TERMINAL INNERVATION OF THE UTERUS AND VAGINA OF THE DOMESTIC HEN

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Summary. The distribution of nervous tissue within the oviduct of the hen has been examined with particular reference to the isthmus, uterus and vagina. The uterus and utero-vaginal junction were well innervated and nerve cells were more abundant in these regions. An extensive mesh of large nerve fibres was evident in the uterus, together with a much finer network of mainly single fibres associated with the muscle cells. Fewer large nerves were found in the utero-vaginal and vaginal regions. Many of the nerves in the isthmus innervated the blood vessels. Several small ganglia were found externally, towards the caudal end of the uterus and surrounding the uterovaginal junction.

The arrangement of the smooth muscle layers of the posterior oviduct is described.

INTRODUCTION

Various manipulative treatments of the distal regions of the oviduct of both mammals and birds can cause disturbances in either ovarian or oviducal function (Meyer, Leonard & Hisaw, 1930; Shelesnyak, 1931; Haterius, 1933; Rothchild & Fraps, 1945; Moore & Nalbandov, 1953; Huston & Nalbandov, 1953; Sykes, 1953; van Tienhoven, 1953; Nalbandov, Moore & Norton, 1955; Donovan, 1961; Donovan & Traczyk, 1962). Current work in this laboratory involving the surgical manipulation of the distal region of the oviduct of the fowl has confirmed previous observations that oviducal function may be disturbed for a long time.

It is well known that the oviduct of the hen is innervated (Johnson, 1925; Mauger, 1941; Hsieh, 1951; Freedman & Sturkie, 1961). Biswal (1954) has also demonstrated ganglion cells in the vaginal musculature and Johnson (1925) briefly mentioned an intrinsic nerve plexus in the whole oviduct. Recently, P. D. Sturkie (personal communication) confirmed the presence of nerve fibres in the uterus. However, no attempt has been made to determine whether the intrinsic nervous tissue is distributed uniformly throughout oviduct. The literature on the general histology of the reproductive tract of the hen is extensive (Brambell, 1925; Richardson, 1935; Romanoff & Romanoff, 1949; Biswal, 1954; Bradley, 1960) but deals primarily with the changes in the secretory mucosa associated with egg formation.

In an investigation of factors which might affect ovulation and the function of the oviduct, it was felt necessary to obtain more detailed information on the extent of the intrinsic innervation and musculature of the oviduct, with particular reference to the uterus (shell gland) and the utero-vaginal junction.

MATERIALS AND METHODS

Brown Leghorns of the Poultry Research Centre flock between the ages of 10 weeks and 18 months were killed by an overdose of sodium pentobarbitone (Nembutal, Abbott Laboratories). An examination of the nerves within the oviduct was made using the following methods.

SCHABADASCH'S (1935) METHYLENE BLUE VITAL STAIN (CITED BY MITCHELL, 1953a)

The appropriate region of the oviduct was dissected out within 5 min after death and either wholly, or with the mucosa removed, immersed for 20 min at 37° C in a buffered 0.1% aqueous solution of methylene blue, pH 5.7. Differentiation was then carried out in phosphate buffer (pH 5.7) and the tissue was mounted entire. Before adopting the pH of 5.7, solutions at pH 5.7, 5.9, 6.0 and 6.2 were examined, since it had been pointed out (Meyling, 1953) that the pH of the methylene blue solution and the pH of the buffer were critical. It was found that pH 5.7 gave the most consistent results in our case. The best results were obtained if the tissue was dealt with promptly *post mortem* and if excess of adipose and connective tissues surrounding the oviduct were removed. Sometimes particular tissue samples failed to stain correctly (Mitchell, 1953b); it was not possible to account for this variability.

The combination of supravital and intravital staining described by Mitchell (1953a) was attempted, but no advantage was gained.

TEXT-FIG. 1. Diagrammatic illustration of the posterior oviduct of the domestic hen. Tissues were taken from Areas 1, 2 and 3 for histological examination.

SILVER IMPREGNATION (WEDDELL & GLEES, 1941)

Tissue blocks were cut from the oviduct regions indicated in Text-fig. 1, and fixed in neutral formol-saline solution for at least 1 week. Frozen sections were cut at 50 to 100 μ , stained, and mounted in DePeX solution.

SILVER CARBONATE METHOD (JABANERO, 1948)

This method was tried but proved unsatisfactory in our hands, as it was more difficult to prevent the staining of tissue elements other than nervous ones.

ANALYSIS OF THE DISTRIBUTION OF THE NERVOUS TISSUE IN THE OVIDUCT

To obtain information about the relative densities of nervous tissue in various regions of the oviduct the following procedure was adopted. Whole mounts of the different regions of one oviduct were stained by the methylene blue procedure (*see above*). The authors selected at random, independently, a standard number of fields of each region for microscopical examination. A number taken from a predetermined arbitrary scale from 0 to 4 was assigned to the amount appearing in each field of (a) large nerves consisting of many fibres, (b) single fibres, (c) ganglion cells associated with the large nerves, and (d) free ganglion cells. For each of the above categories a number was obtained for each region by summing the values from the individual standard fields. Mean totals were obtained from the estimations of both authors. The whole procedure was repeated on several hens and grand mean totals were calculated for the different nervous tissue elements in each region. An expression of their relative abundance in the different regions was thus obtained.

For general histology, different parts of the oviduct (Text-fig. 1) were fixed in Susa for 24 hr. Paraffin sections were stained by haematoxylin and eosin, van Gieson, modified van Gieson (Marshall, 1946) or Picro-Mallory trichrome.

RESULTS

The isthmus is well innervated by both large nerves and single fibres (Table 1), which appeared to be associated mainly with the blood vessels, although some

TABLE 1

DISTRIBUTION OF VARIOUS NERVOUS ELEMENTS IN THE POSTERIOR
REGIONS OF THE OVIDUCT OF THE DOMESTIC HEN

<i>Region</i>	<i>Large nerves</i>	<i>Single fibres</i>	<i>Ganglion cells associated with large nerves</i>	<i>Free ganglion cells</i>
Isthmus	4	10	1	0
Isthmus-uterus junction	18	6	2	0
Uterus	11	8	5	1
Utero-vaginal junction	3	14	1	8
Vagina	4	8	0	1

Each number represents a mean estimate of the relative abundance of the nervous elements.

of the finer branches were found in the muscle, lying parallel to the longitudinal axis of the cells. Very few ganglion cells were seen, but they appeared to increase in number at the isthmus-uterus junction. The cells were always found in the large nerve trunks.

There was more nervous tissue in the uterus than in the isthmus (Table 1) which was particularly evident from an examination of methylene blue preparations of the whole oviduct from young birds (8 to 10 weeks). In the uterus a

complex, irregular network of large nerves was found lying mainly just beneath the serous layer (Pl. 1, Figs. 1 and 2). This network was associated with a second network, mainly of single fibres, lying deeper in the musculature (Pl. 1, Fig. 3); its meshes were elongated in the direction of the long axis of the smooth muscle cells. This distribution was similar to that described by Meyling (1953) and Mitchell (1953 a, b) for various mammalian organs. In some hens, the finest fibres were beaded (Pl. 1, Fig. 4) and it appeared that the extent of beading depended upon the physiological state of the uterus at the time of examination. A few fibres undoubtedly innervated small blood vessels (Pl. 1, Fig. 5), but the majority appeared to be associated directly with the muscle cells. This was especially evident from an examination of silver-stained frozen sections, when nerve fibres were observed within the muscle layers (Pl. 1, Fig. 6) and in places small swellings were seen adjacent to the individual muscle cells (Pl. 1, Fig. 7). These appeared to be similar to the terminal arborizations between the autonomic nerves and smooth muscle cells in mammals (Meyling, 1953).

Although no specialized sensory receptors were observed, a few free nerve endings were noted; on occasions these appeared to enter the submucosa (Pl. 1, Fig. 8). Mitchell (1953b) reported that no endings were found in the uterine mucosa of mammals.

More ganglion cells were found in the uterus than in the isthmus (Table 1). Most of these cells were found, either singly or in groups of two or three, in the large nerves and characteristically at the junctions in the network (Pl. 1, Figs. 1, 9 and 10). They were large and oval, had a prominent nucleus and were usually bipolar, although multipolar types were present. Small numbers of nerve cells were also lying free in the uterine tissue (Table 1).

In the abdominal cavity a small ganglion was always visible lying on the dorsal surface of the caudal end of the uterus. In histological sections it was seen to be adjacent to the pelvic nerve; it had a well-defined capsule and contained several ganglion cells (Pl. 2, Fig. 11). The nuclei of the nerve cells of the fowl were not so easily stained by the techniques successfully used for the mammal.

In methylene blue preparations, it was obvious that the distribution of nerves and nerve cells in the area of the utero-vaginal junction was different from that of the uterus, as there were fewer large nerves and the relatively orderly arrangement of fine single fibres was replaced by a diffuse network (Pl. 2, Fig. 12) associated with the muscle cells. In this network many scattered ganglion cells were observed (Pl. 2, Figs. 12 and 13) which were generally smaller than those found in the uterus. An examination of silver preparations confirmed these observations (Pl. 2, Fig. 14).

On the peritoneal surface at the utero-vaginal junction were several small ganglia (Pl. 2, Fig. 15), each of which was similar in appearance to that described above, at the caudal end of the uterus.

The innervation of the vagina resembled that of the utero-vaginal junction, but there was less nervous tissue (Table 1), with only a few scattered nerve cells (Biswal, 1954) and a less extensive fine terminal network.

In general, the musculature of the hen's uterus was comparable in appearance to that of the mammal, but the smooth muscle was less compact, since there was

more connective tissue between muscle bundles (Pl. 2, Fig. 16). In the relaxed uterus of the hen the mucosa is thrown into numerous folds, each of which contains a few muscle cells. The distribution of the muscle was similar in the isthmus.

As depicted in Text-fig. 1, the vagina is extremely tortuous and the folds are held firmly together, and against the caudal end of the uterus, by a considerable amount of connective tissue. A crescent-shaped projection into the uterine lumen is found partly surrounding the opening of the vagina. In histological sections of the utero-vaginal junction, it was apparent that there was a marked increase in the amount of the internal circular smooth muscle layer (Bradley, 1960). The circular muscle in this region was different in appearance from that in the uterus. In the latter it took the form of large bundles (Pl. 2, Fig. 16) widely interspersed with connective tissue, but in the proximal vagina there was a more compact mass of smaller bundles (Pl. 2, Fig. 17). The thickened muscle was present for some distance caudally along the vagina, indicating that an anatomically localized sphincter was not present.

DISCUSSION

As expected from previous work, the oviduct was found to be well innervated, but it was clear that the distribution of nervous tissue was not uniform throughout. Generally the isthmus and vagina had considerably less nervous tissue than the uterus and utero-vaginal junction and in our experience intrinsic ganglion cells were mainly found in the latter two regions.

The appearance of the intrinsic nervous elements was similar to that described in visceral organs of mammals (Meyling, 1953; Mitchell, 1953 a, b). The nervous tissue presumably forms part of the autonomic nervous system; it was not possible to distinguish between the parasympathetic and sympathetic systems. Weiss & Sturkie (1952) suggested from work using autonomic-mimetic drugs that both systems were present in the fowl uterus.

We have been unable to demonstrate Cajal cells (Meyling, 1953) in our preparations. Some structures similar in appearance to the 'protoplasmic strands' (Jabanero, 1953) were found in silver preparations (Pl. 2, Fig. 18), but these were undoubtedly relatively large nerve fibres, possibly in an abnormal state.

Although no specialized sensory organs were demonstrated conclusively in the regions of the oviduct examined, it is possible that an afferent nervous system is present. The work of Huston & Nalbandov (1953), Sykes (1953) and van Tienhoven (1953) and our own unpublished data have shown that the presence of an irritant in the wall of the oviduct can seriously disturb its function. Van Tienhoven (1953) found that a thread in the isthmus of the oviduct affected ovulation more adversely than did a thread in the magnum. Moreover, Sykes (1953) demonstrated that a thread in the uterus caused the expulsion of soft-shelled eggs. In view of the present results, it is possible that these different effects could be related to variations in the distribution of nervous tissue in the oviduct. The extent to which the intrinsic innervation is involved in the normal functioning of the hen's oviduct remains to be established.

Bradley (1960) refers to a sphincter at the caudal end of the uterus. It has

been pointed out earlier that histologically there was no definitely localized sphincter in the hens of this flock. However, from a consideration of the complex arrangement of the muscles and nervous tissue in this region, the operation of a functional sphincter seems quite possible. A definite pressure can be felt in this area around a finger if it is inserted through the vagina into the uterus. Moreover, during laparotomy, this region is clearly seen to relax immediately prior to oviposition.

It is interesting to consider possible analogies between the mammalian and avian oviducts. Brambell (1925) suggested that the oviduct of the hen was similar to the Fallopian tube and uterus of the mammal from the point of view of secretory activity. It is possible that the function of the utero-vaginal junction is similar to that of the cervix of the mammal, considering their close resemblance in position. Also the innervation of the utero-vaginal junction and the adjacent ganglia could be compared to the paracervical ganglia and the innervation of the cervix (Mitchell, 1953b). However, present evidence is insufficient to justify making a close comparison.

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PLATES 1 AND 2 OVERLEAF

EXPLANATION OF PLATES

PLATE 1

FIG. 1. Part of the nerve network in the uterus. Nerve cells (N) are visible at two junctions in the meshwork of the larger nerves. Note the beaded appearance of the finer fibres (—→). Methylene blue whole mount.

FIG. 2. Large nerves in uterus. Mucosa (M). Serous coat (S). T.S. frozen section. Silver stain.

FIG. 3. Network of small nerve fibres in the uterus. Same field as Fig. 1, but different plane. Methylene blue whole mount.

FIG. 4. Several small nerve fibres in the uterus showing beaded appearance. Methylene blue whole mount.

FIG. 5. Nerve fibres innervating a small blood vessel (B) in the uterus submucosa. T.S. frozen section. Silver stain.

FIG. 6. Terminal nerve fibres within the smooth muscle layer of the uterus. T.S. frozen section. Silver stain.

FIG. 7. A possible terminal arborization of a small nerve fibre in the muscle of the uterus. T.S. frozen section. Silver stain.

FIG. 8. Terminal nerve fibre entering the uterine mucosa (M). T.S. frozen section. Silver stain.

FIG. 9. Ganglion cell at the junction of large nerves in the uterus. The axon (A) is clearly visible. Methylene blue whole mount.

FIG. 10. A junction in a large nerve in the uterus showing two ganglion cells. Methylene blue whole mount.

PLATE 2

FIG. 11. A nerve ganglion at the caudal end of the uterus, containing several nerve cells. H. & E.

FIG. 12. A dense, diffuse network of small nerve fibres on the ventral surface of the utero-vagina junction. Several ganglion cells (—→) can be seen out of focus. Methylene blue whole mount.

FIG. 13. A high magnification of some of the ganglion cells seen in Fig. 12. Methylene blue whole mount.

FIG. 14. A ganglion cell lying in the muscle of the utero-vagina junction. T.S. frozen section. Silver stain.

FIG. 15. Ganglion (G) and nerve (N) situated in the connective tissue surrounding the utero-vagina junction. Longitudinal section. Modified van Gieson.

FIG. 16. Low power picture showing the distribution of the smooth muscle in the uterus containing a fully formed, hard-shelled egg. Inner circular muscle (C). Outer longitudinal muscle (L). L. S. H. & E.

FIG. 17. Low power picture of the vagina showing the increased smooth muscle of the inner circular layer just caudal to the utero-vagina junction. Inner circular muscle (C). Outer longitudinal muscle (L). L. S. H. & E.

FIG. 18. Nerve fibres in the uterus showing vacuolated appearance. T.S. frozen section. Silver stain.

PLATE 1

(Facing p. 48)

PLATE 2