

anesthesia, even after the initial hypotonus of these muscles has apparently subsided, (3) decreased resistance to fatigue of the muscles of this limb, as illustrated by the curves of fatigue of the gastrocnemius muscles when the sciatic nerves are stimulated by means of a tetanizing current.

These results seem to justify the conclusion that the sympathetic innervation is a factor in the maintenance of the tonus of voluntary muscles, although this need not be apparent in the normal activities of the animal. The fact that voluntary muscles in which the sympathetic nerve-fibers have undergone degeneration are more flaccid than the corresponding muscles on the opposite side while the animal is under surgical anesthesia seems to indicate that the tonus of voluntary muscle which is maintained through its sympathetic innervation, like the tonus of involuntary muscle, is less completely eliminated by anesthesia than the tonus which is maintained through its somatic innervation. The decreased resistance to fatigue of the muscles of the hind limb following extirpation of the corresponding lumbar sympathetic trunk, which is more marked after the sympathetic fibers have undergone degeneration than immediately following the operation, as illustrated by the curves of fatigue of the gastrocnemius muscles obtained in the above experiments, seems to indicate that the sympathetic innervation is an important factor in sustaining the activity of voluntary muscles under conditions of stress and that this involves an outflow of nerve impulses directly from the sympathetic ganglion cells. Doubtless, the sympathetic ganglion cells constitute a source of nerve impulses which are also an important factor in maintaining the normal efficiency of voluntary muscles.

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Sex-reversal following ovariectomy in the fowl.

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It has been known for a considerable period of time that the female in many breeds of birds takes on certain male characters following the removal of the ovary, either by ovariectomy or

pathological absorption. In the latter case the reduction of the ovarian mass may be only partial or for a short period of time, followed by recovery and regeneration of the ovary; or the ovary may be permanently reduced and destroyed. In all such cases the female assumes certain male characters, and the extent to which such male characters are developed and their permanency, apparently depends upon the degree of removal or reduction of the ovary.

It is the purpose of this paper to call attention to experiments being carried on in this laboratory which have a very special bearing on some of the work recently reported by Crew¹ in the fowl, and by Riddle² in the pigeon. Investigations along this line were suggested by Professor F. R. Lillie, early in the fall of 1922, and have been carried on under his direction.³

My work thus far has been confined exclusively to the Light Brown Leghorn race. Sexual dimorphism in this breed is very pronounced, and the birds are more active and perhaps somewhat more hardy than are the birds of some of the heavier breeds. Most of the birds were obtained from a reliable breeder immediately after hatching and reared in the laboratory, where operations could be made at various ages and results observed.

The operation in the female is relatively difficult, owing to the proximity of the great blood vessels. In the young female, prior to the age of laying, the ovary is a flat sheet of tissue attached intimately by one surface to the ventral surface of the left iliac and vena cava. It was found that the ovary is most readily and most satisfactorily removed during this stage of its development. If the operation is delayed until the changes preceding the laying season have set in, the ovary has enlarged and has become more extensively vascularized, and its removal is more difficult. As regards the operation, assuming that the preliminary steps have been accomplished and the ovary exposed to view, it was found very essential to carefully remove the dorsal peritoneum which covers the ovary. The ovary is now fully exposed and may be

¹ Crew, F. A. E. 1923. Studies in Intersexuality. II. Sex-reversal in the Fowl. *Proc. Roy. Soc., B*, Vol. 95, No. 667.

² Riddle, O. 1924. A case of complete Sex-reversal in the Adult Pigeon. *Am. Nat.*, Vol. 58, No. 655.

³ This work was aided by a grant from the Committee on Sex Research of the National Research Council.

removed by firmly applying a pair of forceps to the free borders and gently peeling it off. The initial attempts were almost invariably disappointing, owing to fatality or incomplete removal. However, a technique was developed by which the ovary could be completely removed without greatly inconveniencing the bird. After the ovarian mass had been carefully removed, all questionable fragments remaining were thoroughly seared with a hot scalpel. Aseptic methods were carefully followed, and both general and local anesthetics have been employed.

The results of complete castration in the female bird have not been so extensively recorded as the results following castration in the male, owing largely to the difficulties referred to. Some workers have even definitely stated that it is impossible to remove the ovary, that invariably fragments remain behind which regenerate in a short time, thus maintaining the normal ovarian influence. That the operation is exceedingly difficult, and that fragments may remain behind which will regenerate is not denied; but these obstacles may be overcome.

It was found by previous workers that, following the ablation of the ovary, the female takes on the male plumage at the following moult, the spurs begin to develop at once; comb, wattles, and earlobes become pale and small so that they approximate those of the capon in size and general appearance. These ovariectomized birds, though smaller, are very similar to the capon in appearance and are readily mistaken as such by those not knowing their history. While this type of transformation appears in our work it is the exception rather than the rule, and indeed is extremely rare as a definitive condition.

The larger percentage of our birds have assumed additional male characters following ovariectomy until they are practically complete replicas of the male, and, to those not familiar with their history, they are regarded as *unmistakable* males. Thus we find that they assume the complete male plumage, spurs grow as they do in the normal cock, head furnishings increase in size until they cannot be distinguished from those of the normal male. Other birds in the pen regard them as males and when a strange cock is introduced they fight as would other cocks, very frequently assuming the initiative, some of them having been observed to come off victorious in such a combat. Many of these birds crow regularly, and of these, some have been seen making

definite attempts at treading other hens, while one has actually been seen doing so. When aroused by a disturbance, it was found that their reaction is very similar to that of the male; the sound emitted, together with their reaction on such occasions, reminds one very much of the young male just prior to maturity. Some sixty birds have been ovariectomized, of which number approximately twelve are incompletely ovariectomized, while the majority of the others show the above changes in the male direction including the plumage, spurs, and head furnishings.

One set of experiments may be mentioned as an example: Out of one lot of fourteen females of the same hatch, one was kept as control and thirteen were ovariectomized between the ages of six weeks to six months; twelve of these have developed all the characteristics of the male mentioned above, some being completely cock-feathered, while the others are fast becoming so. The other one of the thirteen is very capon-like in appearance except perhaps for size and cannot be readily distinguished from her capon brothers by those not knowing her history. This bird has assumed complete male plumage, is developing spurs; but the comb, wattles, and earlobes are pale and small, resembling those of the capon.

A number of ovariectomized birds have been killed and examined. Bird No. 533 was hatched on April 26, 1923. On June 30, an ovariectomy was performed at which time the ovarian mass was apparently completely removed. The latter part of August it was noticed that the bird had become almost completely cock-feathered and that it was developing the comb and wattles of the male, in which respect it was difficult to distinguish it from its brothers. This development proceeded in the male direction until January 24, 1924, when the bird was killed and examined. At the time of killing, the bird was completely cock-feathered except for a few wing-coverts which were entirely hen-like, showing no modification in the male direction. It had the head furnishings of the male and had developed spurs. Its voice and reactions were decidedly male-like though it had never been observed to crow, but may have done so since no effort had been made to discover this reaction.

When the abdominal cavity was exposed we found on the left, corresponding in position to the normal ovary, a white testis-like organ, quite bulky, being somewhat longer than wide, and slightly

irregular owing perhaps to an encysted mass of cotton which had been left on the site of the removed ovary following the operation. Associated with this mass of testis-like regeneration was an ovarian follicle about the size of a dried pea. Histological examination revealed a tubular mass similar to the seminiferous tubules of the normal testis in the cock. The oviduct was present, though somewhat reduced.

On the right side, corresponding in position to the organ found on the left, was found a similar though larger testis-like organ measuring 2.3 cm. in length and 0.7 cm. in width; leading posteriorly from it was a small white strand resembling an immature vas deferens. The histological examination revealed a tubular mass with the typical structure of the normal immature testis.

Bird No. 543 was hatched on April 26, 1923, and was ovari-otomized on August 7, 1923. The bird was killed and examined on January 14, 1924. This bird was almost completely cock-feathered early in October, and it was noticed that the head furnishings were growing, being deep red in color, comparing favorably in size with those of its brothers though somewhat smaller. The bird at the time of killing was completely cock-feathered, head furnishings were of the male type and it had developed spurs. Its voice and behavior were typically male.

Dissection revealed on the site of the original ovary a testis-like organ similar to that found on the left in bird No. 533 and also associated with it was a small mass of ovarian tissue. The mass was slightly elongated, somewhat irregular and intimately attached by its outer margin to an encysted mass of cotton which had been left at the time of the operation. The ovarian part was found at the anterior outer margin and could be readily distinguished, consisting of a few follicles varying in size. The oviduct was present though considerably reduced. The histological examination revealed immature seminiferous tubules.

On the right side a testis-like organ was found corresponding in position to that on the left. This organ was somewhat larger, measuring 2.3 cm. in length and 0.8 cm. in width, and weighing 450.01 mg. Leading posteriorly from it was a small white strand resembling a vas deferens. This organ was not only larger than that found on the left, but was also much more regular in outline, giving the general appearance of a small immature testis. The histological findings proved it to be an immature testis with the characteristic tubular mass.

Bird No. 619 was hatched the early part of July, 1923, and ovariectomized on January 13, 1924. On May 29, 1924, the bird was killed and examined. At the time of killing the bird was not completely cock-feathered, probably less than half the feathers being cocky. Some of the feathers on the breast showed cocky tips and henney bases very distinctly, and some on the back and saddle, less distinctly. The bird had developed the head furnishings typical of the cock and was developing spurs.

Examination of the original ovarian site revealed no tissue which could possibly be ovarian or testicular, removal had been complete, and no regeneration had taken place. The oviduct was present and approximately normal in size.

On the right side, corresponding in position to the ovary on the left, a testis-like organ was found which was somewhat irregular in shape. The organ measured 2.3 cm. in length and 1.2 cm. in width. Leading posteriorly from it was a small white strand which could be followed to the cloaca and which presumably was an immature vas deferens. The organ has not yet been studied histologically.

The greater number of our ovariectomized birds show equally pronounced external male characteristics. They are being kept a time in order to see what the eventual transformation may be.

The results as here obtained appear to be relatively rare so far as the earlier investigations along these lines are concerned. Goodale⁴ was probably one of the earliest investigators who made extensive observations on completely ovariectomized fowl. Goodale observed a regenerated mass on the right side in some of his ovariectomized birds which resembled histologically early nephrogenous tissue. Pezard⁵ states definitely, in commenting on the work of Zawadowsky, that he never observed regeneration on the right side in any of his work. Zawadowsky⁶ observed regeneration on the right side and correlated with it was a more or less

⁴ Goodale, H. D. 1916. Gonadectomy in relation to the Secondary Sexual Characters of some Domestic Fowl *Carnegie Inst. Wash. Pub.*, No. 243. Goodale, H. D. 1916. Further developments in Ovariectomized Fowl. *Biol. Bull.*, No. 30.

⁵ Pezard, A. 1922. Modifications periodiques ou definitives des caracteres sexuels secondaires chez les gallinaces. *Ann. des Sc. Nat. Zool.*, S. 10, V. 6.

⁶ Zawadowsky, M. 1922. Sex and Development of Sex Characters. Moscow, State edition, Russian with 20 pages of German summary.

complete assumption of male characters. Benoit⁷ reports similar results following ovariectomy.

In contrast to these observations several authors have reported the presence of glandular structures on the right side in birds showing pathological reduction of the ovary, and it is to this category that the cases of sex-reversal reported by Crew for the fowl, and Riddle for the pigeon, belong. Thus in such cases there has been found an ovotestis on the left associated with a testis-like structure on the right (Crew) or these structures may be unilateral, occurring only on one side. These birds very rarely show the complete secondary sexual characters of the male, but more frequently show maleness in only one or two of these characters; thus the bird may show only spurs of the male, or spurs and head furnishings may be male-like, while the plumage is henny, and correlated with these characters there is usually a certain degree of male behavior.

We thus find in the bird, as a result of our ovariectomy experiments, a condition analogous in many respects to that reported by a large number of authors in cases of pathological absorption of the ovary. There is a clear possibility that these ovariectomized individuals may undergo complete sex-reversal. They are certainly more complete replicas of the male at this stage than are the large majority of birds recorded in cases of pathological reduction of the ovary, and coupled with this there is a certain degree of male behavior which often is not pronounced in the pathological individuals. These observations are interesting in view of the attitude taken by Riddle² in a recent paper on "Sex-reversal in the Pigeon", where he suggests that "cases of complete sex-reversal will probably occur only in those adults which develop special diseases in particular or restricted organs."

In some of our cases individuals which have assumed more or less complete male characters as concerns head furnishings, plumage, and spurs, are reverting toward the female type as shown by the female type of plumage reappearing. This would indicate that bits of ovary may regenerate after a time, causing certain of the male characters to revert. If this be due to regenerating

⁷ Benoit, J. 1923. A propos du changement experimental de sexe par ovariectomie, chez la poule. *C. R. de la Soc. de Biol.*, 89, pp. 1326-28. Benoit, J. 1923. Transformation experimentale du sexe par ovariectomie precoce chez la poule domestique. *C. R. de l'acad. des Sc.*, 177, pp. 1074-77.

ovary then we would have to assume, that whenever sufficient ovary regenerates it would prohibit complete sex-reversal in all its characters and it may cause a reversal of some of the already formed male characters in the female direction. This statement is made with some reserve since it is not known at the present time what the internal findings will reveal. However, if ovarian tissue should be found it would seem to show that complete sex-reversal is not possible in cases of pathological reduction or natural ovariectomy unless the ovary be completely destroyed or removed.

Our results indicate that the female in the Brown Leghorn fowl has many potentialities of the male, which are normally inhibited by the presence of the ovary, and that these potentialities can assert themselves approximately fully after the complete removal of the ovary at an early age.

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Bacterial allergy and tissue reactions.

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The bacterial allergies, though they are phenomena of relatively specific hypersusceptibility, differ in certain fundamental respects from true protein anaphylaxis. Our own investigations on this subject have dealt chiefly with the tuberculin reaction in regard to which it seems safe to state that:

1. Tuberculin allergy as manifested by the intracutaneous reaction may occur without general anaphylaxis to tuberculo-protein, and vice versa.
2. The active principle of tuberculin which elicits reactions in the sensitive subject is not a protein.
3. Tuberculin sensitiveness seems inseparably associated with the reactions of the animal tissues to the tubercle bacilli or their constituents; in other words, after prolonged experimentation, we arrive again at the recognition that there is no tuberculin sensitiveness without a tubercle. Here again the fact that the