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# A COMPARATIVE MORPHOLOGICAL STUDY OF THE SOMATIC INNERVATION OF THE ANTEBRACHIUM AND MANUS OF THE DOMESTIC ANIMALS (Bos taurus, Ovis aries, Capra hircus, Sus scrofa domesticus, Equus caballus)<sup>1</sup>

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ABSTRACT. The present study was undertaken in order to obtain detailed information regarding the somatic innervation of the appendages of the ox, sheep, goat, pig and horse and for a better understanding of the domestic species differences, if any. The innervation of the forearm and foot of the forelimb had been studied on 10 forelimbs each for the ox, sheep and pig, 17 forelimbs for the goat, and 15 forelimbs for the horse. Breed, age, sex, and body weight of the animals were not considered in this investigation. Observations were made by dissection on fresh specimens as well as on embalmed specimens. The innervation of the forearm and foot of the ox, sheep, goat, pig and horse was accomplished by: the N. ulnaris, the N. medianus (including the Ramus muscularis distalis of the N. musculocutaneus), the N, radialis and the N. cutaneus antebrachii cranialis n. axillaris. In order to facilitate the comparative morphological study of these regions, the branches of these nerves, according to their topography, were partly renamed. The extent of the cutaneous innervation was also discussed.

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# INTRODUCTION

A knowledge of the innervation and disposition of various nerves supplying the appendages of the ox, sheep, goat, pig, and horse is essential for the sake of "applied" or "practical" anatomy, in order to satisfactorily accomplish various nerve blocks and to perform satisfactory surgery. A specific knowledge about the nerve supply to a particular region may, in turn, aid in the diagnosis of the area of involvement when certain nerves are affected by fracture, trauma, tumor, etc. Diseases of the feet of livestock often impair their utility and cause economic loss to the

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farmer, and indirectly to the country. Therefore, this investigation has both academic and practical application.

Moreover, the pig and the small ruminants (<u>e.g.</u>, sheep and goat) are frequently used as experimental animals in various medical research institutions around the country and abroad. Since very little information, to date, is available in the literature, especially in English, on the nerve supply to the appendages of the pig and in both small and large ruminants, a comparative anatomical study was deemed advisable. There are few references to the innervation of the digital regions in the goat, although it has been assumed that possibly there is no significant difference in the pattern of distribution of nerves in these regions of the goat, ox and sheep. Thus, in order to obtain detailed information in this area and for a better understanding of the domestic species differences, if any, this investigation has been undertaken.

Within the framework of the present investigation, the innervation of the forearm, or "antebrachium," and foot, or "manus" of the forelimb has been studied. At times, information regarding the origin of some nerves supplying these regions has been included in the results to present a more comprehensive picture.

For all practical purposes, the Nomina Anatomica Veterinaria (Pars prima) (1963) was used for the nomenclature of the muscles and the nomenclature recommended by Ghoshal and Getty (1967a), the Nomina Anatomica Veterinaria (1967) and the Nomina Anatomica (1966) for the nerves of the forearm and forefoot of the domestic animals.

#### REVIEW OF LITERATURE

The innervation of the forearm and forefoot in the ox, sheep, pig, and horse is, to some extent, described in standard textbooks and in articles (see bibliography). Because of the number of texts, the inconsistency of descriptions, and the complexity of the area, space does not permit consideration of all these detailed differences in this review, however, a detailed review by Ghoshal and Getty (1967a) has been published previously. Moreover, in order to avoid repetition, references relative to similar as well as dissimilar observations will be incorporated in the results.

# MATERIAL AND METHODS

Ten forelimbs each of the ox, sheep, and pig, twelve forelimbs of the goat and four forelimbs of the horse were dissected for this investigation. In addition, these observations were further verified on another 5 forelimbs of the goat in the freshmen dissection class during the spring quarter of 1965, and on 11 forelimbs of the horse in the freshmen dissection class during the winter quarter of 1966, and the variations, if any, were recorded. Therefore, this study includes a total observation of 10 forelimbs each for the ox, sheep and pig, 17 forelimbs for the goat and 15 forelimbs for the horse.

The animals were sacrificed in the Department of Veterinary Anatomy, College of Veterinary Medicine, Iowa State University, Ames, Iowa, between June, 1964, and June, 1966. Breed, age, sex, and body weight of the animals were not considered in this study. The animals were anesthetized with pentobarbitol sodium and exsanguinated via a canula from the right carotid artery. At times, dissection was accomplished on fresh specimens. When embalmed specimens were used, the following embalming solution was employed: isopropyl alcohol, 60%; formalin, 4%; phenol, 6%; corn syrup, 2.5%; and water, 27.5%.

# RESULTS

Innervation of the forearm, or antebrachium, and foot, or manus, in the ox, sheep, goat, pig and horse consists of the following:

- (1) The N. ulnaris;
- (2) The N. medianus (including the Ramus muscularis distalis n. musculocutanei);
- (3) The N. radialis; and
- (4) The N. cutaneus antebrachii cranialis n. axillaris.

The N. ulnaris (Figs. 1, 2)

The ulnar nerve passed distally in a common sheath with the median nerve (N. medianus) for a variable distance in the region of the arm.

The N. cutaneus antebrachii caudalis was given off by the N. ulnaris from its cranial aspect. It arose within the distal half of the arm in the ox, sheep, and goat, near the medial tuberosity of the humerus in the pig and within the proximal half of the arm in the horse. Frequently, it divided into a cranial and a caudal branch and supplied the fascia and skin on the craniomedial aspect of the carpus, and the caudomedial and lateral aspect of the proximal third of the forearm in the ox. In the sheep and goat, it supplied the fascia and skin on the caudomedial aspect of the proximal one-fourth of the forearm. Reimers (1925b) and Schreiber (1956) described the cranial branch as ending at the level of the carpus and the caudal branch as furnishing the caudal region to the distal end of the carpus in the ox. Reimers (1925b) asserted that the course and area of ramification of the caudal cutaneous nerve of the forearm in the sheep and goat were similar to those of the ox. May (1964) found that it supplied the skin of the volar aspect of the forearm in the sheep. In the pig. it furnished the fascia and skin about halfway down on the medial aspect and the proximal third of the caudal aspect of the forearm. But Schneider and Zintzsch (1962) stated that the cranial branch was distributed in the skin of the volar two-thirds of the medial side of the forearm downwards to the carpal joint. The caudal branch supplied the skin of the volar half of the forearm to the carpal joint. In the horse, it ramified in the fascia and skin on the proximal third of the caudomedial aspect and up to the distal two-thirds of the caudolateral aspect of the forearm. Reimers (1925b) and Grau (1935) observed a similar distribution. But Tagand and Barone (1964) alleged that it ramified behind the carpus and, according to Koch (1965) it supplied almost the entire skin of the lateral surface of the forearm and metacarpus.

In all animals, the muscular branches of the ulnar nerve innervated the M. flexor carpi ulnaris, M. flexor digitorum (digitalis) superficialis and the humeral and ulnar heads of the M. flexor digitorum (digitalis) profundus. Besides, it also supplied, below the carpus, the M. interosseus medius. Grau (1934) asserted that the preceding muscle received the ulnar part of the lateral volar nerve in the horse, named thus due to comparative anatomical reasons. Further, he quoted Zimmerl (1930) as stating that both volar nerves gave off twigs to the M. interosseus medius in the horse. Our findings in the horse concur with Reimers (1925b) that the volar (palmar) branch of the ulnar nerve divided into the superficial ramus and deep ramus at the distal end of the carpus. The deep ramus formed a short trunk which soon entered the M. interosseus medius.

The ulnar nerve divided, usually in the distal half of the forearm at a variable level, into a dorsal and palmar branch.

The dorsal branch (Ramus dorsalis n. ulnaris), in the ox, sheep, and goat gradually descended along the caudolateral aspect of the carpus, detached a small twig to the lateral aspect of the accessory carpal bone and proceeded distally as the N. digitalis dorsalis communis IV. During its distal course, it gave off numerous twigs to furnish the fascia and skin along the dorsolateral aspect of the metacarpus. Near the fetlock joint it split into two twigs (Nn. digitales IV et V dorsales proprii). The palmar twig descended along the palmarolateral aspect of the fetlock joint, where it released a twig (N. digiti V dorsalis proprius) backwards which was directed towards the lateral accessory digit (5th digit) to ramify around the same. The dorsal twig extended downward and forward to ramify in the fascia and skin on the dorsal aspect of the fetlock joint. Subsequently, the dorsal branch of the ulnar nerve extended distally as the dorsal proper digital nerve (N. digiti IV dorsalis proprius) of the 4th digit.

In the pig, the dorsal branch of the ulnar nerve coursed along the caudolateral aspect of the forearm and furnished twigs to the fascia and skin of the region. The main trunk continued distally and, near the carpus, divided into a medial and lateral branch. The lateral branch (Ramus lateralis) of the Ramus dorsalis n. ulnaris continued distally as the dorsal lateral digital nerve (N. digiti V dorsalis lateralis) to the 5th digit. The medial branch (Ramus medialis) joined the ramus communicans of the lateral branch (Ramus lateralis) of the superficial Ramus of the radial nerve at a variable level along the dorsal surface of the metacarpus to form the N. digitalis dorsalis communis IV. This combined trunk finally split into two dorsal proper digital nerves (Nn. digitales IV et V dorsales proprii) of the 4th and 5th digits.

In the horse, the dorsal branch of the ulnar nerve emerged between the tendons of insertion of the M. flexor carpi ulnaris and the M. ulnaris lateralis, about an inch above the accessory carpal bone. At this place it gave off several twigs to supply the fascia and skin of the adjacent area. One of the twigs was of considerable size, and, after coursing lateral to the carpus, ramified in the fascia and skin on the dorsolateral aspect of the carpus. Another twig descended somewhat medial to the accessory carpal bone and furnished twigs to the fascia and skin on the caudal aspect of the carpus and the area immediately distal to it. The dorsal branch extended towards the caudolateral aspect of the carpus, where it furnished a few twigs to supply the fascia and skin. Further, it coursed distally along the dorsolateral aspect of the metacarpus. During its course, it supplied numerous twigs to the fascia and skin of the adjoining area to the dorsolateral aspect of the fetlock joint.

According to Langer and Nickel (1953) and Graeger (1957) the volar

(palmar) branch of the ulnar nerve continued below the oblique ligament of the carpus and, immediately distal to the accessory carpal bone, released an articular branch to innervate the carpal joint capsule in the ox.

The palmar branch (Ramus palmaris n. ulnaris), after having supplied the M. interosseus medius by its deep branch, joined the Ramus communicans of the N. palmaris lateralis to constitute the N. digitalis palmaris communis IV, detached twigs (N. digiti V palmaris proprius) to the 5th digit, in agreement with Chauveau and Arloing (1902), Langer and Nickel (1953) and Dobberstein and Hoffmann (1964); and continued as the N. digiti IV palmaris proprius in the ox and sheep. In the goat, the Ramus palmaris n. ulnaris joined the N. palmaris lateralis and continued as in the ox and sheep. In the pig, the palmar branch divided into a medial and a lateral branch. The lateral branch continued as the N. digiti V palmaris lateralis and the medial branch descended as the N. digiti IV palmaris lateralis. In the horse, the palmar branch joined the N. palmaris lateralis, received a Ramus communicans from the N. palmaris medialis and finally divided into the Ramus dorsalis and the N. digitalis palmaris lateralis s. proprius. The deep ramus of the Ramus palmaris formed a short trunk which soon entered the M. interosseus medius. But Dobberstein and Hoffmann (1964) and Koch (1965) related that the deep volar branch innervated the M. interosseus medius with a fraction of its fibers and divided into two branches, the Nn. metacarpici volares profundi lateralis and medialis (Nn. metacarpei palmares), in the space between the preceding muscle and the volar surface of the third metacarpal bone. They extended to the axial side of the small metacarpals, distal to the button process of the small metacarpals but on the lateral surface of the fetlock joint. They were traceable to the corium of the hoof. Within the framework of the present investigation we could not ascertain the observations of Dobberstein and Hoffmann (1964) and Koch (1965).

The N. medianus (including the Ramus muscularis distalis n. musculocutanei) (Fig. 2)

According to Reimers (1925b), Schreiber (1956), Dobberstein and Hoffmann (1964) and Koch (1965) the Ramus musculocutaneus of the median nerve (N. medianus) should be regarded as the Ramus muscularis distalis of the musculocutaneous nerve (N. musculocutaneus).

The Ramus musculocutaneus apparently arose from the N. medianus at a variable level within the distal half of the arm. It divided into a muscular and a cutaneous branch. The muscular branch was entirely expended inside the M. brachialis, while the cutaneous branch (N. cutaneus antebrachii medialis), in agreement with Langer and Nickel (1953) supplied the fascia and skin on the dorsal and medial aspects of the forearm and carpus, where it exchanged twigs with the Ramus superficialis of the radial nerve in the ox (Fig. 1). Later the superficial branch of the radial nerve divided into a Ramus medialis (N. digitalis dorsalis communis II) and the N. digitalis dorsalis communis III. This was also observed by Schreiber (1956), but he further described that the cutaneous branch of the musculocutaneous nerve may continue distally as the N. digiti III dorsalis medialis. In the sheep, goat, and pig, it supplied the fascia and skin on the proximal third of the dorsomedial aspect of the forearm. According to Graeger (1957) the N. musculocutaneus was the largest contribution for the innervation of the elbow joint by means of filaments which were derived from the so-called Ramus muscularis distalis. In the horse, the medial cutaneous nerve of the forearm ramified in the fascia and skin on the dorsomedial and dorsolateral aspects of the distal half of the forearm, carpus and metacarpus to the fetlock region. One of these twigs innervated the M. pectoralis transversus. This finding is in accordance with Reimers (1925b), Tagand and Barone (1964), Dobberstein and Hoffmann (1964) and Koch (1965).

In all animals the median nerve supplied muscular twigs to the Mm. pronator teres, flexor carpi radialis, flexor carpi ulnaris, flexor digitorum (digitalis) superficialis, and the humeral and radial heads of the M. flexor digitorum (digitalis) profundus. In the horse, we did not observe any contribution of the median nerve, similar to Rooney (1956), to the Mm. flexor carpi ulnaris and flexor digitorum (digitalis) superficialis. However, Martin (1923) and Sisson and Grossman (1953) affirmed that the median nerve gave off a branch to the M. flexor digitorum (digitalis) superficialis. Grau (1934) referring to Zimmerl (1930) and also Sisson and Grossman (1953) stated that the median nerve supplied the M. flexor carpi ulnaris.

One of these twigs coursed along the caudal aspect of the proximal extremity of the radius and was destined to supply the caudal aspect of the capsule of the elbow joint in the ox.

The median nerve divided into a medial and a lateral palmar nerve within the distal half of the metacarpus in the ox, sheep and goat. In the ox and sheep, the N. palmaris medialis divided into the N. digitalis palmaris communis II and the Ramus interdigitalis. The former nerve, near the fetlock joint, split into the Nn. digitales II et III palmares proprii. The N. digiti II palmaris proprius supplied the second digit and, according to Langer and Nickel (1953) also supplied the medial fetlock joint capsule in the ox. The N. digiti III palmaris proprius and the Ramus interdigitalis which continued as the N. digiti III palmaris lateralis descended on either side of the 3rd digit. The N. palmaris lateralis also gave rise to the Ramus interdigitalis and the Ramus communicans. The former continued as the N. digiti IV palmaris medialis and the latter received the Ramus palmaris of the ulnar nerve to form the N. digitalis palmaris communis IV and descended along the palmarolateral aspect of the fetlock joint and, near the latter, the combined trunk detached a few twigs (N. digiti V palmaris proprius) to the fifth digit and continued farther as the N. digiti IV palmaris proprius. In one case, both the N. digiti III palmaris lateralis and the N. digiti IV palmaris medialis of the ox arose from the N. digitalis palmaris communis III-the lateral terminal branch of the medial palmar nerve. In the ox, in agreement with Reimers (1925b) and Langer and Nickel (1953) we observed in four specimens that the N. digitalis palmaris communis II communicated with the N. digitalis dorsalis communis II of the superficial branch of the radial nerve by means of a delicate twig. Further, we observed communication between the Nn. digitales dorsales III et IV proprii and the Nn. digitales palmaris III et IV proprii through the interdigital space in seven specimens. A similar finding was also recorded by Reimers (1925b) and Langer and Nickel (1953) in the ox. In the goat the N. palmaris medialis

divided into the N. digitalis palmaris communis II and the N. digitalis palmaris communis III. The N. digitalis palmaris communis II divided into the Nn. digitales II et III palmares proprii near the fetlock joint. The former innervated the medial accessory digit, while the latter nerve descended as the N. digiti III palmaris proprius. The N. digitalis palmaris communis III gave rise to the Nn. digitales palmares III et IV proprii. The N. palmaris lateralis joined the Ramus palmaris of the ulnar nerve and continued in a similar manner to that of the ox and sheep. May (1964) mentioned that the median nerve in the sheep divided into three branches, medial, middle and lateral, at the junction of the middle and distal thirds of the metacarpus. He described that the medial volar digital nerve detached an anastomotic branch to the axial volar digital nerve of the third digit, which we could not confirm. In the pig the median nerve detached the Ramus medialis near the middle of the metacarpus, which continued as the N. digiti II palmaris medialis. Near the fetlock joint the median nerve gave off the N. digitalis palmaris communis II which divided into the Nn. digitales II et III palmares proprii. The continuation of the median nerve, below the fetlock joint, released the Ramus lateralis, which continued as the N. digiti V palmaris medialis. The rest descended as the N. digitalis palmaris communis III. The latter divided into the Nn. digitales III et IV palmares proprii. In the horse, the median nerve divided into a medial and a lateral palmar nerve at a variable level within the middle third of the forearm. The N. palmaris medialis detached a Ramus communicans for the N. palmaris lateralis, continued distally, and finally divided into the Ramus dorsalis and the N. digitalis palmaris medialis s. proprius. The N. palmaris lateralis joined the Ramus palmaris n. ulnaris above the carpus and received a Ramus communicans from the N. palmaris medialis and finally divided into the Ramus dorsalis and the N. digitalis palmaris lateralis s. proprius.

The N. radialis (Fig. 1)

At a variable level, about the middle of the arm, the radial nerve divided into a superficial and a deep branch.

The superficial branch (Ramus superficialis) emerged between the M. brachialis and the origin of the M. extensor carpi radialis and detached twigs to supply the fascia and skin on the dorsolateral aspect of the forearm. In the ox it exchanged fibers with the medially lying N. cutaneus antebrachii medialis of the Ramus muscularis distalis n. musculocutanei on the dorsal aspect of the carpus. The continuation of the superficial branch of the radial nerve divided into the N. digitalis dorsalis communis II and the N. digitalis dorsalis communis III within the distal half of the metacarpus in the ox, on the dorsal aspect of the carpometacarpal articulation in the sheep and within the proximal one-fourth of the metacarpus in the goat. The N. digitalis dorsalis communis II released some twigs (N. digiti II dorsalis proprius) to the second digit and continued as the N. digiti III dorsalis proprius, while the N. digitalis dorsalis communis III gave rise to the Nn. digitales III et IV dorsales proprii. In the pig, usually near the middle of the metacarpus, the superficial branch of the radial nerve divided into two branches. The medial branch soon divided into a Ramus medialis and the N. digitalis dorsalis communis II.

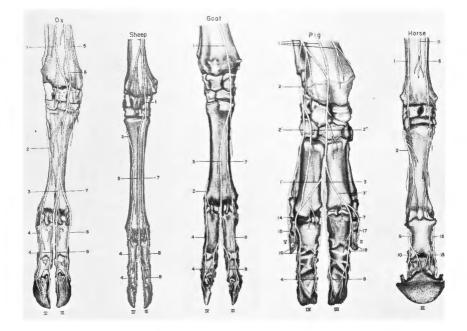


Figure 1. Nerves of the distal part of the right forelimb-dorsal view (schematic).

- 1 Ramus superficialis n. radialis
- l' Ramus communicans n. radialis
- 2 Ramus dorsalis n. ulnaris
- 2' Ramus lateralis n. ulnaris
- 2" Ramus medialis n. ulnaris
- 3 N. digitalis dorsalis communis III
- 3' Ramus medialis n. radialis
- 4 N. digiti IV dorsalis proprius
- 5 N. cutaneus antebrachii caudalis
- 6 N. cutaneus antebrachii medialis
- 7 N. digitalis dorsalis communis II
- 8 N. digiti III dorsalis proprius
- 9 N. digitalis palmaris lateralis

- 10<sup>\*</sup> Ramus dorsalis n. ulnaris
- 11 N. cutaneus antebrachii cranialis
- 12 N. digitalis palmaris medialis
- 13\*\*Ramus dorsalis n. mediani
- 14 N. digitalis dorsalis communis IV
- 15 N. digiti V dorsalis lateralis
- 16 Nn. digitales IV et V dorsales proprii
- 17 N. digiti II dorsalis medialis
- 18 Nn. digitales II et III dorsales proprii

In view of the accompanying blood vessels these should be called as the N. digitalis dorsalis lateralis and N. digitalis dorsalis medialis, respectively.

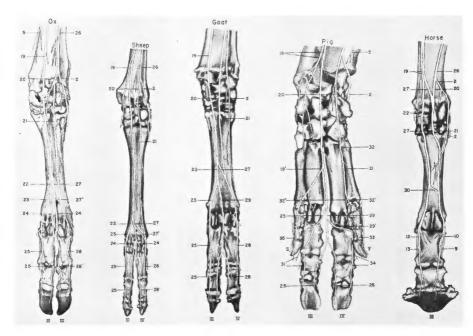


Figure 2. Nerves of the distal part of the right forelimb-palmar view (schematic).

- 2 Ramus dorsalis n. ulnaris
- 5 N. cutaneus antebrachii caudalis
- 9 N. digitalis palmaris lateralis
- 10\* Ramus dorsalis n. ulnaris
- N. digitalis palmaris medialis 12
- 13\*\* Ramus dorsalis n. mediani
- N. medianus 19
- 19' Ramus medialis n. mediani
- Ramus palmaris n. ulnaris 20
- 21 Ramus profundus n. ulnaris
- N. palmaris medialis 22
- 23 N. digitalis palmaris communis II 32" Ramus medialis
- 24 Ramus interdigitalis
- 25 N. digiti III palmaris proprius
- 25' N. digiti III palmaris lateralis
- 26 N. ulnaris
- N. palmaris lateralis 27
- 271 Ramus communicans
- 28 N. digiti IV palmaris proprius

- 28' N. digiti IV palmaris medialis
- 29 N. digitalis palmaris communis III
- 29' Ramus lateralis
- 30 Ramus communicans
- 31 Nn. digitales II et III palmares proprii
- 32 N. digitalis palmaris communis IV
- 321 Ramus lateralis
- N. digiti V palmaris lateralis 33
- 34 N. digiti IV palmaris lateralis et N. digiti V palmaris medialis
- N. digiti II palmaris medialis 35

In view of the accompanying blood vessels these should be called the N. digitalis dorsalis lateralis and N. digitalis dorsalis medialis, respectively. The Ramus medialis continued as the N. digiti II dorsalis medialis, while the N. digitalis dorsalis communis II gave rise to the Nn. digitales II et III dorsales proprii. Similarly, the lateral branch of the Ramus superficialis of the radial nerve divided into the N. digitalis dorsalis communis III and a Ramus communicans. The former divided into the Nn. digitales III et IV dorsales proprii, whereas the Ramus communicans joined the medial branch of the Ramus dorsalis n. ulnaris and descended as the N. digitalis dorsalis communis IV. The latter finally divided into the Nn. digitales IV et V dorsales proprii. In the horse the superficial branch of the radial nerve furnished the fascia and skin of the dorsolateral aspect to the distal third of the forearm, usually about an inch above the carpus. In two instances, we could trace it a variable distance below the carpus. Grau (1935) and Nickel (1941) described it as ramifying in the skin over the muscular bellies of the Mm. extensor digitalis communis and lateralis and, therefore, in the middle of the lateral aspect of the forearm. But Tagand and Barone (1964) alleged that the cutaneous branch of the radial nerve was distributed to the skin of the external surface of the forearm, the craniolateral surface of the carpus, and even of the metacarpus.

In all animals the deep branch (Ramus profundus) gave off the muscular branches which furnished primarily the extensors of the carpus and digit. Besides, Reimers (1925a) asserted that in about half of the cases in the ox and pig the radial nerve gave off a delicate twig to the M. brachialis, close to its insertion. Reimers (1925b), Grau (1934), Nickel (1941), Sisson and Grossman (1953) and Koch (1965) described an inconstant twig for the M. brachialis from the radial nerve in the horse. We observed this radial innervation to the M. brachialis only in the pig. The M. brachialis is a flexor of the elbow joint, whereas the radial nerve, in general, supplies the extensors of the carpus and digit. This innervation allows us to recognize the remnant of the previously present M. brachioradialis which is fused with the M. brachialis.

The N. axillaris (N. cutaneus antebrachii cranialis) (Fig. 1)

In all animals, the axillary nerve furnished the flexors of the shoulder joint and the Pars clavicularis (Reimers 1925b) or Pars cleidobrachialis (Koch 1965) of the M. brachiocephalicus. The latter was originally a part of the M. deltoideus, which is a flexor of the shoulder joint. Moreover, Bruni and Zimmerl (1951) described an axillary twig innervating the M. brachialis in the horse which we could not demonstrate in our specimens.

The cutaneous branch of the axillary nerve, the N. cutaneus antebrachii cranialis, frequently emerged between the two parts of the M. deltoideus in the ox, sheep, goat, and pig, while in the horse it emerged between the M. deltoideus and the long head of the M. triceps brachii. In the ox and sheep it was sometimes observed to emerge between the M. deltoideus and the lateral head of the M. triceps brachii. In the ox the cutaneous branch ramified in the fascia and skin along the craniomedial aspect of the forearm. In the sheep, goat, and pig it furnished the fascia and skin around the dorsolateral aspect of the elbow joint. In the horse it was distributed in the fascia and skin to the middle twothirds on the dorsal aspect of the forearm.

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# A COMPARATIVE MORPHOLOGICAL STUDY OF THE SOMATIC INNERVATION OF THE CRUS AND PES OF THE DOMESTIC ANIMALS (Bos taurus, Ovis aries, Capra hircus, Sus scrofa domesticus, Equus caballus)<sup>1</sup>

Nani Gopal Ghoshal<sup>2</sup> and Robert Getty<sup>3</sup>

ABSTRACT. The present study was undertaken in order to obtain detailed information regarding the somatic innervation of the appendages of the ox, sheep, goat, pig, and horse for a better understanding of the domestic species differences, if any. The innervation of the leg and hind foot had been studied on 10 hind limbs each for the ox, sheep, and pig, 19 hind limbs for the goat and 15 hind limbs for the horse. Breed, age, sex, and body weight of the animals were not considered in this investigation. Observations were made by dissection on fresh specimens as well as on embalmed specimens. The innervation of the leg and hind foot of the ox, sheep, goat, pig, and horse was furnished by: the N. cutaneus surae plantaris lateralis, the N. fibularis (peroneus) (N. peroneus communis of the N.A.V.), the N. tibialis and the N. saphenus. In order to facilitate the comparative morphological study of these regions, some of the branches of these nerves, according to their topog-raphy, were partly renamed. The extent of the cutaneous innervation was also discussed.

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## INTRODUCTION

The objectives and importance of this investigation have been indicated in the previous article concerning the innervation of the forearm and foot of the ox, sheep, goat, pig and horse (Ghoshal and Getty 1968) and, therefore, a repetition is deemed unnecessary here.

Within the framework of the present investigation, the innervation of the leg, or "crus," and foot, or "pes" has been studied. At times information regarding the origin of some nerves supplying these regions has been included in the results to present a more comprehensive picture.

For all practical purposes the Nomina Anatomica Veterinaria (Pars Prima) (1963) was used for the nomenclature of the muscles, and the

<sup>&</sup>lt;sup>1</sup> Taken in part from a 1966 Ph.D. thesis by Dr. Ghoshal, from the Department of Veterinary Anatomy, Iowa State University, Ames, Iowa.

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nomenclature recommended by Ghoshal and Getty (1967a), the Nomina Anatomica Veterinaria (1967), and the Nomina Anatomica (1966) for the nerves of the leg and hind foot of the domestic animals.

# REVIEW OF LITERATURE

The innervation of the leg and hind foot in the ox, sheep, pig, and horse is, to some extent, described in the standard textbooks and in articles (see bibliography). Because of the number of texts, the inconsistency of descriptions, and the complexity of the area, space does not permit consideration of all these detailed differences in this review; however, a detailed review by Ghoshal and Getty (1967a) has been published previously. Moreover, in order to avoid repetition references relative to similar as well as dissimilar observations will be incorporated in the results.

#### MATERIAL AND METHODS

Ten hind limbs each of the ox, sheep, goat, and pig and 4 hind limbs of the horse were dissected for this investigation. In addition, these observations were further verified on another 9 hind limbs of the goat in the freshmen dissection class during the spring quarter, 1965, and on 11 hind limbs of the horse in the freshmen dissection class during the winter quarter, 1966, and the variations, if any, were recorded. Therefore, this study includes a total observation of 10 hind limbs each for the ox, sheep, and pig, 19 hind limbs for the goat and 15 hind limbs for the horse.

The animals were sacrificed in the Department of Veterinary Anatomy, College of Veterinary Medicine, Iowa State University, Ames, Iowa, between June, 1964, and June, 1966. Breed, age, sex, and body weight of the animals were not considered in this study.

The animals were anesthetized with pentobarbitol sodium and exsanguinated via a canula from the right carotid artery. At times, dissection was accomplished on fresh specimens. When embalmed specimens were used, the following embalming solution was employed; isopropyl alcohol, 60%; formalin, 4%; phenol, 6%; corn syrup, 2.5%; and water, 27.5%.

#### RESULTS

Innervation of the leg, or crus, and foot, or pes, in the ox, sheep, goat, pig, and horse consists of:

(1) The N. cutaneus surae plantaris lateralis;

(2) The N. fibularis (peroneus)(N. peroneus communis of the N.A.V.);

(3) The N. tibialis; and

(4) The N. saphenus.

The N. cutaneus surae plantaris lateralis (Figs. 1, 2)

The N. cutaneus surae plantaris lateralis arose directly from the caudal aspect of the ischiatic nerve, usually slightly below the trochanter major of the femur in the ox. In the sheep and goat, the origin of this nerve was variable. In the majority of cases, it arose directly from the

ischiatic nerve, as in the ox, but sometimes it also originated from the fibular or tibial nerve. Schreiber (1956) stated that this nerve arose from the tibial nerve and, according to McLeod (1958), from the posterior border of the sciatic nerve in the ox. May (1964) related that in the sheep it originated from the tibial nerve. In the pig it arose frequently from the fibular nerve, but Schneider and Zintzsch (1962) asserted that the N. cutaneus surae plantaris was given off of the tibial nerve. It originated from the tibial nerve in the horse. According to Sisson and Grossman (1953), and Tagand and Barone (1964) it arose from the sciatic nerve in the horse, whereas Dobberstein and Hoffmann (1964) and Koch (1965) described it as arising from the tibial nerve. It extended downward and forward, lateral to the popliteal lymph node (Ln. popliteus) in the popliteal fossa. In this position it was related superficially to the M. biceps femoris and deeply to the lateral head of the M. gastrocnemius. About the middle of the leg it appeared superficially.

It ramified in the fascia and skin on the caudolateral aspect of the tarsus and proximal third of the metatarsus in the ox, whereas in the sheep and goat it was distributed in the fascia and skin on the lateral aspect of the tarsus. In the pig it furnished the fascia and skin on the lateral aspect of the tarsus and to a variable extent of the metatarsus. Montané and Bourdelle (1920) described an important branch of the N. cutaneus surae plantaris lateralis on the anteromedial side of the tarsus and pes to join the medial branch of the superficial fibular nerve in the formation of the dorsal collateral nerves of the two medial digits. We did not find this branch in our investigation. In the horse it ramified in the skin on the dorsolateral and caudal aspects of the tarsus, middle two-thirds of the lateral aspect of the metatarsus, and sometimes around the dorsolateral aspect of the fetlock joint. This assertion is in agreement with Grau (1935) and Bruni and Zimmerl (1951). Sisson and Grossman (1953), Tagand add Barone (1964), and Koch (1965) described the N. cutaneus surae plantaris lateralis as ramifying under the skin on the lateral surface of the tarsus and metatarsus, but Tagand and Barone (1964) further stated that some of its filaments may extend as far as the lateral side of the toe.

The N. fibularis (peroneus) (N. peroneus communis of the N.A.V.) (Fig. 1) Following separation from the tibial nerve (N. tibialis), the fibular

nerve proceeded downward and forward beneath the tibial part of the M. biceps femoris and was deeply related to the lateral head of the M. gastrocnemius. According to Dambock (1955) a relatively slender anastomotic branch of the fibular nerve (N. cutaneus surae lateralis) to the external saphenous nerve was not always present in the ox. In the ox the division of the fibular nerve into a superficial and a deep branch was extremely variable. It usually resulted about an inch below the lateral tibial condyle. But Schreiber (1956) described this division as occurring at the level of the head of the fibula. In the sheep, goat, pig, and horse, this division resulted in the vicinity of the lateral tibial condyle. May (1964) asserted that this division occurred in the sheep in the region of the head of the fibula. In the horse, according to Dobberstein and Hoffmann (1964) and Koch (1965), the fibular nerve divided into a superficial and a deep branch near the fibular head.

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The superficial fibular nerve (N. fibularis superficialis) (N. peroneus superficialis of the N.A.V.) was, relatively, the stronger of the two. It descended somewhat superficially between the M. fibularis longus cranially, and the M. extensor digitorum (digitalis) lateralis (M. extensor digiti IV) caudal to the middle of the leg and subsequently crossed the deep face of the tendon of the M. fibularis longus in the ox, sheep, goat, and pig. In the horse it descended in the groove bounded by the M. extensor digitorum (digitalis) longus and the M. extensor digitorum (digitalis) lateralis, beneath the deep fascia of the leg.

In the ox the superficial fibular nerve, at a variable level on the dorsal surface of the tarsus, gave off the lateral branch (N. digitalis pedis dorsalis communis IV) (N. digitalis dorsalis communis IV of the N.A.V.) which furnished a few twigs to the fifth digit (N. digiti V pedis dorsalis proprius) and continued as the N. digiti IV pedis dorsalis proprius. The est of the superficial fibular nerve continued distally and, near the middle of the metatarsus in the ox and sheep and, slightly below in the goat, gave off the medial branch (N. digitalis pedis dorsalis communis II) (N. digitalis dorsalis communis II of the N.A.V.) which released a few twigs to the second digit (N. digiti II pedis dorsalis proprius) and descended as the N. digiti III pedis dorsalis proprius. The continuation of the superficial fibular nerve (N. digitalis pedis dorsalis communis III) (N. digitalis dorsalis communis III of the N.A.V.) received a slip (Ramus communicans cum nervo fibulare superficiale) (R. communicans cum n. metatarseo dorsali of the N.A.V.) from the N. metatarseus dorsalis III and finally terminated as the Nn. digitales III et IV pedis dorsales proprii. Preuss and Wunsche (1965) referred to the medial and lateral branches of the superficial fibular nerve, similar to us, as the N. digitalis pedis dorsalis communis II and the N. digitalis pedis dorsalis communis IV, respectively, because they reasoned that the second and fifth digits were displaced on the planta and the original position of the nerves could be established by replacing the accessory digits below on the abaxial side of the main digits.

In the ox the N. digitalis pedis dorsalis communis III received a slip from the third dorsal metatarsal nerve on the dorsal aspect of the fetlock joint. In agreement with Chauveau and Arloing (1902) and Schreiber (1956) we found that the combined trunk immediately divided into a dorsal and a plantar (communicating) branch. The dorsal branch soon divided into the Nn. digitales III et IV pedis dorsales proprii. The plantar (communicating) branch extended backwards through the interdigital space, supplied twigs to the adjacent surfaces, and later joined the dorsal (communicating) branch of the N. digitalis plantaris communis III of the medial plantar nerve or its terminal branches (Ramus communicans cum n. digitali plantari proprio III laterali et Ramus communicans cum n. digitali plantari proprio IV mediali of the N.A.V.). A similar observation was also made by Reimers (1929), Schreiber (1956) and Preuss and Wunsche (1965). An identical relationship was noticed in the sheep and goat, although May (1964) did not mention the connection between the dorsal proper digital nerves and the plantar proper digital nerves in the sheep.

In the pig the superficial fibular nerve detached the medial branch on the dorsal aspect of the tarsus. Below the tarsus the medial branch received a contribution from the saphenous nerve (N. saphenus) and formed the N. digitalis pedis dorsalis communis II (N. digitalis dorsalis communis II of the N.A.V.) which later terminated as the Nn. digitales II et III pedis dorsales proprii. This finding concurs with that of Bruni and Zimmerl (1951), but Montané and Bourdelle (1920) described a branch of the external saphenous nerve (N. cutaneus surae plantaris lateralis) as joining the medial branch of the superficial fibular nerve, which we could not reveal in our investigation. Subsequently, the rest of the superficial fibular nerve divided into a medial and a lateral branch on the dorsal aspect of the tarsometatarsal articulation.

Immediately below the tarsus, the lateral branch gave off the N. digiti V pedis dorsalis lateralis. About the middle of the metatarsus it gave off the N. digitalis pedis dorsalis communis IV (N. digitalis dorsalis communis IV of the N.A.V.) which finally terminated into the Nn. digitales IV et V pedis dorsales proprii. The continuation of the lateral branch continued distally and, at the fetlock joint, received a slip(Ramus communicans cum nervo fibulare superficiale) from the third dorsal metatarsal nerve and constituted the N. digiti IV pedis dorsalis medialis. The medial branch of the superficial fibular nerve extended distally and, near the fetlock joint, received a slip (Ramus communicans cum nervo fibulare superficiale) from the N. metatarseus dorsalis III and descended as the N. digiti III pedis dorsalis lateralis. Therefore, a N. digitalis pedis dorsalis communis III (N. digitalis dorsalis communis III) was missing, as the innervation of the 3rd interdigital space was effected separately by both medial and lateral branches of the superficial fibular nerve. But Chauveau and Arloing (1902), Montané and Bourdelle (1920), Bruni and Zimmerl (1951), and Schneider and Zintzsch (1962) described three dorsal metatarsal branches which provided sensory supply to the adjacent dorsal sides of the corresponding digits of the second, third and fourth interdigital spaces. In the horse the superficial fibular nerve furnished the fascia and skin on the dorsomedial aspect of the tarsus, and the dorsal, dorsomedial and dorsolateral aspects of the metatarsus on or around the fetlock joint. Martin(1923) reported that the superficial fibular nerve ramified on the fetlock joint; Reimers (1929) mentioned it as terminating on the dorsal surface of the third metatarsal; Grau (1935) referring to Zimmerl (1930) related that it extended to the first phalanx; and Ellenberger and Baum (1943) stated that it supplied the skin lateral to the leg, tarsus and metatarsus. The superficial fibular nerve ramified in the skin on the dorsal surface of the metatarsus and first phalanx (Bruni and Zimmerl 1951), supplied the skin along its course in the leg and foot (Dobberstein and Hoffmann 1964), ramified as far as the dorsal face of the tarsus and metatarsus, where it expended in the skin, but some filaments extended as far as the fetlock joint (Tagand and Barone 1964) and supplied the skin dorsal and lateral to the leg, dorsal to the tarsus, and dorsolateral to the metatarsus to the fetlock joint (Koch 1965).

The deep fibular nerve (N. fibularis profundus) (N. peroneus profundus of the N.A.V.), after its separation from the superficial fibular nerve, passed below the M. fibularis longus and gave off several muscular rami which, in all animals, supplied the flexors of the tarsus and extensors of the digit(s). It descended along the longitudinal groove on the dorsal aspect of the metatarsus as the N. metatarseus dorsalis III,

beneath the M. extensor digitorum (digitalis) brevis. Near the fetlock joint it contributed a slip to the N. digitalis pedis dorsalis communis III in the ox, sheep, goat, and two separate twigs in the pig. In the horse the deep fibular nerve divided into the N. metatarseus dorsalis II and the N. metatarseus dorsalis III. Both of them gave rise to the N. digitalis dorsalis III medialis and the N. digitalis dorsalis III lateralis (N.A.V.). They terminated in the fascia and skin around the fetlock joint. Reimers (1929) asserted that it continued in the N. digiti III pedis dorsalis tibialis and the N. digiti pedis dorsalis fibularis and ramified on the medial and lateral sides of the dorsal surface of the third digit, respectively. Bruni and Zimmerl (1951) described the deep fibular nerve as terminating in the skin on the dorsal and medial surfaces of the metatarsus to the fetlock joint; supplying the skin on the anterior and lateral faces of the metatarsus and the lateral face of the fetlock joint (Sisson and Grossman 1953; Tagand and Barone 1964). Tagand and Barone (1964) further stated that some of its filaments may possibly extend to the dorsal face of the phalanges towards the pad. Dobberstein and Hoffmann (1964) alleged that the deep fibular nerve supplied the periosteum and corium of the hoof. Koch (1965) asserted that the lateral branch extended distally and entered the corium of the hoof. It united with the N. metatarsicus plantaris profundus medialis through an exchange of fibers.

#### The N. tibialis (Fig. 2)

The tibial nerve, after its separation from the fibular nerve (N. fibularis), stretched distally through the popliteal fossa, somewhat medial to the popliteal lymph node (Ln. popliteus). In all animals the tibial nerve supplied, in general, the extensors of the tarsus and flexors of the digit(s). Besides, it also furnished the M. popliteus. The tibial nerve usually divided, at a variable level, close to the tuber calcanei in a medial and a lateral plantar nerve in all animals. In agreement with Chauveau and Arloing (1902) and Bruni and Zimmerl (1951) we could not find a transverse anastomosis between the plantar nerves in the ox, but Preuss and Wünsche (1965) stated that a Ramus communicans is present between the lateral and medial plantar nerves in exceptional cases.

The medial plantar nerve, slightly above the fetlock joint, divided into the N. digitalis plantaris communis II and the N. digitalis plantaris communis III in the ox, sheep, and goat. The former detached a few twigs to the second digit (N. digiti II plantaris proprius) and continued as the N. digiti III plantaris proprius, while the latter nerve descended as the Nn. digitales III et IV plantares proprii. Some of its twigs extended dorsally through the interdigital space and joined the N. digitalis pedis dorsalis communis III or its terminal branches. In the pig the medial plantar nerve, slightly above the fetlock joint, divided into a medial and a lateral branch. The Ramus medialis gave off the N. digiti II plantaris medialis and continued as the N. digitalis plantaris communis II, which soon split into the Nn. digitales II et III plantares proprii. The Ramus lateralis gave off a communicating twig which joined the medial branch of the lateral plantar nerve to constitute the N. digiti IV plantaris proprius. It continued farther as the N. digitalis plantaris communis III which divided into the Nn. digitales III et IV plantares proprii. A similar finding was recorded by Reimers (1929), Bruni and Zimmerl (1951) and Schneider

and Zintzsch (1962). In the horse the medial plantar nerve gave off a Ramus communicans to the lateral plantar nerve and continued distally, following division near the fetlock joint, as the Ramus dorsalis and the N. digitalis plantaris medialis s. proprius.

The lateral plantar nerve continued as the N. digitalis plantaris communis IV which furnished a few twigs to the fifth digit (N. digiti V plantaris proprius) and descended as the N. digiti IV plantaris proprius in the ox, sheep, and goat. In the pig the lateral plantar nerve, below the tarsus, gave off the N. digiti V plantaris lateralis and continued as the N. digitalis plantaris communis IV which, near the middle of the metatarsus, divided into a medial and a lateral branch. The medial branch joined the communicating branch released by the Ramus lateralis of the medial plantar nerve and continued as the N. digiti IV plantaris proprius. The lateral branch of the N. digitalis plantaris communis IV continued as the N. digiti V plantaris proprius. This finding is in agreement with Reimers (1929) and Schneider and Zintzsch (1962). Montané and Bourdelle (1920) stated that the lateral plantar nerve constituted the collateral concentric of the lateral accessory digit and a division destined to the formation of the eccentric collateral plantar of the lateral principal digit. In the horse the lateral plantar nerve released a strong twig to supply the M. interosseus medius and coursed similar to medial plantar nerve. According to Dobberstein and Hoffmann (1964), as in the forelimb, the Nn. metatarsici plantares profundi (Nn. metatarsei plantares) extended from it. Koch (1965) asserted that the Nn. metatarsia plantares profundi lateralis amd medialis resemble the deep metacarpal nerves, except that the N. metatarsicus plantaris profundus medialis exchanged fibers with the medial branch of the Ramus profundus of the fibular nerve within the region of the first phalanx. According to Grau (1935) the Ramus communicans, between the medial and lateral plantar nerves, is absent only in 30% of the cases. Ellenberger and Baum (1943) referred to Schreiber (1917), stating that the communicating branch between the plantar nerves is absent in  $12^{\sigma_0}$  of all cases. Grau (1935) further alleged that the anatomical areas of ramification of the plantar nerves do not correspond to the area of numbness observed in experiment. Practically, we have double innervation of the skin of the hind digits in a higher quality than in the fore digits.

# The N. saphenus (Figs. 1, 2)

The saphenous nerve appeared along the caudal border of the M. sartorius near the distal third on the medial aspect of the thigh. Its mode of branching was variable amongst various species and also between different specimens within the same species.

In the ox, sheep, and goat the saphenous nerve ramified in the fascia and skin in the medial aspect of the thigh, craniomedial aspect of the middle two-thirds of the leg and caudomedial aspect to the tarsus and the area immediately distal to it. In the pig it supplied twigs to the fascia and skin in front of the stifle joint, the medial aspect of the thigh and leg, and the dorsomedial aspect of the tarsus. At the distal third of the tibia, the saphenous nerve divided into medial and lateral branches. The lateral branch gave off a slender branch to join the Ramus medialis of the superficial fibular nerve to form the N. digitalis pedis dorsalis communis II. This finding is in agreement with Bruni and Zimmerl (1951), whereas

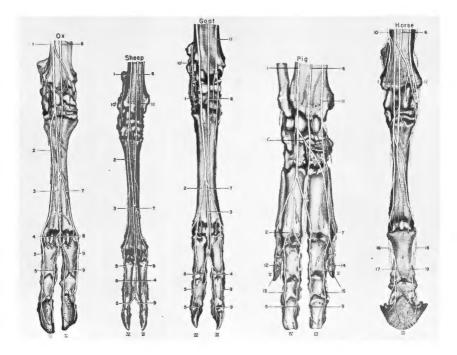


Figure 1. Nerves of the distal part of the right hind limb-dorsal view (schematic).

- 1 N. fibularis (peroneus) superficialis
- 1' Ramus medialis
- 2 N. digitalis pedis dorsalis communis IV
- 3 N. digitalis pedis dorsalis communis III
- 4 Ramus communicans cum n. digitali plantari proprio III laterali et Ramus communicans cum n. digitali plantari proprio IV mediali
- 5 N. digiti IV pedis dorsalis proprius
- 6 N. fibularis (peroneus) profundus
- 7 N. digitalis pedis dorsalis communis II
- 8 Ramus dorsalis

- 9 N. digiti III pedis dorsalis proprius
- 10 N. cutaneus surae plantaris lateralis
- 10' Ramus cranialis of 10
- 11 N. saphenus
- 11' Ramus cranialis of 11
- 12 N. digiti V pedis dorsalis lateralis
- 13 Nn. digitales IV et V pedis dorsales proprii
- 14 N. digiti II pedis dorsalis medialis
- 15 Nn. digitales II et III pedis dorsales proprii
- 16 N. digitalis plantaris lateralis
- 17\* Ramus dorsalis
- 18 N. digitalis plantaris medialis
- 19\*\*Ramus dorsalis

In view of the accompanying blood vessels these should be called as the N. digitalis pedis dorsalis lateralis and N. digitalis pedis dorsalis medialis, respectively.

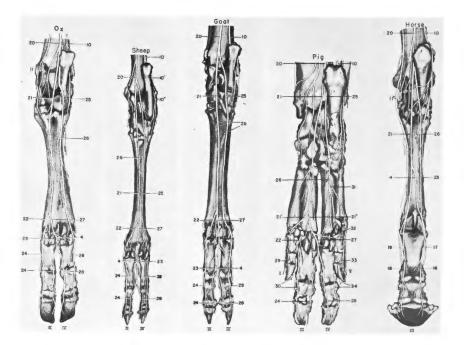


Figure 2. Nerves of the distal part of the right hind limb-plantar view (schematic).

- 4 Ramus communicans cum n. digitali plantari proprio III laterali et Ramus communicans cum n. digitali plantari proprio IV mediali
- 10 N. cutaneus surae plantaris lateralis
- 10' and 10'' Ramus cranialis and Ramus caudalis of 10
- 11 N. saphenus
- 11" Ramus caudalis of 11
- 16 N. digitalis plantaris lateralis
- 17 Ramus dorsalis
- 18 N. digitalis plantaris medialis
- 19\*\*Ramus dorsalis
- 20 N. tibialis
- 21 N. plantaris medialis
- 21' Ramus medialis
- 21" Ramus lateralis

- 22 N. digitalis plantaris communis II
- 23 Ramus plantaris
- 24 N. digiti III plantaris proprius
- 25 N. plantaris lateralis
- 26 Ramus profundus
- 27 N. digitalis plantaris communis III
- 28 N. digiti IV plantaris proprius
- 29 N. digiti II plantaris medialis
- 30 Nn. digitales II et III plantares proprii
- 31 N. digitalis plantaris communis IV
- 32 Ramus communicans
- 33 N. digiti V plantaris lateralis
- 34 Nn. digitales IV et V plantares proprii

In view of the accompanying blood vessels these should be called as the N. digitalis pedis dorsalis lateralis and N. digitalis pedis dorsalis medialis, respectively.

Montané and Bourdelle (1920) stated that the external saphenous nerve gave off the anastomotic branch to the medial branch of the superficial fibular nerve. The medial branch of the saphenous nerve descended as the N. digiti II pedis dorsalis medialis. Similar observations were also made by Chauveau and Arloing (1902), Montané and Bourdelle (1920) and Bruni and Zimmerl (1951). But, on the contrary, Reimers (1929) and Ellenberger and Baum (1943) described that the saphenous nerve ramified on the medial side of the tarsus and metatarsus by means of twigs. In the horse the saphenous nerve furnished the fascia and skin on the medial aspect of the stifle joint, mediodorsal aspect of the distal third of the leg, and the dorsal and mediodorsal aspects of the tarsus and extended to the medial aspect of the fetlock joint. Reimers (1929) stated that the saphenous nerve ramified on the medial side of the tarsus and metatarsus by means of twigs. Martin (1923), Ellenberger and Baum (1943) and Sisson and Grossman (1953) mentioned that it supplied the distal half of the medial surface of the thigh, the medial surface of the leg and the medial surface of the metatarsus. Ellenberger and Baum (1943) further stated that it extended to the fetlock joint. Bruni and Zimmerl (1951) stated that it released twigs along its course in the region of the thigh and leg to the anterior surface of the tarsus. Dobberstein and Hoffmann (1964) alleged that the saphenous nerve extended to the coronary border of the hoof.

From the above description, it is quite evident that there are several discrepancies regarding the extent of various nerves in different species as observed by various authors. Simple morphological investigation will not provide solutions for all these disagreements, but will lay down the basis for other experimental works. Experimental investigations, like neurectomy, conduction anesthesia or narcosis, electrophysiological stimulation, etc. should be employed to complement the morphological studies which, in turn, would eliminate some inconsistencies existing in the literature.

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# REVIEW OF GENUS <u>ORECTODERUS</u> UHLER WITH A KEY TO THE SPECIES (HEMIPTERA, MIRIDAE)

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ABSTRACT. A key is provided for ten species of <u>Orec-toderus</u> of which six species are new. New species are <u>O. bakeri</u>, <u>O. cockerelli</u> and <u>O. salicis</u> from Colorado; <u>O. utahensis</u> from Utah and Colorado; <u>O. montanus</u> from Wyoming and Idaho; and <u>O. ruckesi</u> from Wyoming.

The genus <u>Orectoderus</u> is known only from North America, being erected by Uhler (1876) for a single new species, <u>O. obliquus</u> Uhler. The genus is most closely related to <u>Teleorhinus</u> Uhler which may be distinguished by the more strongly clavate second antennal segment, by the flat and indistinct calli, and with females always macropterous. The females of <u>Orectoderus</u> are brachypterous, with hemelytra very short, the apices acuminate and turned to a vertical position. Thus far females have been collected in such limited numbers it has been impossible to associate them positively with males of the same species. One exception is <u>O. obliquus</u> Uhler which appears to be the only species found in the northeastern states, from Michigan to Maine. The genera <u>Orectoderus</u> and Teleorhinus are placed in subfamily Phylinae, tribe Hallodapini.

Genus Orectoderus Uhler

# Key to the species, males only

1.	Hemelytra without red coloring
2.	Corium with inner half subtranslucent to white, the white color extending as a band around inner apical angle of corium, across paracuneus to join white area at base of cuneus 3 Corium with fuscous to black on inner apical angle, white band not evident

 Pronotal disk shining black, transversely depressed just behind calli; length of antennal segment II, 2.24 mm, greater than width of pronotum at base plus width of vertex (1.97 mm); length 6.7-7.0 mm.

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	Pronotal disk not shining, surface with a dull pruinose sheen, basal edge of calli scarcely impressed; length of antennal segment II, 1.93 mm, not equal to width of pronotum plus width of vertex (2.37 mm); length 6.8-7.5 mm O. <u>bakeri</u> n.sp.
4.	Clavus fuscous to black on inner half for full length of the commissure
5.	Calli distinctly swollen, disk of pronotum somewhat depressed just behind the calli
6.	Rostrum not reaching to posterior trochanters.       7         Rostrum reaching upon posterior trochanters; length 5.8 mm.       7
7.	Length of antennal segment II not equal to twice the width of head. 8 Length of antennal segment II greater than twice the width of head across the eyes; length 6.8 mm <u>O</u> . <u>utahensis</u> n.sp.
8.	Width of pronotum at base subequal to width of head plus dorsal width of an eye; length 6.2 mm.O. montanus n.sp.Width of pronotum not equal to width of head plus dorsal width of an eye; length 5.7 mm.O. cockerelli n.sp.
9.	Pronotum and hemelytra polished or shining; color uniformly black, or with basal half of corium and of cuneus, pallid to white; length 6.8-7.6 mm <u>O. obliquus</u> Uhler Pronotum and hemelytra alutaceous and rugulose; length 7.5 mm <u>O. ruckesi</u> n. sp.
	Orectoderus obliquus Uhler
18	76 Orectoderus obliquus Uhler, Bul. U.S. Geol. Surv. 1:319, n. gen.,

n. sp. 1941 Orectoderus obliquus Knight, Ill. Nat. Hist. Surv. Bul., <u>22(1)</u>:23, 52, figs.

In the original description one specimen was recorded from Colorado, while other specimens were mentioned from Mass., Conn., Pa., Ill., Kans. and southern Canada. Uhler states that "immature specimens are chestnut brown" in color and the writer has also found this to be true. Uhler also states that "all specimens thus far observed, of both sexes, have fully developed hemelytra and wings." The present writer can only say he has never seen a macropterous female specimen.

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# REVIEW OF GENUS ORECTODERUS

Definite records: MAINE:  $3^{\sigma\sigma}$  June 24, 1934;  $\sigma'$  July 1916, Cumberland Co. (A. Nicolay).  $\sigma'$  June 23, 1936, Bar Harbor (A.E. Brower). MASSACHUSETTS:  $\sigma'$  July 6, 1925, Woods Hole (E.D. Ball). NEW YORK:  $\sigma'$  July 2, 1915, Half Way Hollow Hills, Long Is. (W.T. Davis).  $6^{\sigma\varphi}$  July 4-7, 1915, Bayshore, Long Is. (Chris E. Olsen). MICHIGAN:  $\sigma'$  July 1, 1949, Crawford Co. (R.R. Dreisbach). SOUTH DAKOTA:  $\sigma'$  June 25, 1923, Whitewood (H.C. Severin).  $3^{\sigma\sigma'}$  July 20, 1928, Black Hills, alt. 5000 ft. (A.A. Nichol). WYOMING:  $\sigma'$  July 30, 1927, Sundance (H.H. Knight). COLORADO:  $9^{\sigma\varphi}$  June 1900, Fort Collins (E.D. Ball). NEW MEXICO:  $\sigma'$  June 14, Taos Canyon;  $2^{\sigma\sigma'}$  July 15, 1956, Tesque (R. & K. Dreisbach). CANADA:  $\sigma'$  June 21, 1919, Edmonton, Alberta,  $2^{\varphi\varphi}$  July 29, 1926, Penobsquis, New Brunswick (C.A. Frost).

# Orectoderus longicollis Uhler

# 1895 Orectoderus longicollis Uhler, Bul. Colo. Agr. Exp. Sta. 31:47.

In the last three lines of the original description Uhler stated: "Only a single specimen, a male, has thus far been brought to my notice. The female is a desideratum requiring the attention of collectors." "Steamboat Springs, July 14th (Baker)" The last line gives data for the single male specimen.

Some years ago, in 1926, I spent the summer months working at the U.S.N.M., and one objective was to locate any specimens that might be types of Uhler species. Under the label of <u>Orectoderus longicollis</u> Uhler, there was a single male specimen. The pin labels read: "Colo. 1341," "Det. Uhler" and bottom label "C.F. Baker collection." This specimen agrees with the description of <u>O</u>. <u>longicollis</u> Uhler, and I am confident it refers to the species described. However, the label "Colo.1341" when translated by the numbers in Dr. Gillette's "Accession Book," reads: Fort Collins, Colo. 8/15/93, C.F. Baker, in foothills." So it is not the type specimen recorded at end of the original description: "Steamboat Springs, July 14th (Baker)."

In July of 1964, I spent a week, July 11 to July 16 inclusive, collecting Miridae around Steamboat Springs, Colorado. My chief objective was to collect Miridae on the same days of July as C.F. Baker did just 70 years earlier, in 1894. The spring season of 1964 in Colorado was about one week late according to people who follow such matters. The people operating the Western Motel at Steamboat Springs said the peony plants were blooming one week later than the previous year, 1963. So I was a week too early for best results, but several species emerged to the adult stage while I was there. Although I did collect some 18 of the species taken by Baker around Steamboat Springs, I was too early for 6 or 7 others, particularly species reported on willows (Salix).

New records for O. longicollis Uhler are:  $2^{d'd}$  July 11, 1964, alt. 6700 ft., 1 mi. S. E.:  $4^{d'd'}$  July 15, 1964, alt. 6900 ft., Steamboat Springs, Colorado (H. H. Knight), taken about 500 yards east of the skee jumping platform. Some specimens were taken on Artemisia tridentata and others by sweeping the herbaceous vegetation in open spaces between the Artemisia shrubs. Specimens of O. longicollis were so scarce the species cannot be considered phytophagous, but more likely a predatory form, or perhaps having some special relationship with ants since the females are mimetic in form.

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## Orectoderus schuhi Knight

1964 Orectoderus schuhi Knight, Iowa State J. Sci. 39:149.

This species is the only known member of the genus having red on the hemelytra. It was described from Oregon but now we have specimens from Nevada. NEVADA: 2<sup>d</sup> June 26, 1956, Spruce Mountain, Elko Co. (W. C. Russell).

## Orectoderus arcuatus Knight

# 1927 Orectoderus arcuatus Knight, Ent. News 38:302.

In the key this species runs in a couplet with O. bakeri n. sp., but is distinguished by the polished surface and the transverse depression on pronotal disk just behind the calli. In the original description, one specimen, "S Colo. 1690," was recorded as a paratype; in the present paper this specimen is placed with the new species O. bakeri, with full data given for the label. At the present it appears that O. arcuatus Kngt. is known only from the State of Washington.

# Orectoderus bakeri new species

Runs in the key to the couplet with <u>arcuatus</u> Kngt., but distinguished by the flat pronotal disk, the calli on the same level, not depressed just behind; the surface pruinose, not shining.

Male. Length 7.2 mm, width 2.2 mm. Head: width 1.12 mm, vertex . 47 mm; black. Antennae: segment I, length . 41 mm, brownish black, paler on apical half; II, 1.97 mm, apical two-fifths incrassate (.17 mm thick), black, basal half dusky yellow, narrowly blackish on base; III, 1.09 mm, yellowish brown, narrowly black on base and apex; IV, broken. Rostrum, length 2.5 mm, reaching to apex of middle coxae. Pronotum, length .98 mm, width at base 1.60 mm; lateral margins as viewed from above only slightly excavate; disk forming a level surface from collar across inner angles of calli to basal margin, outer half of calli slightly impressed on basal margins, disk transversly rugulose, alutaceous and pruinose, not shining; collar nearly flat but slightly elevated on anterior margin. Mesonotum broadly exposed, scutellum moderately convex, nearly smooth but alutaceous and pruinose. Hemelytra finely wrinkled, alutaceous, pruinose in certain angles of light; clavus black, outer margins or exterior to claval vein, white; corium and embolium black, but basal third of corium and extending as a broad band parallel to claval margin, and extending around inner apical angle, across paracuneus to basal angle of cuneus, pallid to white; cuneus black but inner angle white; clothed with recumbent, and in part appressed, simple golden yellow pubescence. Membrane rather uniformly dark fuscous, the veins nearly black. Ventral surface black, somewhat shining, ostiolar peritreme black, venter pruinose on sides and beneath. Legs yellowish to orange colored, bases of coxae black; tibiae more yellowish, spines and tarsi black.

Holotype: July 11, 1964, alt. 6700 ft., on the heights above Steam -

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boat Springs, Colorado (H.H. Knight). <u>Paratypes</u>: July 15, 1964, alt. 6900 ft., July 16, 1964, alt. 7000 ft., on the mountain above Steamboat Springs, Colorado (H. H. Knight), all in sight of the town; swept from mixed vegetation between patches of <u>Artemisia tridentata</u>. Also a male "Colo. 1690," the record book states: Jackson Co., Grizzly Creek, alt. 9500 ft., Colorado (C.F. Baker), swept from Artemisia tridentata.

No doubt this is a rare, predacious species, that occurs in limited numbers at the higher elevations. Named in honor of the first collector, Prof. Carl F. Baker, who collected type material for many Colorado species of Miridae; and who in later years developed the Department of Entomology at the Agricultural College, Los Banos, Philippine Islands.

## Orectoderus montanus new species

Allied to O. longicollis Uhler but differs as shown in the key; calli not so strongly swollen, or disk so strongly depressed behind calli.

Male. Length 6.2 mm, width 1.5 mm. Head: width 1.12 mm, vertex .51 mm. Antennae: segment I, length.41 mm, yellowish to brown, more fuscous beneath; II, 2.1 mm, pale dusky brown, apical half gradually thicker (.136 mm thick), brownish black; III, 1.2 mm, brownish; IV, .71 mm, brownish black. Rostrum, length 2.4 mm, reaching near tips of middle coxae, dark brown. Pronotum, length .98 mm, width at base 1.39 mm; lateral margins of disk moderately excavate as viewed from above, basal half flaring sharply to wider; calli slightly swollen, basal margins indicated by an impressed line; disk finely alutaceous and transversely rugulose; basal angles rounded, basal margin moderately excavate on middle third. Mesonotum broadly exposed, black, alutaceous. Scutellum nearly flat, slightly convex on middle, transversely rugulose, black, alutaceous.

Hemelytra with costal margins subparallel, surface not shining, but appearing alutaceous in some angles of light; clothed with recumbent and appressed, simple, golden yellow pubescence; color dark fuscous to black, basal half of corium and outer margin of clavus except near apex, pallid to white, the pallid area nearly wedge-shaped, forming an oblique line where pallid color meets black across the middle of corium but not reaching to apex of clavus; basal half of cuneus and part of paracuneus white; membrane and veins rather uniformly dark fuscous. Ventral surface dark brownish black to black, shining, venter more alutaceous on ventral surface. Legs yellowish brown, coxae darker, hind tibiae more reddish brown, tarsi dark fuscous; spines black, length not exceeding thickness of tibia.

Holotype: & August 8, 1927, Yellowstone National Park, Wyoming (H. H. Knight); author's collection. Paratypes: WYOMING: & August 12, 1918, Canyon Camp, Yellowstone National Park (A. L. Melander). 2 & July 20, 1925, Yellowstone National Park (A. A. Nichol). IDAHO: & July 12, 1936, Henry's Lake, alt. 7000 ft. (R. E. Miller).

#### Orectoderus utahensis new species

Allied to <u>O</u>. montanus, but distinguished by the shorter rostrum which reaches only to base of mesosternum; second antennal segment longer, length exceeding twice the width of head.

Male. Length 6.8 mm, width 1.56 mm. Head: width 1.10 mm, vertex .54 mm; black. Antennae: segment I, length .41 mm, yellowish orange; II, 2.4 mm, apical two-fifths clavate (.17 mm thick), black, basal half more slender than segment I, dusky yellow, base sometimes darker; III, broken. Rostrum, length 2.24 mm, only reaching to base of mesosternum. Pronotum, length 1.02 mm, width at base 1.36 mm; as viewed from above the lateral margins deeply excavate, anterior half with margins; disk transversely depressed behind calli, then more convex on basal half, surface transversely rugulose, alutaceous, somewhat pruinose; basal angles rounded, basal edge line forming a slightly excavated arc across central area; color black. Mesonotum broadly exposed, flat; scutellum moderately convex, transversely rugulose, black. Hemelytra surface finely wrinkled, alutaceous, pruinose in certain angles of light; black, clavus with outer half or exterior to claval vein, basal third of embolium and clavus, pallid or white; basal half of corium with margin of the white area angling back across middle to meet clavus just before apex; cuneus white, apical half black, the white area invading paracuneus slightly; membrane and veins uniformly dark fuscous or black. Ventral surface black, mesosternum shining; venter pruinose, two basal segments shining. Legs fuscous brown, coxae nearly black, tibiae more yellowish brown, spines and tarsi black.

Holotype: d. 1928, Heber, Utah (C.J.D. Brown); author's collection. Paratypes: 4dd July 9, 1938, 4 mi. S. Grand Mesa, Colorado. (R. Bauer and U. Lanham).

# Orectoderus salicis new species

Distinguished by the small size and the long rostrum that reaches upon posterior trochanters.

Male. Length 5.9 mm, width 1.87 mm. Head: width .98 mm, vertex Antennae: segment I, length . 37 mm, fuscous; II, 2.0 mm, .47 mm. Slender at base (.07 mm thick), tapering to clavate on apical half (.13 mm thick), fuscous brown, apical half reddish brown, clothed with suberect, short pubescent hairs; III, 1.10 mm, slender, cylindrical, reddish brown; IV, .68 mm, reddish brown. Rostrum, length 2.3 mm, reaching upon the posterior trochanters, dark reddish brown. Pronotum, length .98 mm, width at base 1.36 mm; brownish black, basal angles not wide enough to cover base of clavus; lateral margins as viewed from above, deeply excavate, margins parallel on anterior half, then flaring sharply on basal half to form the basal angles; basal margin sinuate, excavate on middle one-third; calli moderately convex, basal and inner margins indicated by an impressed line; disk transversely rugulose, surface slightly pruinose, finely alutaceous; collar area flat, slightly elevated; sides of pronotum shining, coxal cleft distinct, carried high up to point just under the moderately indicated lateral margin which is just beneath the calli. Mesonotum widely exposed, scutellum rather flat, disk strongly, transversely rugulose; black, surface pruinose.

Hemelytra slightly shining, but with pruinose reflections in some angles of light; moderately clothed with appressed and recumbent, pale to yellowish simple pubescence; costal margins nearly straight, subparallel; color dark fuscous to black, basal angles of corium and outer margin of clavus except apical one-third, forming a pallid to white spot which narrows apically and does not reach apex of clavus; cuneus pallid on basal half, shading gradually into fuscous on apical half; membrane and veins rather evenly shaded with dark fuscous. Ventral surface dark reddish brown to black, moderately shining, but with pruinose areas in some angles of light. Legs dark reddish brown, tibiae more pallid, spines black, length of spines about equal to thickness of segments. Genital segment distinctive of the genus, with details of structure distinctive of the species.

Holotype: July 17, 1964, alt. 11, 325 ft., Berthoud Pass, Colorado (H. H. Knight). Paratypes: J, taken with the type; also many nymphs were taken and released, as the species was just beginning to emerge to the adult stage. Nymphs and adults were taken only on mountain willow, apparently Salix scouleriana Barratt, which grew in patches on the south side of Highway 40 in Berthoud Pass, just opposite the extensive parking ground where the commercial area is developed. Numerous nymphs were collected while sweeping for adults but these were carefully returned to the willow shrubbery, that the species might have a better chance to survive and supply future collectors. The bugs were restricted to the willows where numbers of aphids were also taken, but no other insects were noticed. I believe that this species of Orectoderus must be predatory on the aphids. The altitude at this spot as shown by a sign just across the highway, is 11, 325 feet, so this must be about the highest type locality for a Mirid bug that we know of for North America.

# Orectoderus cockerelli new species

In the key this species runs in the couplet with <u>montanus</u>, but may be distinguished by the narrower pronotum, and viewed from above the lateral margins more nearly parallel.

Male. Length 5.8 mm, width 1.4 mm. Head: width .95 mm, vertex . 47 mm. Rostrum, length 2.4 mm, reaching to middle of intermediate coxae. Antennae: segment I, length. 37 mm, fuscous brown; II, 1.8 mm, more slender on basal half, gradually thickened on apical half (.12 mm thick), yellowish brown, dark reddish brown on apical third; III, 1.10 mm, fuscous brown; IV, missing. Pronotum, length .92 mm, width at base 1.19 mm; calli moderately swollen, basal margins barely evident; as viewed from above the lateral margins only slightly excavate in outline, more nearly parallel than in O. montanus; disk finely alutaceous, slightly shining, basal half somewhat rugulose, color brownish black. Mesonotum broadly exposed, dark brown; scutellum moderately convex, finely transversely rugulose, brownish black. Hemelytra with costal margins subparallel, surface not shining, finely wrinkled, minutely alutaceous; clothed with simple, recumbent, in part appressed, pallid to yellowish pubescence; color fuscous to brownish black, basal half of corium and outer margin of clavus except near apex, pallid to white, the pallid area nearly wedge-shaped, forming an oblique line where pallid meets black across the middle of corium, but not reaching to apex of clavus; basal half of cuneus and part of paracuneus, white; membrane and veins uniformly fuscous brown. Ventral surface brownish black, somewhat shining. Legs uniformly dark brown, spines black.

Holotype: July, 1916, Florissant, Colorado (T.D.A. Cockerell), on Potentilla fruticosa. Named for the collector, Dr. T.D.A. Cockerell, who was a noted entomologist and specialist with Hymenoptera.

## Orectoderus ruckesi new species

Allied to O. <u>obliquus</u> Uhler, but distinguished by the nearly flat pronotal disk and calli; not at all excavate behind the calli; as viewed from above the lateral line of disk nearly straight.

Male. Length 7.6 mm, width 2.3 mm. Head: width 1.05 mm, vertex . 47 mm. Rostrum, length 2.4 mm, reaching upon hind coxae. Antennae: segment I, length . 38 mm; II, 2.24 mm, slender at base (.07 mm thick), tapering gradually to thicker on apical one-third (.136 mm thick), dark fuscous brown, brownish black on apex; III, 1.36 mm, brownish black; Pronotum, length 1.10 mm, width at base 1.63 mm; as IV, missing. viewed from above the lateral margins form a slightly excavate line, nearly straight except flaring somewhat to form basal angles; calli nearly flat with the disk, the margins not clearly defined; dorsal surface alutaceous, slightly pruinose in some angles of light, central area of disk rugulose. Mesonotum widely exposed, brownish black, finely alutaceous; scutellum finely rugulose, black. Hemelytra somewhat elongated, brownish black, the dark areas finely alutaceous, somewhat pruinose in certain angles of light; basal half of corium and narrowly on edge of clavus, white, apical one-fourth of clavus and adjoining part of corium, brownish black; basal one-third of cuneus white; membrane and veins uniformly brownish black. Ventral surface brownish black, in part shining, pruinose in certain angles of light. Legs dark brown to reddish brown, tibiae more pallid, spines black; tarsi brownish black. Genital segment with distinctions, but differences appear small and not practical to use without dissection. The lack of material limits such studies at this time.

Holotype: <sup>o</sup> August 1-8, 1935, Green River Lake, alt. 8500 ft., Wind River Mts., Wyoming (H. Ruckes); Knight Collection. Named for the collector, Dr. Herbert Ruckes.

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#### LUTEINIZING HORMONE ACTIVITY IN BOVINE PLASMA<sup>1, 2</sup>

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ABSTRACT. Peripheral blood was collected from 10 Angus heifers at different reproductive stages. The plasma was concentrated by precipitating the proteins with ethanol and acetone. These plasma protein fractions were assayed for luteinizing hormone (LH) activity by the ovarian ascorbic acid depletion method. During the estrous cycle a peak in LH activity occurred about 4 hours after the onset of estrus, and from  $\frac{day}{T}$  to  $\frac{day}{16}$ , the hormone level was also elevated. At other times in the cycle, the LH level was markedly lower. Luteinizing hormone levels in blood from heifers which were in the early stages of pregnancy, or after hysterectomy or bilateral ovariectomy were determined.

An increase in blood levels of luteinizing hormone activity (LH) has been associated with the occurrence of ovulation in several species. According to Zaki et al. (1964), serum levels of LH activity were higher at estrus than any other stages of the estrous cycle in the cow. Anderson and McShan (1966) also reported an increase in plasma levels of LH at estrus in the cow, but little, if any, activity during the remainder of the cycle.

The experiments reported here were conducted to obtain information on the levels of luteinizing hormone in peripheral blood plasma during the estrous cycle, early pregnancy and after hysterectomy or bilateral ovariectomy in the cow. The ovarian ascorbic acid depletion assay was used for the determination of LH activity. It was necessary to concentrate hormone activity in the plasma because of the limits of the sensitivity of this bio-assay.

#### MATERIALS AND METHODS

Animals. Jugular vein blood was collected from 10 Angus heifers, 18-24 months old, at different stages of the estrous cycle, pregnancy,

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after hysterectomy or after bilateral ovariectomy. The day of estrus was designated day 1. Estrous cycles of 18-23 days were established before experimental use. The heifers were checked twice daily for estrous behavior with a vasectomized bull. Two heifers (no. 41 and 72) were observed frequently for evidence of estrous behavior. In these animals the onset of estrus (male acceptance) was established within 2 hours of its occurrence.

Collection of Peripheral Blood. The blood samples (0.6-1.0 liter) were obtained by free flow and collected in chilled flasks containing heparin (panheparin, Abbott). Blood used for the determination of lute-inizing hormone activity was collected at the following reproductive stages:

a. Within 6 hours after onset of estrus. Samples were obtained from heifers no. 41 and 72 at about hourly intervals after the onset of estrus. They were collected at two consecutive estrous cycles from heifer no. 72.

b. During the estrous cycle. These blood samples were obtained in two seasons, the first during November, 1966 (Experiment 1) and the second during March, 1967 (Experiment 2). Blood was collected at several stages of the estrous cycle from heifers no. 42, 68, 72 and 76 in Experiment 1, and from heifers no. 68 and 72 in Experiment 2.

<u>c.</u> Pregnancy. Heifers no. 37 and 75 were bled at the 20th and 30th day after artificial insemination.

<u>d</u>. After hysterectomy. Heifers no. 39 and 77 were hysterectomized at  $\frac{day 9}{days}$  of the estrous cycle and each was bled 10 and 20 days after surgery ( $\frac{days}{days}$  20 and 30 after the last estrus).

e. After bilateral ovariectomy. Heifer no. 9 was ovariectomized at day 19 of the cycle and bled 87 days later.

Plasma Protein Fractionation. Plasma protein fractionation was started immediately after centrifuging the blood at 18,000 x g for 20 minutes at 5°C. The methods used for precipitation of plasma protein fractions in this investigation were those described by Cohn et al. (1946) and Cartland and Nelson (1937). The fractionation procedure was carried out in a cold room (3-5°C). The plasma was stirred gently during the addition of sodium acetate-acetic acid buffer for adjustment of the pH to 7.2. An ethanol-water mixture (53%) by volume) was added with stirring to attain a final ethanol concentration of  $8^{\sigma_0}$ . After 24 hours the precipitate was removed by centrifugation. The pH of the supernatant was adjusted to 6.9 and ethanol-water mixture was added to attain a final ethanol concentration of 25%. The second precipitate was removed 24 hours later, suspended in 100-150 ml of water and the pH was adjusted to 6.9. The following day the supernatant was removed by centrifugation, an equal volume of acetone was added and the pH was adjusted to 6.0 with HCl. after 24 hours the precipitate was removed and lyophilized. The dried samples were stored at -10°C.

Bio-assay of Plasma Proteins. Luteinizing hormone activity was determined by the ovarian ascorbic acid depletion (OAAD) assay as described by Parlow (1961), Five rats were injected at each dose level of the reference and plasma protein preparations. There was a two-fold difference between the two dose levels of each preparation. The plasma protein preparations were injected on the basis of volume rather than concentration because the biological activity in several preparations was low even though the solution was viscous. The reference preparation (NIH-LH-B5) was given at two volumes having the same concentration of the hormone and at one volume having two concentrations of the LH. Each of these two dose levels gave similar results (relative potency = 1.03 and 0.09). The lower dosage of the reference preparation was 0.3 or 0.4 ug/rat.

The results are expressed as ug-equivalents of NIH-LH-B5/100 ml unfractionated plasma. The potencies of each sample were calculated by the method described by Finney (1952) for parallel line assay and the confidence limits were calculated by the method of Cox and Ruhl (1966). All bio-assays reported here are valid, with an index of precision = 0.207 + 0.012.

#### RESULTS

Luteinizing hormone activity increased (p < 0.05) in two of the three estrous cycles in which blood was collected hourly during the first 6 hours after the onset of estrus (Fig. 1). In the subsequent estrous cycle of heifer no. 72, however, the LH activity remained low during the period of 6 hours after estrus. The peaks of hormone activity (35.7 and 6.2 ug LH/100 ml plasma) occurred at 3-5 hours after the onset of estrus. These heifers ovulated 12-16 hours after the end of the estrous period. Ovulation was determined by rectal palpation.

During the estrous cycle plasma levels of luteinizing hormone activity increased from 6 hours after the onset of estrus (day 1) through the early luteal phase (days 7 and 9) in heifers no. 42, 68, 72 and 76 (Fig. 2; Experiment 1). This LH activity increased from about 5 to 15 ug/100 ml plasma in these animals. During the latter part of the cycle (days 12 to 21) LH activity declined to about 5 ug/100 ml plasma. In heifer no. 76 the activity increased between days 13 and 19. In a second series on heifers no. 68 and 72 (Experiment 2), plasma levels of LH doubled between days 5 and 8 of the estrous cycle (4.5 and 9.5 ug/100 ml). This was a significant increase (p < 0.05) in heifer no. 68. By day 16 the LH activity had declined (p < 0.05) to 3 ug/100 ml plasma in this animal. Plasma levels of LH activity in heifer no. 72 continued to rise to a peak of 15.5 ug/100 ml at day 16 and then they declined to 4 ug/100 ml by day 20. This peak of LH activity was higher (p < 0.05) than those levels at days 1,3,5 and 20.

Results of bio-assays of peripheral plasma from heifers in different reproductive states are presented in Table 1. During early pregnancy, LH activity remained low (1 ug/100 ml) in heifer no. 37, whereas it was higher (10 ug/100 ml) in heifer no. 75 at these two stages. After hysterectomy or bilateral ovariectomy, plasma levels of LH ranged from 2.7 to 5.5 ug/100 ml.

## DISCUSSION

An ovulatory peak of plasma LH activity at about 4 hours after the onset of estrus agrees with the observations of Jubb and McEntee (1955) in that the delta basophilic cells of the adenohypophysis become degranulated

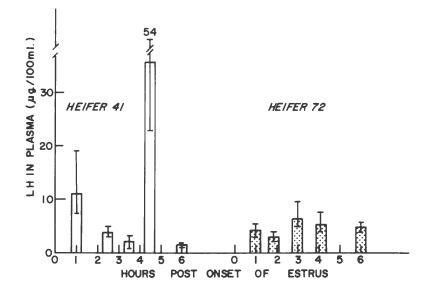


Figure 1. Luteinizing hormone activity in peripheral plasma from two Angus heifers during the first 6 hours after the onset of estrus. The values are expressed as the means and standard errors and equivalent to NIH-LH-B5.

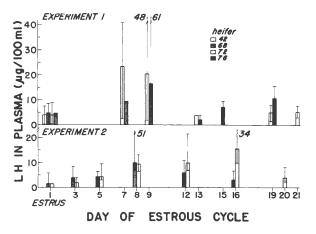


Figure 2. Luteinizing hormone activity in peripheral plasma from Angus heifers during the estrous cycle. The blood was collected from the animals in Experiment 1 during November, 1966 and from the heifers in Experiment 2 during March, 1967. The values are expressed as the means and standard errors and equivalent to NIH-LH-B5.

Animal no.	Physiologic state	LH*/100 ml plasma	95% Confidence limits			
37	day 20 of pregnancy	1.5	0.2 - 3.9			
37	day 30 of pregnancy	1.2	0.2 - 5.6			
75	day 20 of pregnancy	10.1	2.8 - 88.7			
75	day 30 of pregnancy	10,9	1.0 - 18.7			
39	10 days after hysterectomy**	5.1	2.7 - 9.4			
39	20 days after hysterectomy***	5.5	2.9 - 8.6			
77	10 days after hysterectomy**	4.4	3.2 - 6.0			
77	20 days after hysterectomy***	2.7	1.8 - 4.6			
9	87 days after ovariectomy	4.7	2.0 - 8.2			

TABLE 1. Luteinizing hormone activity in plasma from

Angus heifers in various physiologic states

\*NIH-LH-B5 µg equivalents.
\*\*Twenty days after last estrus.
\*\*\*Thirty days after last estrus.

during the first few hours of estrus. This finding differs, however, from that of Anderson and McShan (1966) who found elevated levels of LH activity 6 to 17 hours later in estrus.

The steepness of the ovulatory LH peak of heifer no. 41 suggests that hormone release is abrupt and short lived. The rapid drop in plasma LH activity thereafter indicates both a sharp decline in LH release and a circulating half-life of less than 20 minutes. The failure to find a high ovulatory LH peak in heifer no. 72 in two cycles may have resulted from release of LH from the pituitary gland during the time elapsing between blood collections. This peak may also have occurred before or after the 6-hour collection period.

Similar trends in LH level were shown during the cycle by the four heifers studied (Fig. 2). The variability in levels among animals may represent consistent among-animal differences. However, there may be a between-cycle variability within each heifer. The difference in LH activities between Experiment 1 and Experiment 2 may have resulted from seasonal effects. The trends are similar, however, during these two experimental periods.

In contrast to the acute release of LH at estrus there is the continuous output beginning by day 7 and lasting for about 10 days. If the half-life of LH is as short during this period as at estrus, the pituitary gland would be releasing large amounts of the hormone, in the order of 100 mg per day. During this period the pituitary gland content of LH increases according to Rahka and Robertson (1965), indicating that storage and secretion may occur simultaneously. Release of gonadotrophic activity during the mid-luteal phase of the cow is also indicated by increased follicular development (Rajakoski 1960) and estrogen production (Hansel et al. 1949). The decline in plasma LH in middle to late luteal phases may be associated with the failure of the corpus luteum, since administration of LH on day 16 extends the luteal lifespan (Donaldson and Hansel 1965).

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#### THE ALGAE IN A MILK-WASTE STABILIZATION POND\*

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ABSTRACT. Twenty genera and 28 species of algae were observed in a milk-waste stabilization pond. Fourteen species found in the pond have not been previously reported in other waste stabilization ponds. <u>Anabaenopsis elenkinii</u>, <u>Ankistrodesmus falcatus var. acicularis</u>, and <u>Kirchneriella lunaris var. irregularis</u> were dominant at some time during the sampling period with the Euglenales, especially <u>Euglena</u> acus, being the most prevalent. The general belief that species of <u>Chlorella</u> and <u>Scenedesmus</u> are universal constituents of the algal flora in stabilization ponds should be re-evaluated in light of further studies.

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## INTRODUCTION

The history of sewage stabilization ponds has been well documented (Caldwell 1946; Smallhorst 1960; Svore 1960; Towne et al. 1957; Towne and Horning 1960).

Algae in sewage stabilization ponds received very little attention until Silva and Papenfuss(1953) initiated a study to alleviate the lack of knowledge, as well as to supplement laboratory investigations into the role of algae in the purification process (Ludwig et al. 1951; Oswald et al. 1953 a, b.).

Allen (1955) and others (Neel and Hopkins 1956; Neel et al. 1961) have made extensive studies of the algal flora with respect to its seasonal variation and relative abundance in selected ponds. Other studies concerned with algae and their relationship to other environmental factors have been reviewed by Fitzgerald and Rohlich (1958), Heukelekein(1961), and Okun (1962).

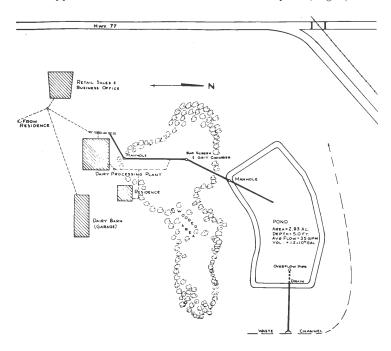
Most of the work reported has been concerned with domestic sewage ponds, but there are reports on industrial ponds and the affects of industrial wastes entering domestic ponds (MacKenthun and McNabb 1961; Maloney et al. 1960; Ryan 1950; Silva and Papenfuss 1953). Very few of these reports have been concerned with the algal flora of industrial ponds, and no one has made an extensive study of the algae in a milk-waste stabilization pond. It was the main purpose of this investigation to study the algal flora and its periodicity as well as certain environmental factors in a milk-waste stabilization pond.

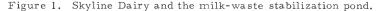
<sup>\*</sup> Received for publication December 15, 1967.

## RONALD L. RASCHKE

#### THE POND

The pond studied is located north of Skyline Dairy which is approximately 3 miles south of Lincoln, Nebraska, on highway 77. The pond area is 2.93 acres with a water-holding capacity of 1, 221, 860 gallons, and an average inflow of 35 gpm (gallons per minute). Due to the steep slopes, the 5-foot depth of the pond is attained near the edge. The influent pipe extends to the interior of the pond and the effluent pipe is a spill-over apparatus located at the east end of the pond (Fig.1).





Although ice and snow covered the pond from December, 1961, until March, 1962, it was used throughout the year. The pond receives both dairy and domestic wastes. The dairy wastes are usually the result of cleaning procedures, but cheese production was initiated on August 6, 1962, thus contributing to the amount and type of waste entering the pond. An average of 25 persons per day contribute to the domestic waste.

## MATERIALS AND METHODS

On each of the designated sampling days, two sampling stations were chosen at random. Samples were obtained in the morning between 8:30 and 9:30 a.m. and in the afternoon between 3:30 and 4:30 p.m. At each station two plankton samples were obtained: one near the surface (1') and

the other near the bottom (4') with the Horizontal Sampler (Fig. 2). Benthic and periphyton samples were collected in the morning near the edge of the pond with pipettes and tweezers.



Figure 2. Horizontal Sampler.

Dissolved oxygen (DO) was determined by the Alsterberg (Azide) modification of the Winkler Method (Standard Methods, 1961). The pH was measured with a Beckman pocket Model pH meter. Oxygen, pH, and water temperature were measured immediately after all eight samples were collected.

The algae procured from the field were usually examined the same day. If the examination could not take place immediately, the samples were preserved in Transeau's six-three-one solution (Prescott 1962). Algal enumerations were made according to Standard Methods (1961).

### RONALD L. RASCHKE

# **RESULTS AND DISCUSSION**

Twenty genera and 28 species of algae were observed in the milk-waste pond (Table 1). The Euglenales and Chlorococcales were represented by the greatest number of species. All of the species listed in Table 1 were representative of the phytoplankton except <u>Cladophora glomerata</u>, <u>Gomphonema montanum</u>, <u>Oscillatoria tenuis</u>, and <u>Arthrospira jenneri</u>. <u>Cladophora was found along the edge of the pond</u>, attached to weeds; and <u>Gomphonema</u>, a diatom, was attached to the <u>Cladophora</u>. <u>Oscillatoria tenuis</u> and <u>Arthrospira jenneri</u> were the predominant algae growing on the bottom and edge of the pond, and were the major constituents of blue-green mats on the surface of the pond.

A comparison of Table 1 with previously published data is of interest. The Volvocales, Chlorococcales, Euglenales, and Oscillatoriales are reported to comprise the greater portion of the phytoplankton in sewage ponds, and the composition of the algal flora in the milk-waste pond is in general agreement with these data. Repeated observations indicate that many species of chlamydomonads and euglenoids, as well as certain chlorococcalean species are restricted to aquatic environments which are rich in organic matter (Silva and Papenfuss 1953). Fourteen species found in the milk-waste pond (Table 1) have not been previously reported in other ponds. Species of Chlorella were not found in the pond although they are commonly found in such ponds (Allen 1955; Maloney et al. 1960; Neel et al. 1961; Silva and Papenfuss 1953). Neel and Hopkins (1956) found that they were not predominant in the Kearney, Nebraska, lagoon.

In the spring <u>Chlamydomonas</u> species and <u>Euglena</u> acus were the first algae to appear in the pond (Table 1). <u>Chlamydomonas</u> was dominant from April 2 to April 14. Other organisms dominant at some time during the sampling period are <u>Anabaenopsis</u> elenkinii, <u>Ankistrodesmus</u> falcatus var. <u>acicularis</u>, and <u>Kirchneriella lunaris</u> var. <u>irregularis</u>. The <u>Eugle-</u> ales, especially <u>Euglena</u> acus, appeared to be the most prevalent group throughout the investigation.

Neel and Hopkins (1956), Neel et al. (1961), and Silva and Papenfuss (1953) found that species of Chlamydomonas and Euglena were usually the earliest arrivals in the ponds; the data in Table 1 agree with their observations. Chlamydomonas and Euglena appeared after a period of oxygen depletion. Neel and Hopkins (1956) noticed in the Kearney, Nebraska, pond that Chlamydomonas almost unfailingly appeared after each anaerobic period. They reported that changes in dominant organisms appear to follow a pattern of seasonal succession, but also pointed out that the occurrence of Chlamydomonas populations showed that pond conditions also played a major role.

During the investigation, Water temperature ranged from  $1^{\circ}$ C to  $31.5^{\circ}$ C (Table 2). On May 18 water temperatures over  $20^{\circ}$ C occurred, and on the same date a three-fold increase in the number of species was recorded (Table 1). Temperature may not have been the main factor which caused this increase, but it probably was one of the contributing factors.

Temperature was usually uniform during each sampling period. The greatest vertical difference in temperature on the surface (1') and at the bottom (4') at any one sampling station was 5°C.

Species	Date Total no. Algae/ml.	10/29/61	12/15/61 0	1/14/62 0	2/16/62 0	3/3/62 0	3/18/62 0		4/14/62 582,508	5/1/62 181,068	5/18/62 95,999	6/11/62 11,694		7/23/62 208,463		
Anabaenopsis Élenkinii Miller Cladophora glomerata (L.) Kuetzing		D**									Р		P	Р	Р	Р
duglena acus Ehrenberg Chlamydomonas spp.		0						O D	O D	F	М	F	0	F	0	0
Ankistrodesmus falcatus var. acicularis (A. Br.) G.S. West										D	0	0	F	Α	F	0
Oscillatoria subbrevis Schmidle Scenedesmus dimorphus (Turp.) Kuetzing										0 0	A O	A O	0 F	0	F	0
Scenedesmus obliquus (Turp.) Kuetzing										0	0	0	F	0	F	0
Actinastrum gracilimum G.M. Smith											A	М	0	0	F	0
Arthrospira jenneri:Stizenb. ex Gomont											Р	Р	Р	Ρ	Р	Ρ
Euglena elastica Prescott Gloeocystis gigas (Kuetz.) Lagerheim											F	O F	F F	F	0	0
Gomphonema montanum Schumann											Р	Р	р	Р	р	Р
Lepocinclis fusiformis (Carter) Lemmermann											0	F	Α	М	0	0
Micractinium pusillum Fresenjus Oscillatoria tenuis C.A. Agardh Phacus sp. Scenedesmus quadricauda											0 P 0	O P O	O P F	O P O	P O	P F
(Turp.) de Brebisson Mitzschia palea (Kutz.) J. Smith											0 0	0	F	0 0	0	
Gloeocystis ampla (Kuetz.) Lagerheim												F	м	0		
Cryptoglena pigra Ehrenberg Kirchneriella lunaris var. Lrregularis G.M. Smith														0 A	D	D
Pandorina morum (Muell.) Bory														0	F	0
thabdomonas incurvum Tresenius														0	F	0
Cuglena proxima Dangeard Phacus pleuronectes (Muell.) Dujardin															0 0	0 0
Phacus pusillus Lemmermann Pyrobotrys stellata Korshikov															0	0

⊳ LGAE

Ē ⊳

MILK-WASTE

POND

<sup>\*</sup> Species which have not been reported in sewage stabilization ponds \*\* The letter following each component denotes relative abundance (number not volume) according to the following scheme

D Dominant (nearly a unialgal population)

M Most abundant (in a population where two or more components are abundant)

A Abundant

F Frequent

<sup>0</sup> Occasional

P Present, but no estimate of relative abundance

Physical and Chemical Factors	Sampling Dates														
	10/29/61	12/15/61	1/14/62	2/16/62	3/3/62	3/18/62	4/2/62	4/14/62	5/1/62	5/18/62	6/11/62	7/2/62	7/23/62	8/13/62	9/5/62
ater Temperature in <sup>O</sup> C															
Maximum	13.0	4.0	4.0	4.5	4.0	6,0	9.0	13.0	17.0	25.0	27.5	31.0	30.0	31.5	25.0
Minimum	15.0	3.0	2.0	3.5	1.0	1.0	5.0	9.0	12.0	23.0	22.0	24,2	26.0	24.0	19.5
H															
Maximum	8.0	7.1	7.1	7.4			7.4	7.7	7.7	8.0	8.1	8.4	8.1	8.4	7.8
Minimum	7.4	7.0	7.0	7.2			7.2	7,3	7.1	7.6	7.2	6.9	6.8	7.2	7.2
0 in mg/L															
Maximum	9.2	0.0	0.0	0.0	0.0	0.0	0.6	9.8	17.5	9.1	12.3	15.1	14.5	26.0	10.9
Minimum	0.8	0.0	0.0	0.0	0.0	0.0	0.0	7.0	6.6	0.7	0.0	0.0	0.0	0.0	0.0

#### Table 2. Physical and chemical data.

The pH ranged from 6.8 to 8.4 during the sampling period (Table 2). From June 11 to August 13 the lower pH values were found on the bottom, and the higher pH values on the surface.

Of all the chemical substances in ponds, oxygen is one of the most significant. It is significant both as a regulator of metabolic processes of organisms within the pond, and as an indicator of pond conditions (Ried 1961). During the sampling period, the DO of the pond ranged from 0 to 26 mg/L (Table 2). In the spring oxygen was not measurable until April 2, 1962, the same time the algae appeared for the first time that year.

The occurrence of a measurable DO at the 4-foot level on October 29, April 14, May 1 and May 18 could have been due to wind action.

Based upon a recognition of the importance of several biological processes, Oswald (1960) has distinguished three classes of ponds; Anaerobic, facultative, and aerobic. Anaerobic ponds were defined as those ponds in which the major fraction of the applied biochemical oxygen demand (BOD) is decomposed through methane fermentation. The term facultative was proposed for stabilization ponds most commonly described in the literature. Ideally BOD removal occurs as a result of both aerobic processes in the supernatant and anaerobic processes in the bottom sludge layers. In aerobic ponds organic matter is decomposed solely through the mechanism of aerobic oxidation. On the basis of DO, pH and general pond conditions, the milk-waste pond would be considered facultative.

This investigation and the sparse information on industrial ponds (Porges 1963) although incomplete, points out the great need for further investigations on industrial ponds.

The fact that 14 previously unreported organisms were discovered in this pond points out the incompleteness of our knowledge concerning the algal flora in sewage stabilization ponds. The general belief that <u>Chlorella and Scenedesmus</u> are universal constituents of the algal flora in these ponds will have to be re-evaluated in the light of further investigations. Information concerning the organisms inhabiting other types of industrial ponds and their periodicity are certainly incomplete. Benthic and epiphytic organisms in particular have not been extensively investigated, and the interrelationships between particular algae and other organisms in the pond are little known.

#### ACKNOWLEDGMENTS

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