

after injection, and 5 minutes after the drug was administered. The results of this study are presented in Table 11.

Table 11. Effect of a single intravenous injection of curare[1] on feather pulling force of feathers pulled from the dorsal feather tract of White Leghorn hens

No. of birds	Dosage (mg/kg)	Mean feather pulling force ± standard error (gms) Before admin.	Immed. after	Per cent change	5 min. after	Per cent change
5	0.0267	622 ± 55	602 ± 57	-0.03	618 ± 43	-0.01
3	0.05	584 ± 12	567 ± 28	-2.9	622 ± 15	+6.5

1. Tubocurarine chloride.

From the data presented, it is apparent that curare had very little effect on feather pulling force. No significant differences were found between any of the observations when tested against the value observed before drug administration. Paralysis of the skeletal muscles of the leg was produced immediately after the injection of the drug at both levels. The higher level also paralyzed the respiratory muscles. At the five minute observation, the degree of paralysis caused by the lower level of the drug became variable to the extent

that three of the five birds were capable of body movement. On the higher level all birds were still completely paralyzed at the five minute observation. Thus, it is suggested that the skeletal muscles do not play a role in the release of the feather from its follicle.

GENERAL DISCUSSION

A. Anatomical studies.

The findings obtained in this part of this investigation reveal that the feather follicles of domestic turkeys and fowl are tubular structures, embedded into the dermis layer of skin and connected to one another by a complex system of involuntary muscles. The latter fact was not surprising in view of the evidence presented by Langley (1904) on the muscles which move the feathers. Further confirmation of the observation by Langley (1904) was provided by the fact that these muscles were attached to the feather follicle by elastic tendons. In the present study, the elastic fibers were observed to continue in conjunction with connective tissue around the feather follicle, thus forming the outermost layer of the follicular wall. The fact that the outermost layer of the feather follicle was found to be connective tissue is in disagreement with Lillie (1940) who reported that the feather follicle was enclosed by circular muscle. It should be pointed out, however, that the detailed histological structure of this tissue bears a marked similarity to that of smooth muscle.

In fact, when a general stain such as hematoxylin and eosin was used, it was virtually impossible to differentiate this tissue from the smooth muscle of the feather follicle. Consequently, unless a specific connective tissue stain is used, it is quite probable that these two tissues could be confused. As previously noted, when Masson's connective tissue stain was employed, there was a marked contrast in the staining reaction between muscle and connective tissue. This particular stain colors connective tissue blue and muscle fibers red. In view of the fact that the layer in question stained an intense blue, it was concluded that it consisted of connective tissue. That this layer is connective tissue instead of smooth muscle was also noted by Lucas (1962) and Stettenheim (1962).

The histological findings also clearly demonstrate that the muscles of the feather follicle have a rich supply of nerves. In view of the fact that these nerves are so closely associated with the muscles of the feather follicles, they undoubtedly consist of nerve fibers which are motor in origin and which thereby function in feather movement. As to whether these nerves also consist of fibers which are sensory in origin is not known. It appears, however, that

they do since numerous sensory nerve endings (Vater-Pacini corpuscles) were observed in close association with the feather follicle. This view is substantiated by the fact that nerve fibers of the Vater-Pacini corpuscles run chiefly in the sensory fibers of mixed nerves supplying muscles, tendons and blood vessels (Best and Taylor, 1961). Thus it is conceivable that the nerves observed in the muscles of the feather follicles are mixed nerves, i.e., consisting of both motor and sensory fibers.

From the histological studies, it is difficult to determine whether these nerves represent the sympathetic or parasympathetic division of the autonomic nervous system. On the evidence of Langley (1904), however, it seems more than likely that at least the sympathetic system is present. This would correspond with the innervation of the arrectores pilorum of the mammalian hair follicle. Some evidence that the parasympathetic system may be present stems from the present histochemical studies. Although these studies were somewhat limited, the presence of cholinesterase activity was quite obvious. It is also quite possible that both systems may be present. This is indicated by the physiological studies of this investigation which will be discussed later.

The fact that these nerves were not observed to terminate in motor end-plates is consistent with the findings of Winkelmann (1960). Also in agreement with Winkelmann (1960) was the observation of a network of nerve endings. According to Winkelmann (1960) this network is not a motor-end-plate in the sense that they appear in striated muscles.

The demonstration of the Vater-Pacini corpuscles was not surprising in view of the fact that they were long known to exist in the skin of birds (see Winkelmann and Myers, 1961 for review). These corpuscles, previously regarded as the Herbst corpuscles, are anatomically similar to those of the mammal. On the other hand, they display chemical properties which separate them very distinctly from those of mammals (Winkelmann, 1960). In the mammal these corpuscles are considered as pressure receptors, while in the bird there is some controversy as to their function. They were formerly thought to function as tactile receptors; however, recently they have been considered to be vibration receptors (Marshall, 1961). In view of the fact that they are sensory end-organs it is assumed that they do not function in the release of the feather from its follicle. Although these corpuscles were observed to be in close association with the feather

follicle in the present study, they are also known to be present in the non-feathered skin, beak, tongue and genital regions (Winkelmann and Myers, 1961).

Another histological observation which is worthy of consideration is that of the follicular cavity. From the semi-diagram presented by Lillie (1940) it can be seen that this cavity exists between the sheath of the feather and the epidermal lining of the follicular wall. In the present study, this cavity was observed in many sections. On the other hand, there were also many sections in which this feature was not observed. It therefore becomes interesting to raise the question as to whether this is a normal phenomenon or an artifact due to histological processing. The author accepts the latter view since tiny strands of keratinized epithelium (microscopic bridges) were seen to connect the two epithelial layers when a cavity was present. It is suggested that these microscopic bridges are formed as the feather itself shrinks away from the keratinized layer of epidermis.

A most interesting finding was that skin removed from an anesthetized bird underwent changes which allowed feathers to return to their tightened state. Just what the nature

of these changes were was not determined. The fact that they occurred, in the absence of innervation, however, strongly implies that the smooth muscles of the feather follicles are involved. In particular, tonus may have been regained. Physiologically, tonus may be of several origins: 1) it can be created by nerve impulses of low frequency; 2) it can be brought about by asynchronous activity in different parts of the muscle; or 3) it can originate within the muscle fiber itself. When the skin is left intact on an anesthetized bird, the degree of tonus is undoubtedly depressed by the action of the anesthetic. When, however, the tissue is removed from the bird, it becomes conceivable that tonus may be regained through either the second or third mechanism listed above. Consequently, feathers become tight in their follicles.

Although lack of skin tissue containing feathers in the loosened state makes it difficult to assess the histological changes involved in feather loosening, it is nevertheless interesting to speculate on how some of the structures observed might function in this phenomenon. As previously stated, the present theory is based on the fact that heat denatures proteins which are present in the muscle of the

feather follicle, thus allowing muscular relaxation and feather loosening. In view of the large number of muscles which were observed to be associated with the feather follicle in this study, as well as their complexity, one must agree with the above theory that the muscles are in some way involved. Other constituents of the feather follicle, however, should not be overlooked. Of particular importance are the elastic fibers which are located in the outermost layer of the follicular wall. These fibers undoubtedly function in feather loosening and tightening, although the exact way in which they function is not known. Since they are continuous with the tendon connecting the muscle to the follicle, it is suggested that they function synonymously with the muscles of the feather follicle in the feather release phenomenon.

As to whether or not the large number of nerves observed in the muscles of the feather follicles are involved in feather release remains obscure. It is well known, however, that the nervous system can in some way cause the release of the feather from its follicle. If one makes the assumption that these nerves are involved in feather release, and in view of the physiological studies of this investigation

there is every reason to do so, one also must make the assumption that they function in this phenomenon indirectly, as in no case were nerves observed surrounding the feather proper. It is suggested that the part played by the nervous system in feather release is simply to stimulate or depress the muscles of the feather follicle which, in turn, play the active role in this phenomenon.

The actual site at which the feather separates from its follicle appears to be at the keratinized layer of epithelium. This is evidenced from the fact that when a feather was plucked from its follicle, the keratinized layer of epithelium remained attached to the follicular wall.

B. Physiological studies.

The significance of the results obtained in this portion of the investigation is principally that of providing basic information through which one might contemplate the physiological mechanisms involved in the release of the feather from its follicle. In general, three observations provided information which demonstrated conclusively that the nervous system was involved in this phenomenon: 1) feathers loosened when certain parts of the brain were pierced, but remained tight when other areas were pierced; 2) various drugs which function by acting on the nervous system resulted in feather loosening; and 3) spinal transection resulted in feather loosening posterior to the level of the transection.

In regard to the first of these observations, it is apparent from the data (Table 2) that a center which influences feather release may exist in the brain. Thus, this finding is in agreement with the long recognized view that feather loosening will occur if the proper part of the brain is pierced (King, 1920; Weaver, 1936; Klose et al., 1962). The general part of the brain which is involved in feather release could not be determined from the data obtained in Experiment I, simply because of the fact that usually more

than one part was involved in the stick. The data (Table 2) would seem to indicate, however, that the cerebellum or medulla is the essential part.

In this connection, it is of interest to reiterate the fact that both the medulla and the cerebellum were reported to contain a center for feather loosening. As previously mentioned, King (1920) reported that the medulla was the essential part, whereas Weaver (1936) was of the opinion that the center was located in the cerebellum. In view of the results obtained in Experiment II with general anesthetics, the reports of both King and Weaver became somewhat questionable. It will be recalled that both of these workers studied the part of the brain involved in feather loosening in birds which are under the influence of anesthesia. In the case of King, ether was used, whereas Weaver employed chloroform. Since in the present experiment both of these drugs reduced feather pulling force from the value observed in the conscious state, it raises the question as to whether the feathers on birds which the above two workers were studying were loosened by the anesthetic or by the destruction of certain brain tissue. It is of interest to note that King (1920) reported that ether had no effect on the muscles of the feather follicle.

Although the data (Table 2) seem to indicate that the general part of the brain involved in feather release is the medulla or the cerebellum, the possibility that the diencephalon may be the essential part should not be overlooked. Evidence to support this contention is contained in the data obtained from the effect of tranquilizing drugs on feather pulling force (Table 4). The fact that chlorpromazine and promazine reduced feather pulling force was not completely surprising since other tranquilizing agents were previously shown to produce this same effect (Sturkie et al., 1958; Klose et al., 1961, 1962; Knapp and Newell, 1961). The exact manner in which these tranquilizing agents cause feather loosening is not known. One explanation may be the fact that the peripheral effect of chlorpromazine and promazine are due to a depression of sympathetic nervous activity (Beckman, 1958). This view is substantiated by the present finding that the sympatholytic agent, yohimbine, decreased feather pulling force (Table 6). Whether chlorpromazine and promazine depress sympathetic nervous activity centrally or peripherally is not known. It is known, however, that the tranquilizing agent, reserpine, decreases sympathetic outflow at the hypothalamic level of the

diencephalon (Beckman, 1958; Drill, 1958). Thus, in view of this fact, it is possible that tranquilizing agents may cause feather loosening by depression of the sympathetic center in the hypothalamic area of the diencephalon. It is conceivable therefore, that during brain-sticking the sympathetic center in the hypothalamic area of the diencephalon may be damaged which, in turn, results in feather loosening. It is interesting to note that during brain-sticking via the roof of the mouth the knife generally passes through this area.

Although the above explanation attributed feather loosening to a decrease in sympathetic nervous activity, it is also apparent from the data presented in Table 6 that suppression of the parasympathetic nervous system will result in a reduced feather pulling force. Hence, this finding is in agreement with those of King (1920) who reported atropine and scopolamine to cause feather loosening. Feather loosening induced with atropine may be attributed to a paralysis of the parasympathetic nerve fibers in the muscles of the feather follicle.

The finding that either yohimbine or atropine resulted in feather loosening suggests that the muscles of the

feather follicles are innervated by sympathetic and parasympathetic fibers of the autonomic nervous system. Moreover, it is apparent that these nerves must be depressed in order for feather loosening to occur, a fact which was also indicated by the data obtained from studies with anesthetics and tranquilizers.

Further evidence that suppression of the nervous system is necessary for feather release is contained in the spinal transection study. It is obvious from the data obtained on the spinal transected birds, that when motor impulses from a higher brain center were interrupted by severing the spinal cord, feather loosening occurred posterior to the level of the transection.

It may be argued, however, that feather loosening observed in spinal transected birds may have been due to spinal shock. It is well known that when the spinal cord is severed, spinal shock occurs. Although no experiment was carried out to study the effects of spinal shock, it was assumed by the author to have an insignificant effect on feather pulling force. The basis of this assumption was two-fold. First, spinal shock may be regarded as a general phenomenon, i.e., it affects the entire body. Thus, if spinal shock affects

feather pulling force, it should do so over the entire body. Since feather pulling force anterior to the level of transection remained approximately the same, as opposed to a marked decrease posterior to the transection, it is taken as evidence that the results observed were not influenced by spinal shock. The second basis for this assumption originates from the following data that were obtained from a bird which was kept four days after the spinal cord was severed.

Table 12. Effect of spinal transection at the thoracic level on feather pulling force of feathers pulled from the dorsal feather tract of a White Leghorn hen

Relationship to spinal transection	Mean feather pulling force (gms)			
	Before	1 hour	2 days	4 days
Anterior	724	868	860	829
Posterior	844	172	218	294

It is obvious from these data that feather pulling force posterior to the transection was markedly reduced from that observed anterior to the transection, thus again suggesting that spinal shock had very little, if any, effect on feather pulling force.

The fact that reduced feather pulling force observed in the live bird could not be carried over into the early post-mortem state when the bird was sacrificed by bleeding is a most interesting finding. No doubt this was due to the effects of struggling. Klose et al. (1962) also reported that feather pulling force increased very rapidly following death by bleeding. It cannot be stated, however, that struggling is the only factor involved in causing feather pulling force to increase after death. It was shown in this study and previously by Klose et al. (1962) that if struggling was minimized by virtue of anesthesia, feather pulling force also increased following death, although at a much slower rate. This again suggests that some change occurs in the skin which allows the feathers to return to their tightened state.

SUMMARY AND CONCLUSIONS

The first part of this investigation was undertaken to characterize the anatomical features of the feather follicle and its immediate surroundings in the tightened and loosened state. Both gross and histological studies were conducted. The macroscopic anatomy was studied in skin taken from the dorsal feather tract of a "cooked" turkey, whereas the microscopic studies were of tissues generally taken from the dorsal feather tract of chickens. From these anatomical studies the following conclusions were drawn:

1. The feather follicle is a tubular structure and is embedded well into the dermis layer of skin.
2. The feather follicle is supplied with a complex system of smooth muscles which arise within the dermis. These muscles attach to the feather follicle by tendons which, in turn, are replete with elastic fibers.
3. The outermost layer of the follicular wall is composed of collagen connective tissues.
4. A rich supply of nerves was demonstrated in the connective tissue stroma of the skin and muscles of the feather follicles. The nerves apparently terminate in free nerve

endings. Histochemically, cholinergic nerves were shown to be present.

5. Numerous Vater-Pacini corpuscles were present in the feather area of the skin. They were observed to be in close association with the feather follicle.
6. Numerous contraction bands were observed in the muscles surrounding the feather follicle. They were observed in tissue taken from birds in the anesthetized, unanesthetized and scalded state.
7. No appreciable histological differences were observed between feather follicles present in skin taken from birds in the anesthetized and unanesthetized state. This undoubtedly was due to the fact that the feather follicle returned to its normal physiological state before complete fixation occurred.
8. Sections of skin from birds which were subjected to scalding were too distorted for any detailed histological analysis to be made.
9. The technique of freeze-drying was not satisfactory for the study of the feather follicle in this investigation. Frozen sections of un-fixed tissue were also unsatisfactory for any detailed histological studies to be made.

The second portion of this investigation concerned a series of experiments in which various physiological phenomena were evaluated for their effect upon the release of the feather from its follicle. Feather pulling force, as measured by a spring scale, was used as the criterion. The following conclusions were drawn from these physiological studies:

1. The exact part of the brain that is involved in the release of the feather from its follicle was not completely determined. The data indicate that the diencephalon, medulla or cerebellum may be the essential part.
2. All of the general anesthetics, sodium pentobarbital, chloroform and ether, significantly decreased feather pulling force from the value obtained in the conscious state. The local anesthetic, procaine, also had a significant effect on decreasing feather pulling force immediately around the site of injection.
3. Tranquilizing drugs, chlorpromazine and promazine, significantly reduced the force required to pull an individual feather from its follicle.

4. Atropine and yohimbine, blocking agents of the parasympathetic and sympathetic system respectively, significantly decreased the force required for feather removal. None of the neuromimetic drugs used, however, significantly altered feather pulling force.

5. All levels of spinal cord transections significantly reduced feather pulling force of feathers pulled from the dorsal feather tract posterior to the level of the transection. In contrast, feather pulling force of feathers pulled anterior to the spinal transection remained relatively unchanged.

6. Feathers pulled from the femoral feather tract showed a significant reduction in pulling force immediately and 25 minutes after the spinal cord was severed regardless of the level of transection. However, only spinal transections anterior to the second lumbar vertebra had a consistent effect on reducing feather pulling force at the 60 minute observation.

7. Spinal transections at the level of the sixth thoracic vertebra and posterior to this level, failed to have a loosening effect on the feathers of the pectoral feather tract.

8. Skeletal muscles do not influence the release of the feather from its follicle.
9. Reduced feather pulling forces increased rapidly to near their normal value following death of birds by bleeding. When birds were sacrificed by a lethal dose of sodium pentobarbital, feather pulling forces also returned to near their normal value. The return, however, was much slower than that observed for birds sacrificed by bleeding.

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