THE OPOSSUM: ITS AMAZING STORY

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The opossum (*Didelphis virginiana* Kerr) was the first marsupial discovered by Western Europeans and is the only marsupial found in the United States. In spite of its fascination to scientists, the opossum is one of the most misunderstood mammals native to this country. This is due in part to its physical appearance. The opossum has a narrow, tapered snout and jaws that contain fifty teeth. When encountered the startled opossum will open its large mouth to expose these formidable teeth and growl to warn potential threats or predators. Because of this behavior, which is in fact a bluff, the opossum is considered by many to be a dangerous creature that is a threat to man and domestic animals. In addition, a long, naked, rat-like tail characterizes the opossum. Thus, the opossum has acquired the reputation of being a repulsive, aggressive, rat-like creature that should be avoided.

Generally, the only contact most individuals have with an opossum is a fleeting glimpse in a backyard or along a roadway during the late evening hours or perhaps as an encounter at night if pets are being fed outside. However, the opossums witnessed by most are generally road kill victims. The opossum and most other marsupials, with the exception of Australian kangaroos and koalas, are rarely exhibited in zoos or animal parks. As a result, only a few have been privileged to closely examine this fascinating animal. The Krauses have studied this marsupial since 1967 and the observations have resulted in the publication of over one hundred articles including an extensive bibliography on this species.

They have written this short, contemporary, well-illustrated book on the opossum specifically for those individuals with a limited scientific background but who are interested in learning more about this much-maligned species. The book is designed to be a useful text so that specific information with regard to the opossum can be located quickly and with ease. The most obvious change is in the organization of the material within each chapter. The subject matter has been broken down into small units and important points are emphasized in the text by **boldface type.** The narrative of the text has been developed in response to the most frequently asked questions from the general public gathered during a series of presentations given on the opossum.

It is their hope that after becoming more familiar with the life and ways of the opossum the public will begin to understand and appreciate that the opossum is not the fearsome, repulsive creature they assume it to be. Rather it is one of the most fascinating mammals native to the United States.

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Introduction

The common opossum or Virginia opossum (*Didelphis virginiana* Kerr) is one of the most fascinating mammals of North America and has intrigued biologists since its initial observation by Western Europeans. Although a wealth of information has been published on the opossum, these articles are scattered throughout a very diverse scientific literature with regard both to discipline and time. Yet, in spite of its fascination by scientists, to the general public the opossum is perhaps one of the most misunderstood mammals native to the United States. This is due perhaps, at least in part, to its physical appearance. The opossum has a narrow, tapered snout the jaws of which contain fifty fairly large, pointed teeth. When encountered the startled opossum will open its large mouth to expose these formidable teeth and growl to warn potential threats or predators. Because of this behavior, which is in fact a bluff, the opossum is considered by many to be a dangerous creature that is a threat to man and domestic animals. In addition, a long, naked, rat-like tail characterizes the opossum. Indeed, because of this feature, some believe the opossum to be a relative of the rat and therefore is a dirty, filthy creature. Thus, due primarily to its physical appearance, the opossum has acquired the reputation of being a repulsive, aggressive, rat-like creature that should be avoided if possible and if it is encountered should be killed like other vermin.

Generally, the only contact most individuals have with an opossum is a fleeting glimpse in a backyard or along a roadway during the late evening hours or perhaps as an encounter at night if pets are being fed outside the household. However, the opossums witnessed by most are generally road kill victims, the crushed bodies of which are found along roadways. The appearance of their greasy, flattened carcasses hasn't helped their reputation or status among mammals as far as the public is concerned. Indeed, the public has coined the phase "road pizzas" for these unfortunate victims of the automobile. With the exception of Australia, living marsupials are rarely exhibited in zoos or animal parks and this is particularly true of the opossum. As a result, only a few have been privileged to examine this fascinating animal closely.

We have written this short contemporary, but well illustrated book on the opossum specifically for those individuals with a limited scientific background but who are interested in learning more about this much-maligned species. The reader of this book undoubtedly will be struck by its format, which departs rather considerably from that of a typical book on wildlife. The book is designed to be a useful text so that specific information with regard to the opossum can be located quickly and with ease. Thus, the most obvious change is in the organization of the material within each chapter. Here the subject matter has been broken down into smaller units and important points are emphasized in the text by **boldface type**. The narrative of the text has been developed around and in response to the most frequently asked questions from the general public gathered during a series of lectures given on the opossum.

The initial chapter describes how the opossum fits into the current scientific classification scheme and explains the need for such a system. This chapter then traces the discovery of the opossum by Western Europeans and explains how the opossum came to be named. The

following chapters then describe the appearance of the opossum, trace its evolution, examine its distribution, and present the most interesting aspects of its natural history. Later chapters emphasize the opossum's reproductive biology because **it is this feature that make the opossum unique** in comparison to other mammals native to the United States. Throughout the text the opossums interrelationship with man is considered as well as its economic and scientific importance. The book finishes with a few tips on the temporary care of orphaned young opossums as well as some common myths with regards to the opossum. It is our hope that after becoming a bit more familiar with the life and ways of the opossum the public will begin to understand and appreciate that the opossum is not the fearsome, repulsive creature they assume it to be. But rather one of the most fascinating mammals native to the United States, which is in fact, one that has a very gentle demeanor.

1. Classification and Its Meaning

Like most other known animals and plants, the opossum has been given two basic types of names: a common name and a scientific name. **Common names** are usefully only within a single language or dialect and therefore are of limited value. Furthermore, many living and/or extinct organisms do not have common names in any language. An additional problem with common names is that this type of nomenclature may be used to refer to more than one type of closely related plant or animal. In contrast, **scientific names** are names applied to organisms by scientists in order to communicate clearly and precisely across linguistic and cultural boundaries. A complete scientific name consists of two parts that are called **binomials** (that means two names). Sometimes the scientific name of an animal or plant is referred to as its **binomial nomenclature**. Scientific names usually are derived from Latin and only one scientific name is ever applied to a given plant or animal. The scientific name is then followed by the name or an abbreviation of the name of the individual who first described and named the plant or animal in question. Once named the plant or animal is given a position within one or more standardized hierarchical systems of classification or categorization.

The opossum is a type of animal classified as a **metatherian mammal** or **marsupial**. The complete classification scheme (according to the established rule of the international commission on Zoological nomenclature) of the opossum is as follows:

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Mammalia
Subclass:	Theria
Infraclass:	Metatheria
Order:	Didelphimorphia
Family:	Didelphidae
Genus:	Didelphis
Species:	virginiana

This method of scientific classification informs the reader that the opossum is an animal that during development has a notochord (a stiff rod of tissue that provides the primary axial support for the back of the developing body; in the adult vestiges remain as part of the intervertebral disks) and a dorsally positioned central nervous system (**Chordata**). In addition, the spinal cord portion of the central nervous system is contained within a segmented spinal column or backbone made up of vertebrae (**Vertebrata**). Furthermore, the opossum is placed into are large category of animals referred to as **mammals**. All mammals have three characteristics not associated with other types of vertebrate animals: **mammary glands**, **hair**, and **three separate middle ear bones**.

Mammals feed their newborn young with **milk**, a secretion produced by the mammary glands. Milk is a nutritive fluid rich in proteins, carbohydrates, and fats. The mammary glands generally are located on the ventral (under) surface and may number from two to a dozen or more dependent on the mammalian species considered. They usually are located somewhere along two ventral lines, called mammary lines, that extend from the chest area to the groin. Mammary glands are modified sweat glands that develop from the epidermis of the skin and are specialized for the production of milk. The root of the term **Mammalia** is in reference to this gland.

All mammals have hair during some phase of their life cycle even though it may not be obvious in all adults. Mammalian hair consists of a protein called **keratin** and is the product of hair follicles located in the skin.

Hair of mammals performs a variety of functions, which include thermal regulation, protection from abrasion, sensory functions, camouflage or a conspicuous warning to predators, or may be modified into quills, spines or horns to deter predators.

Mammals hear sounds in the environment by transmitting the vibrations of sound waves from the eardrum to their inner ear mechanism (cochlea) by a chain of three tiny bones called **ossicles** (malleus, incus, and stapes) contained within a middle ear cavity. The malleus and incus are derived from bones that form the jaw articulation of most other vertebrates.

Mammals can be further subdivided into three groups or Infraclasses: Prototheria, Metatheria, and Eutheria. Prototherian mammals are egg-laying mammals that today are few in number and restricted in distribution to Australia and New Guinea. The mammals that make up this group are the duckbilled platypus and the echidnas. **Metatherian mammals** are generally referred to as the **marsupials** and are grouped into seven orders, including **Didelphimorphia**, which contains the majority of American marsupials.

The Metatherian mammals or marsupials consist of about 275 different species. Today, marsupials are restricted in distribution to the Australasian region (about 200 species) and to North, Central, and South America (about seventy-five species). Eutherians form the largest group (about 90% of all mammals) and are the mammals with which the general public is most familiar. Cats, dogs, and farm mammals (cows, pigs, sheep, and horses) fall into this category. The Subclass **Theria** is used to group together the two Infraclasses, Metatheria and Eutheria, as therian mammals give birth to live young. This subclassification is used to distinguish live bearing mammals from the egg laying mammals, the monotremes.

Marsupials differ from eutherian mammals in a number of subtle ways with regard to their anatomy. For example, the seven or eight cheek teeth usually present on each side of the upper and lower jaws in most mammals are divided into three premolars and four molars in the majority of metatherian mammals. This is in contrast to the four premolars and three molars typical of eutherian mammals. This feature together with differences in tooth structure makes it possible to distinguish between the jaw fragments of remains or fossils of eutherian and marsupial mammals. The lower jaws of many marsupials do not have the same number of

incisors as the upper jaws. The pattern of tooth replacement, milk (deciduous) teeth by adult (permanent) teeth, also differs between these two groups of mammals. In addition, marsupials have a distinctive pair of epipubic (marsupium) bones attached to the pelvic girdle in both sexes. An important and obvious difference between marsupials and eutherian mammals is with regard to their reproductive biology. In marsupials the reproductive tract of the female is fully doubled. They have two ovaries, two oviducts; two completely separate uteri and left and right vaginae, which terminate in a common vestibule called the urogenital sinus. Birth of the young takes place through a median canal that develops at the time of delivery called the **pseudovaginal canal**. Marsupials **do not** have a vaginal delivery. But by far the most conspicuous difference between eutherian mammals and marsupials is the **degree of development** of their young **at the time of birth**. Marsupials are born after a very short gestation period that is less or equal to the length of the estrus cycle. Compared to eutherian newborns, newborn marsupials are embryonic in appearance and organs are only in the initial stages of development. In the majority of, but not all marsupials, most development takes place in a **pouch** or **marsupium** that houses the mammary glands and the lactation period is prolonged. The term **Marsupialia** (marsupials) is from the Latin word marsupium (meaning pouch) and is in reference to those metatherian mammals that have a pouch. The term marsupium is derived from the Greek word marsupion, which means, "little purse". It should be emphasized, however, that in the overwhelming majority of metatherian mammals native to Central and South America a distinctive pouch may be absent or consist only of two thin longitudinal folds of skin separated at either end. In these marsupials, the young firmly attach to the teats of the associated mammary glands and hang suspended from the ventral surface of the mother, like a cluster of grapes, without the protection of a pouch. Incredibly, once attached to a teat, most survive this phase of their life cycle.

Thus, the **reproductive strategy of marsupials** is based primarily on the lactation (nursing) phase of their development and is in contrast to eutherian mammals where intrauterine development of the young is the primary reproductive investment. Although the reproductive process of marsupials was at one time considered less advanced and not as efficient when compared to eutherian mammals, it actually has a major advantage. Marsupials invest relatively few resources in their young during the brief gestation period. Their major commitment is during lactation, which is more easily terminated if adverse conditions arise. Thus, a marsupial that loses its young is able to make a second attempt at reproduction more quickly and will be in better physical condition than a eutherian mammal in a comparable situation. This is particularly true for *Didelphis*, which normally produces two litters per year, and this high birth rate not only assures the survival of this species but also has contributed to the expansion of its range.

The common North American or Virginia opossum is placed in a taxonomic Family or category known as the **Didelphidae**. There are approximately seventy-five different species (types) in this specific group or family, which are known as the didelphid marsupials. Didelphid marsupials are distributed widely in the Americas and attain maximum diversity in tropical South America.

The **scientific name** or the **Genus and species designation** for the Virginia opossum is *Didelphis virginiana*. The genus name *Didelphis* is derived from the Greek prefix *di* (two) and the Greek word *delphys* (womb) in reference to the reproductive tract of the female opossum, which is paired in its entirety. The species name, *virginiana*, is the Latinized word for "of Virginia" and refers to the state in America in which the first specimen was collected and described scientifically by the Western Europeans.

However, even the establishment of the scientific name in the classification of this marsupial species was not without controversy. Linnaeus, one of the most celebrated zoologists of that time, misspelled the generic name which he developed to describe and classify this mammal, substituting an "i" for the "y" if the Greek derivation had been accurately followed. He meant to use the designation "a mammal with two uteri (di=two; delphys=uterus)" as the genus classification for this animal. The genus name, **Didelphis,** stands today as Linnaeus originally created it, as he developed the first scientific classification scheme in which to place this newly discovered mammal. It wasn't until 1792 that Robert Kerr provided the first accurate classical description of the opossum that finally determined the official scientific name of the Virginia opossum, which is *Didelphis virginiana* Kerr. Current scientific evidence with regard to the geographic distribution of the genus, *Didelphis,* suggests that this genus is actually made up of four separate, distinct species: *Didelphis virginiana* of North and Central America (but the only opossum native to the United States), *Didelphis marsupialis* of Central and South America, *Didelphis albiventris* found in the highlands of South America, and *Didelphis aurita* native to the Brazilian Atlantic forest.

Discovery and Naming

Although this animal was obviously known to the indigenous people of the Americas, the opossum was the first marsupial that European explorers encountered. The discovery of this mammal and how it was named is in itself a very interesting story. In the late 1400s the Spanish explorer Vicente Yáñez Pinzón (commander of Christopher Columbus ship, the *Niña*) found a female opossum with young in her pouch after landing off the coast of Brazil. Pinzón brought this strange mammal back with him to Spain and presented it at court to King Ferdinand and Queen Isabella, the Spanish monarchs that ruled Spain at this time. The two monarchs and those at court that examined the opossum were astonished that the pouch of the opossum could be opened by finger pressure alone and that the pouch contained young attached to teats. It would be over one hundred years later, in the early 1600s, before Captain John Smith and other settlers of the Jamestown colony would write the first English description of the North American opossum.

It was John Smith who gave this marsupial the common name **"opossum"** by which it is known today. The term opossum is an anglicized construction of the American Indian (Algonquian) word for this species "apasum" which means, "white animal". The opossum rapidly became the symbol of the natural wonders held by the American colonies and was dissected and discussed at length by Europe's leading scientists. At that time the opossum played an important role during the period of transition from medieval to enlightenment in science.

However, some confusion exists even today with regard to the use of the common names **opossum** and **possum**. The reason for such confusion once again can be traced historically to the original naming of this group of mammals, the marsupials. In 1768 the British navigator, Captain James Cook, set out on a ship, the *Endeavour*, and in his exploration of the Pacific Ocean was the first European to encounter Australia. On board was naturalist, Sir Joseph Banks, who headed the scientific staff. It was Banks and his staff that applied the common name opossum to several newly discovered Australian marsupials. Although these mammals were indeed marsupials, they belonged to an entirely different group of marsupials that are classified today as belonging to the Family: **Phalangeridae**. Several Australian marsupials fall into this category and the vernacular name "possum" is used almost exclusively in the Australasian region to refer to many members of this Family of marsupials. The term "phalanger" refers to the adaptation of the first two toes (digits or phalanges) that are opposable to the other three toes of their fore paws. This adaptation aids these aboral marsupials in climbing trees. To avoid confusion the "**o**" was dropped from opossum and the term possum used to refer to the phalangers of the Australasian region.

Today, also using the common type of nomenclature, an adult male opossum is referred to as a **Jack**, an adult female opossum is called a **Jill**. A pouch young opossum of either sex is called a **Joey,** as are kangaroo pouch young. There is no name for a group of opossums such as a "herd" of bison or "mobs" of kangaroos as adult opossums are primarily solitary animals.

2. General Appearance

Opossums are about the size of a large house cat with a weight that ranges between 1.8 and 5.5 kilograms (4 to 12 pounds). Opossums range in length between 609 mm and 914 mm (2 to 3 feet). Males are generally larger than females. The large range in size is thought to be due to two different groups within the general opossum population. If, for example, two females with litters of roughly the same age are caught during the early spring and their litters raised in captivity the following observations have been made. The very large females tend to have large offspring; the small females tend to have small offspring. Juveniles from the larger female may be twice the size of those from the small female at weaning. Indeed, prior to this observation it was assumed that the larger juveniles were older, either from the first litter of the current spring or may have even come from the previous year. This discrepancy in size continues throughout their lifespan.

The head of the opossum is elongated with a slender snout that ends with a pink nose. The hair on the face is short and white. Head markings represented by three dark streaks are sometimes present: one streak running along the midline of the crown and one running through and behind each eye.

An example of a light, gray phase female opossum is shown in the **left** *photograph. An example of a dark phase male opossum is shown at the* **right**. *In addition to the overall dark appearance, note the dark steaks of fur passing through the eyes and the dark streak running along the midline of the head.*

Opossums are characterized at a distance by a long, rat-like, tapered, scaly tail that bears only a few scattered hairs. The tail usually measures less than 90% of head and body length. It is usually covered in fur that is black in color at its base and naked along the rest of its length. The latter region ranges in color from yellowish white to pink white.

A photograph of an opossum tail illustrates its rough, scaly appearance. Although usually described as hairless, tiny light colored hairs do occur between the patches of epidermal keratin. The region of the tail nearest the body is usually pigmented and covered by fur.

Photographs of a female opossum climbing among the branches of a tree. Note in particular how the opossum uses its prehensile tail to wrap tightly around a branch to provide stability and aid in climbing. Note also the use of the hind feet, which are able to tightly grasp branches. Because of the opposable large toe, the hind foot is used very much like a human hand.


The opossum (left) can use its tail to wrap around larger structures to aid in climbing. This opossum is climbing down a tall pole using it tail much as a telephone lineman uses a safety belt when climbing. A gray phase juvenile opossum (right) eating a grasshopper it has just caught. Note the dexterity of the digits of the forepaws to hold its prey while feeding.


The opossum to the **left** *was photographed while walking along the branch of a tree. This photograph illustrates that the digits of the forepaw can easily be spread over a 180-degree area. The ability to spread the digits over such a wide area enables the opossum to firmly grasp and hold prey items when feeding or to firmly grasp branches when climbing as shown in the photograph to the* **right**.

The **tail is prehensile** (able to grasp) and is used as a safety device when climbing. The opossum can hang and support its own weight with the prehensile tail, but only for a short period of time. During walking or running the tail functions in balancing.

In addition, there have been several observations of opossums carrying bundles of grasses, leaves and other matter within a coil of their tails as they transport these materials to line dens or temporary sleeping areas. Thus, opossums appear to have the ability to voluntarily control the use of their tails to accomplish specific tasks. As a result of such voluntary behavior, the opossums' tail is considered by many as a fifth appendage or hand.

Opossums have a large mouth that can be opened quite wide and the jaws have more teeth than most other mammals. The jaws contain **fifty teeth** with the following dental formula: I5/4, C1/1, Pm3/3, M4/4 X 2. This formula means that the upper jaw contains ten incisors, two large canines, six premolars, and eight molars. The lower jaw contains eight incisors, two large canines, six premolars, and eight molars. This dental formula is diagnostic for all opossums of Didelphidae. The **last premolar** is the **only deciduous tooth** of the opossum. In other words, the opossum has only one set of teeth (the permanent or adult teeth) except for the last premolar, which has a preceding baby (deciduous) tooth that is lost and later replaced by a permanent tooth. All the teeth are rooted and sharp. The molars are multicuspid and indicative of their omnivorous diet.

Opossums have large, well-developed salivary glands that like other mammals drain into the oral cavity or mouth. These glands produce saliva important for the digestive process and keeping the mouth clean. Opossums salivate or drool considerably, so much so, that saliva literally drips from their mouths most of the time.

The snout has long whiskers or vibrissae. The vibrissae are arranged primarily into two groups on each side of the face. One group is located in the cheek area; the other vibrissae are located on the snout. The base of each vibrissa, located deep within the skin, is provided with an elaborate network of touch-sensitive nerve endings that are extremely sensitive to the slightest touch. Each vibrissa of the opossum is capable of independent movement and used to provide sensory input from the external environment particularly around the region of the head. Touching the vibrissae also sets off appropriate defensive or avoidance reactions.

The opossum does not see well as can be demonstrated by passing an object in front of its head but within reach. The opossum usually will not snap at or avoid the object. If, however, one of the vibrissae is touched, the reaction is incredibly swift. The vibrissae are not unique to opossums and are highly developed in other mammalian species.

The eyes of the opossum are black and prominent and appear somewhat exophthalmic (pop eyed) reminiscent of those of a mouse. Captive animals that are fat, usually because of overfeeding, often appear cross-eyed due to accumulation of fat behind the eyeball. The jet-black appearance of the eyes is due to the fact that the eye is extremely dilated and primarily a large pupil characterizes the front of the eye. The iris is usually not seen except if the opossum is examined in direct sunlight. It is believed that the wide-open pupil is an adaptation of this spe-

Close inspection of the head of an opossum illustrates its elongated snout that ends with a pink nose. The hair on the face is short and white. The snout has long vibrissae (whiskers) arranged in two groups on each side of the face. The bases of the vibrissae are provided with numerous touch-sensitive nerve endings that are extremely sensitive to the slightest touch. Eyes of the opossum are prominent, somewhat exophthalmic (pop eyed) and black in color. The ears are short, hairless and leathery in texture.

Two young juvenile opossums on a tree limb. The individual toward the rear has its head tipped upward to sniff the air and get a better olfactory reading of its environment. Note the large, thin, hairless ears that are leathery in consistency. The majority of the ear is black in color and tipped with a pinkish-white band of variable width. The white tipped band of the ears of the opossum to the rear is prominent whereas the ears of the opossum in the foreground show only a small area tipped by white. The ears of some opossums are entirely black. These opossums are littermates and illustrate the biological variation that occurs with regard to ear markings.

cies to its nocturnal (nighttime) habits. Vision of the opossum does not appear to be very acute as it responds poorly to most visual stimuli.

The external ears (pinnae) are short and hairless, thin and leathery in consistency, and bluish black in color. They are often tipped with a pinkish-white band of variable width. However, the ears of some opossums may be entirely black in color.

The ears of opossums in the northern regions of their range often appear ragged due to frost-bite. The thin membranous ears are folded when sleeping. When aroused the ears may remain wrinkled, but quickly smooth out, as the opossum becomes active. Opossums are extremely sensitive to sound from its environment as can be demonstrated by simply snapping a finger.

The body of the opossum is stocky or stout supported by relatively short legs. The hind legs are slightly longer than the forelegs. The fur tends to be darker on the front and hind legs and the toes are often white. The five toes on the forefeet have nails (claws). The forefeet are quite dexterous, allowing the opossum to grasp branches or other objects and hold food items while

The naked palmar surface (left) of an opossum forepaw shows six tori separated by a deep midline groove. The pollex (thumb) bears a nail and diverges much less than does the hallux of the hind paw. Note that the central digit is in line with the midline groove. These structural features enable the opossum to grasp and spread the digits over 180 degrees. The dorsal surface of the hind paw (right) illustrates the sharply diverging hallux (big toe), which lacks a nail. The remaining four digits of the hind paw have nails. The back of the paw is furred to the base of the digits. (Cutts and Krause, Anat.Anz. 154: 1983).

The plantar (under) surface of the hind paw (left) is hairless and shows five tori separated by grooves. Note the prominent groove that separates the hallux and its torus from those of the remainder of the paw. The plantar surface of the hind paw (right) has been inked to illustrate the pattern of dermatoglyphs (friction ridges or fingerprints) on the tori and digital pads. The friction ridges provide additional traction for grip when climbing or holding an object. (Cutts and Krause, Anat.Anz. 154: 1983).

feeding. The **hind feet** of the opossum **are unusual** in that the **big toe or hallux** stands out in that it looks like a thumb. The big toe is opposable and can be used to touch the tips of the other four toes. Thus, the opossum's hind foot is shaped somewhat like a human hand or a primate foot with an opposable big toe. Primates, together with the opossums, are the only mammals with opposable first toes. The big toe **lacks a nail** or claw but the other four digits of the hindfoot have nails. The **nails** or claws of both the forefeet and hind feet are **non-retractable**, a feature that makes it possible for the opossum to pick up objects or grasp thin branches better than most other mammals. The nails of the opossum have never been observed to be used as weapons as those of cats or some other group of mammals. The hind feet are used for grasping and holding onto things as the opossum moves about, particularly when climbing in trees. This adaptation together with the use of the prehensile tail makes the opossum an adept tree climber for both gathering food and escaping from predators. **Friction ridges** or **fingerprints** are present on the plantar (under) surfaces of both the forefeet and hind feet that aid in providing a firmer grip.

The feet of the opossum are **plantigrade**, that is, shaped so that the opossum walks on the sole of its foot (plantar surface) with the heel touching the ground rather than on its toes as is true of many mammals. The planter surface of each foot is without fur and naked. The plantigrade type of movement is readily apparent if one examines the **tracks (footprints)** of an opossum. Opossum tracks occur in pairs with each pair having the imprint of one front and one rear foot close together. The five toes of the front feet are usually spread wide apart. The most characteristic imprint is that of the hind foot and is unlike the track of any other mammal native to the United States. The imprint looks very much like a small hand made it. The imprint of the hallux of the hind foot is perpendicular to the direction of travel. Dependent on the position of the tail at the time the tracks are made, it may or may not leave drag marks. The **opossum walk** on the ground or a surface has been described as a waddle but is, in actual fact, **a trot**. The faster the trot the more exaggerated the waddle.

Footprints (tracks) of the left hind foot and forefoot of the opossum. (Modified from D. Hunsaker, The Biology of Marsupials, Academic Press, 1977).

Opossum tracks occur in pairs with each pair consisting of the characteristic rear footprint and a footprint of the forepaw from the same side. The two imprints of each pair are always close together and may overlap. Each pair is separated by several inches. How far they are separated is dependent on the size of the opossum and how fast it was traveling.

The **coat (fur or pelage)** and skin color of opossums varies considerably from different regions of North America. In northern regions, opossums have a relatively thick undercoat, which is white nearest the skin, and is usually tipped with black. The underfur is overlain with a thin covering of pale guard hairs giving the majority of opossums a gray, grizzled appearance. Opossums with this type of coloration are called **gray phase opossums**. The guard hairs are thought to be protective and reduce the amount of abrasion on the underfur that functions to keep the opossum dry and warm. Because of the oiliness of the important dense undercoat, rain or mist generally does not wet the opossum's skin. The guard hairs are usually tipped white. The greater the extent of the black color along the shaft of the guard hair the darker the

opossum. If the black extends more than two-thirds down the guard hairs and hairs forming the undercoat, the opossum is much darker in color and referred to as a **dark phase opossum**. Opossums from the southern regions of the United States generally have a sparser, dark underfur and show a greater number of dark guard hairs giving the population of animals in this area a darker appearance. It is estimated that in the Deep South the dark phase opossums outnumber the gray phase about five to one. Gray and dark phase opossums may occur within the same litter. Pure **white opossums** with black eyes and ears also have been observed. Likewise, typical pink-eyed **albino opossums** have been found but are a rare occurrence. A **cinnamon phase opossum** also has been described and is thought to be a genetic variation as this phase is reported to lack the overlying guard hairs.

Eyes at Night

The eyes of the opossum and other nocturnal mammals "glow" or are reflective in the dark if illuminated by the headlights of an automobile or some other bright light. This is due to a structural adaptation within the eyeball of these creatures to improve their night vision. The superior (upper) half of the opossum retina is unusual in that it represents a modification of the pigmented portion of the retina called the **reflective tapetum lucidum**. In the tapetal region of the opossum retina, the epithelial cells of the pigment layer are enlarged, relatively free of melanosomes (black pigment granules) and instead are filled with reflective lipoidal (cholesterol-containing) spheres. Similar retinal tapeta are found in reptiles and fish but are not usually associated with mammalian eyes. The tapetum acts as a light-reflective surface that reflects light back to the photoreceptors of the retina to enhance vision that occurs under conditions of poor illumination. The tapeta of eutherian mammals such as ungulates (cattle, deer, and sheep) are fibrous whereas in carnivores it is more cellular in composition. However, in both instances, the tapetum is located behind the retina rather than being a part of the retina as it is in the opossum. The tapetum of the opossum reflects a yellow/green color when activated by light at night.

Construction of the Pouch

The pouch or marsupium is often considered to be a hallmark feature of marsupials; however, most marsupial species from the Americas lack a well-defined pouch. The female Virginia opossum has a well-developed pouch or marsupium on her ventral (under) surface that usually contains thirteen nipples arranged in two arches of six with one nipple located centrally. It is within the protection of this pouch that the female opossum carries her offspring during the first ninety days following their birth. The overall dimensions of the pouch change depending on the reproductive status of the female. When young are absent it is relatively small and shallow in appearance. The space within the pouch expands with the first litter to accommodate the developing young. Near the end of lactation it can be distended to a considerable size and it may house as many as a dozen rat-sized young. The expansion is temporary and after weaning the pouch becomes much reduced in size but never returns to the original dimensions prior to the first pregnancy. The construction of the pouch is basically an invagination of skin through a sheet of cutaneous abdominal musculature called the **panniculus carnosus**. This

Four photographic illustrations of the pouch (marsupium) of the Virginia opossum. The upper left figure shows the closed pouch opening located on caudal ventral surface of the body wall. The pouch orifice shown at upper right has been opened by gentle finger pressure exposing young opossums contained within the pouch. The figure at the lower left illustrates the lips of the pouch and the amber color of fur within the pouch. The color is the result of secretions of sudoriferous (modified sweat) glands within the pouch. A litter of larger pouch young (lower right) within a pouch illustrates that the pouch expands considerably to accommodate the size of the litter.

sheet of voluntary muscle occurs in the skin of many mammals and its action readily seen in animals such as horses when it is contracted in an attempt to shake off flies. The thirteen mammary glands and teats associated with the female opossum are located in the dorsal wall of the pouch integument (skin) next to the abdomen. The panniculus carnosus muscle is well developed in the pouch region of the female opossum and can be subdivided into three regions: the pars dorsalis, the pars thoracoabdominalis, and the pars pudenda. The pars pudendal subdivision lies beneath the skin of the abdominal midline and in the female passes around the opening of the pouch. Some of its muscle fibers run between the two layers of skin

forming the edges of the pouch wall to insert into the base of the skin that borders the opening of the pouch. The female opossum can voluntarily close or open the entrance to the pouch by contracting the skeletal muscle fibers forming the pars pudenda and this region is often called the **sphincter of the pouch**. The skeletal muscle fibers do not extend into the region of the mammary glands. A pouch is **absent** in the male opossum.

The pars pudendum is highly developed in the semi-aquatic water opossums (*Chironectes*) of South America that are the only marsupials adapted to an aquatic environment. They have webbed hind feet and a waterproof pouch. The mother can go swimming with young in her pouch when feeding on crayfish, shrimp, and fish because of the well-developed sphincter muscle in the margin of the pouch. The contraction of this muscle plus the secretion of glands in the skin located along the lips of the pouch coverts the pouch into a watertight compartment. In addition to mammary glands, the skin lining the opossum pouch interior contains hair (fur), sebaceous (oil) glands, and apocrine sweat (modified scent) glands. Both types of glands increase in size or hypertrophy with the advance of pregnancy. The reddish oily secretion of the apocrine sweat glands in the pouch may provide olfactory cues that guide young opossums to the safety of the pouch after birth and/or may even have bactericidal properties that keeps microbial growth within the pouch in check as has been shown in an Australian marsupial, the quokka.

The skeleton of the opossum has a **pair** of **marsupial** or **epipubic bones** that extends forward from the pelvic girdle. Although these bones were earlier thought to be a supportive element for the pouch, the epipubic bones are now thought to act as supporting structures for the ventral abdominal body wall. Both male and female opossums have epipubic bones. The only other mammals that have epipubic bones associated with their skeletons are the monotremes.

3. Evolutionary History

Opossum Fossils

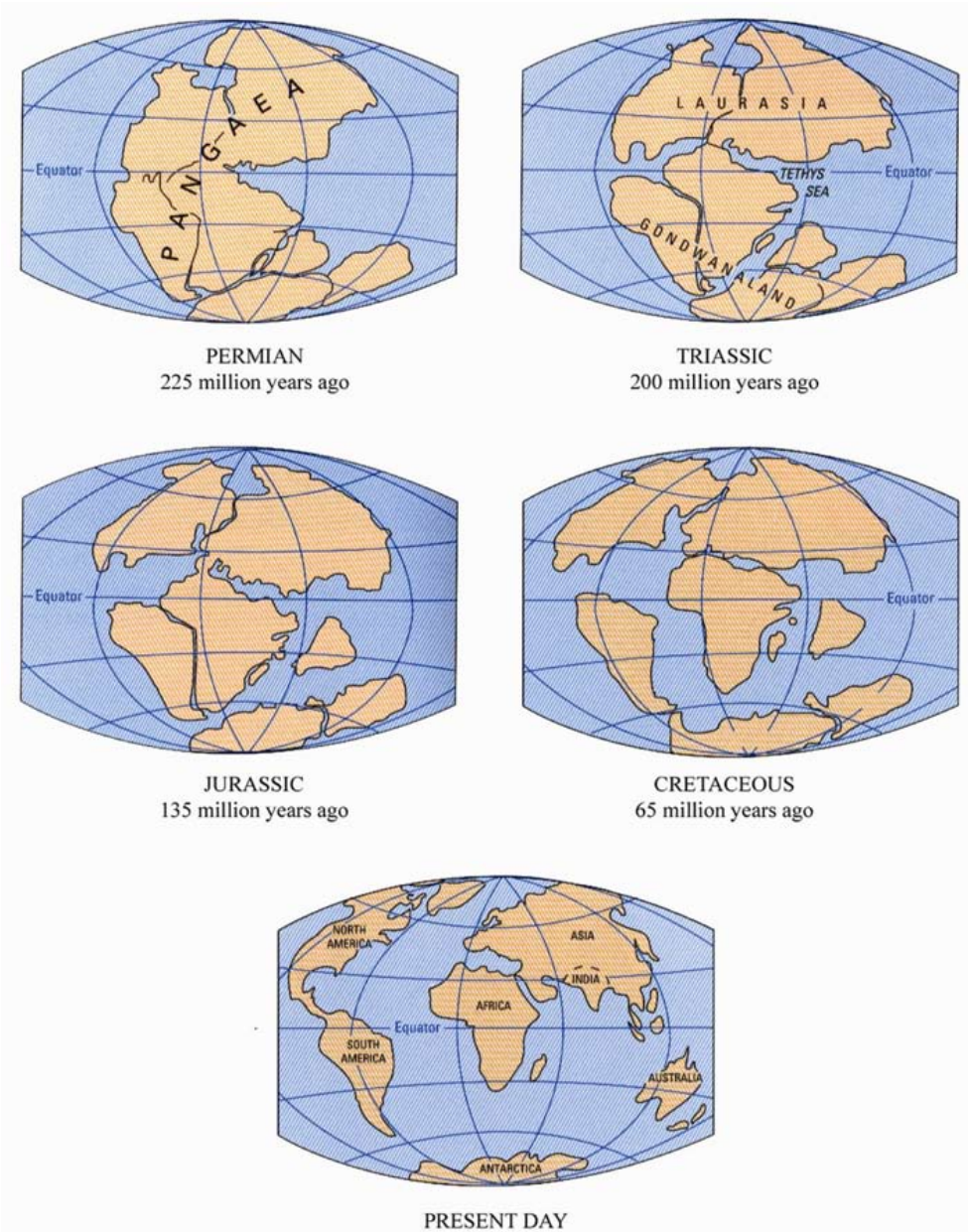
At the present time fossilized remains of marsupials have been found in Africa, Antarctica, Australia, Europe, North America, South America, and Asia. The "opossum-like" fossil marsupials found in the Americas are of particular interest to scientists because some of the features associated with these fossils are thought to approximate those of early therian mammals. It is believed that both marsupial and eutherian mammals arose simultaneously from a common ancestor sometime during the Late Jurassic or Early Cretaceous periods. This therian mammal represents the ancestor of all metatherian mammals. The early stock of marsupials that arose from this creature gave rise to the current diverse populations of marsupials found in the Americas and Australasia today. It should be emphasized that very large gaps exist in the current known fossil record and that the precise details of the origins of these mammals and where they originated should be discussed with great caution. Being aware of this limitation and based on the fossil information that does exist, the last common ancestor of marsupials and eutherian mammals is thought to have lived at least 130 million years ago. Some of the oldest marsupial fossils found thus far are from the Upper Cretaceous Milk River Formation found in Montana and the southern most portion of the Province of Alberta in western Canada. These marsupial fossils are estimated to be about **110 million years old**. Recently, fossil marsupials approximately of the same age were found in Bryce Canyon National Park (Dakota Formation) in southwestern Utah. Most are astonished to learn that The Milk River marsupials lived nearly 45 million years before extinction of the dinosaurs and prior to the age of mammals. The Cenozoic Era is often referred to as "the age of mammals" and encompasses the last 65 million years of earth history. However, it should be pointed out that fossils from mammal-like vertebrates have been shown to exist in the Jurassic, which is over 145 million years ago. The early marsupials were small animals and thought to have resembled members of the modern opossum-like marsupials (the Didelphidae) found in South America as well as the larger Virginia opossum. The extinct Milk River and Dakota Formation marsupials mark the beginning of a record of early marsupials in North America and several specimens have been found and documented. Most were found in the eastern most Rocky Mountain region and adjacent parts of the Great Plains. A few have been found in Baja California, Mexico; the only early marsupial fossils discovered along the Pacific Rim of North America.

The recent discovery (2003) of an intact marsupial fossil called *Sinodelphys szalayi* in the fossil beds of China's northeastern Liaoning province strongly suggests that marsupials originated in a northern continent; either present day North America or Asia. *Sinodelphys* was a mouse-sized marsupial with a long tail that lived 125 million years ago. It is by far the **oldest marsupial fossil** discovered to date. The discovery of this marsupial suggests that the divergence between metatherian and eutherian mammals occurred much earlier than originally thought.

To better understand how marsupials evolved and found their way to various continents, one must realize that the major landmasses of the world have not always been where they are today. At one time (about 200 million years ago) the continents were joined together in a landmass called **Pangaea**. Over time Pangaea separated into two large land masses: a northern land mass known as **Laurasia** (which would become North America, Europe, and Asia); and a southern land mass known as **Gondwanaland** (which would become South America, Africa, Madagascar, the Indian subcontinent, Antarctica, and Australia). As Gondwanaland separated over the next 100 million years, Africa and India moved northward while Antarctica and Australia remained connected to South America as a single landmass. This entire region continued to enjoy a temperate climate and as a result land animals are thought to have moved back and forth between what would become South America, Antarctica, and Australia until the early Tertiary.

It should also be remembered that today's oceans did not form until the Jurassic. The South Atlantic Ocean did not begin to form until during the Cretaceous; the North Atlantic Ocean did not form until the old Tertiary. This means that South America and Africa were still connected together during the older Cretaceous period and North America was still connected with Europe during the early Tertiary. The fossil evidence for marsupial opossums in the early tertiary of North Africa suggests that there may have been a direct distribution of marsupials from South America to Africa. Current fossil evidence suggests that marsupial mammals arose in North America or Asia and then spread to Europe and South America and from South America to Africa. After the separation of these continents from one another, the marsupial species occupying North America, Europe, Africa, and Asia became extinct. The only early marsupials that survived were found in what would become South and Central America. Eventually the continents forming Gondwanaland separated from one another. Australia moved away from the Gondwanaland landmass of Antarctica and South America between 38 and 45 million years ago. As the landmass separated Australia took with it Gondwanaland animals and plants which then developed in isolation for about 30 million years. Some of the early Gondwanaland marsupials evolved into the 200 different species of marsupials associated with Australia today as well as several extinct forms including the Mega-fauna. Thus, the native (current) marsupials and monotremes of Australia are the descendants of similar mammals from Gondwanaland. Indeed, not only have the fossilized remains of marsupials been found on Antarctica but also those of large form of duckbilled platypus have recently been discovered near the tip of South America in present day Argentina. This duckbilled platypus lived about 16 million years ago. These findings provide additional scientific support that the native animals of Australia took their origins in what is now known as South America.

The movement of continents "plate tectonics" in the dispersal of marsupials is further complicated by the global rise and fall of sea levels between continents, particularly North and South America. **Ones species of marsupial later re-invaded North America** from South America as these two major continents were reunited by the Central American land bridge. That species would become known as the **Virginia or North American opossum *(Didelphis virginiana* Kerr).**



According to the continental drift theory, the super continent of Pangaea began to fragment about 225 million years ago eventually forming the continents recognized today. (Courtesy of J.M. Watson, U.S. Department of the Interior, U.S. Geological Survey).

Many of the early marsupials were formidable and fossils of a group of giant marsupials referred to as the Mega-fauna of Australia have been found. Many of these giants were larger varieties of several of the marsupials alive today. For example, there were cow-sized wombats, rhinoceros-sized palochestids characterized by a tapir-like trunk, marsupial lions, and giant kangaroos. Some of these giants continued to exist until about 50,000 years ago and were still living after the arrival of humans on the continent of Australia.

A Living Fossil?

The family Didelphidae is a current classification scheme that contains a sizable array of living and fossil opossum-like marsupials from the Americas. Some of the living taxa forming this group are thought to have retained features of the early ancestral forms of the Metatheria. The most ancient known marsupial opossums are from the Upper Cretaceous of North America and China's northeastern Liaoning province. The opossum-like marsupials disappeared from North America and Europe in the Miocene (22-5 million years ago) and from Africa presumably as early as the old Tertiary. They survived until recent times only in South and Central America from where they migrated to the north. The new dispersal of opossums to North America occurred not before the recent Pleistocene (1.8 million- 11,000 years ago) with the Genus *Didelphis.* The earliest marsupials are believed to have resembled members of the modern opossum family, Didelphidae, including *Didelphis virginiana*. Because many of the earliest known marsupials are believed to have resembled *Didelphis*, which has retained several features of these early marsupials, it is often referred to as a living fossil. In spite of retaining some of the primitive features of the earlier forms, however, *Didelphis virginiana* Kerr is perhaps one of the most recent marsupials to evolve.

Arrival in North America

It is surprising to some that the Virginia opossum did not originate or evolve in North America even though fossil remains of a smaller, similar appearing or closely related marsupial species have been found in both the United States and Canada. Instead, the direct ancestors of the Virginia opossum are believed to have evolved in South America.

During the Pliocene Epoch, about two to five million years ago, the North and South American landmass were reunited after being separated for several millions of years. During this period of separation the early marsupials of South America evolved and diversified into a rich variety of species. As animals (eutherian mammals) that evolved in North America invaded South America, a single South American marsupial (thought to be similar to *Didelphis marsupialis* or a closely related species) moved north. This marsupial continued to expand its range northward and in less then one million years diverged into what is recognized today as the Virginia opossum, *Didelphis virginiana* Kerr.

It is only during this last century that the Virginia opossum has expanded its range into southern Canada and the pacific states. The ability of the opossum to expand its range during the last one hundred years to the Far West and northern regions of the United States and into southern Canada can be directly linked to the inadvertent or deliberate intervention of

humans. Because of its adaptability to eat almost anything and create a den almost anywhere, the opossum has been able to expand its range considerably since the colonialization of North America. Prior to European settlement in North America, the Virginia opossum was found primarily in the southeastern United States and based on studies of several archeological sites, was a food source for the indigenous people that inhabited this region. Primarily cold winter temperatures limited the northward expansion of the opossum and its westward expansion was limited by arid conditions. As farms, villages, and small towns were established, the opossum was able to adapt to human habitation and actually use its ability to live in the shadows of modern man to extend its range into southern Canada and along the Pacific coast. The opossum is able to feed on a variety of foods including spillage and/or left over food provided for domestic animals and pets as well as human garbage items. Its ability to use portions of human dwellings and buildings (attics, spaces between walls, barns) in establishing temporary dens was also helpful in expanding its range into the Northernmost extremes of its range. The more recent extension of its range also is undoubtedly related to the development of the automobile and the elaborate roadway system within North America.

As the speeds for travel increased so did the number of animals killed on the roadways, thereby providing an abundant food source for this adaptable, opportunistic feeder. Indeed, it is usually at night, along a roadway, that the majority of people actually see a live opossum caught in the headlights of a vehicle. The reason opossums are often seen with greater frequency along roadways is because they are feeding on other animals (reptiles, birds, and mammals) killed while crossing or traveling along the roadways. The opossum has a keen sense of smell and is searching for carrion on which to feed. Inadvertently, the opossum also may become a victim if startled while feeding or searching for food by a fast approaching automobile and temporarily blinded by the headlights. More often than not the opossum seems to dart into the pathway of oncoming traffic and becomes a casualty of the roadway.

The opossum has recently expanded its range into the Great Plains as far as southern Minnesota, southeastern South Dakota, and eastern Nebraska and Kansas. In this region of the United States they are restricted primarily, but not exclusively to, the drainages of major rivers such as the Mississippi, Missouri, North Platte, and Niobrara and their tributaries.

Perhaps the most interesting story is **how the opossum came to California** and the Pacific Coast, expanding its range as far north as southern British Columbia. Before human intervention, the hostile environments associated with the mountain ranges and surrounding deserts kept the opossum from spreading to the West Coast. However, in 1890 the Virginia opossum was introduced into southern California near Los Angeles. This population became well established and expanded into adjacent Ventura County by 1924. Immigrants, originally from Tennessee, imported an additional group of live opossums from that state into central California (near San Jose) in 1910. The live opossums were sent as food items as individuals from this region of the United States considered opossums a delicacy at the time. Several escaped their hutches over time and provided one of the initial populations of opossums into this region of California. Another individual introduced an additional documented group of opossums from South Carolina to a farm near Visalia, California, in an attempt to raise opossums as fur-bear-

ers. Opossum fur at that time was being used as an inexpensive fur trim for some garments and hats. After several years of failure, the fur farmer abandoned this enterprise and many of the animals were simply released into the surrounding countryside. Since this initial introduction, the opossum has prospered along the coast and expanded its range over a considerable area of California, in particular those regions associated with agriculture.

At the time the opossum was rapidly expanding its range in California (the early 1900s), the Virginia opossum was also introduced into the states of Oregon and Washington. Between 1910 and 1920 opossums were released as liberated pets or some kept as exotic pets simply escaped. Today the range of the opossum extends up the coast of the western United States into southern British Columbia. In several counties of north central California, opossums are quite abundant and frequently seen as road-kill victims on the roadways.

Likewise, the opossum has been introduced into the arid states of Arizona, New Mexico, Colorado, and Idaho by humans for sport and is now continuing to expand its range in some regions of the southwest where water is available.

The success of the opossum in continuing to expand its range in North America is due in part to its adaptability to several types of habitat. The **opossum is** considered **a generalist** rather than a specialist adapted to a specific environment, and therefore very adaptable to changing conditions and opportunities that it continues to exploit. The range of the opossum seems to be restricted only by a lack of water and extremely cold temperatures of long duration.

4. Natural History

Current Distribution

The Virginia opossum is the **only marsupial native to the United States**. The current distribution of the opossum is over most of the United States except for some of the northern and

The approximate distribution of the Virginia opossum in North America with regard to time. (Modified from D. Hunsaker, The Biology of Marsupials, Academic Press, 1977).

western states as shown on the following map.

Home Range (Territory)

The Virginia opossum is not territorial in the strict sense of the term, but represents a solitary species that excludes other individuals from its area when they are encountered. An opossum's territory is highly variable and depends on the availability of food and on an individual

opossum tendency to wander. They generally have elongated rather than circular territories as most follow the edges of streams or rivers. If food is plentiful the range be may very small. If food is scarce the opossum may travel up to two miles in search of food. The average home range of an adult male opossum is about 300 acres that it appears to wander through during its lifetime. That of the female opossum is thought to be considerably smaller, about 150 acres, and the boundaries more permanent. Individual ranges tend to overlap considerably. The life of the adult male is totally solitary and is in contrast to the female, which has young with her during much of the year. A late, second litter of young may stay with the female during the winter until the following spring.

The Virginia opossum **is primarily a terrestrial species** and spends the majority of its time on the ground. The opossum's **preferred habitat** is deciduous woodlands in areas close to water. However, opossums are extremely adaptable creatures and are often found in prairies, marshes, and farmlands. They generally keep to the woody vegetation along rivers and streams, a behavior that has permitted them to move into treeless grasslands and some desert environments. They often will live in very close proximity to human habitation. This adaptable mammal is very successful at surviving or even thriving not only in agricultural areas but also in residential and suburban areas. Here it adapts buildings, woodpiles, or accumulations of other materials as potential sites to den and is able to feed on human refuse or food provided for pets or domestic animals.

Nests or Dens

The opossum is primarily a **nocturnal mammal** (active at night) and forages for food shortly after dark, a behavior that continues until dawn. However, opossums may become active during daylight hours during cold weather (winter usually) when food is more difficult to obtain and its metabolic need is greater. The opossum, as is the case for most other mammals active during cold weather, needs a greater caloric intake in order to survive. Like most nocturnal animals, the opossum must build a rough nest or find a den in which to rest during the daylight hours. Depending on the location, the **opossum den** can take a variety of forms: an abandoned burrow or underground tunnel, cavities in hollow trees, abandoned squirrel nests, crevices in rocks, or crawl spaces under houses, in attics or in some other dark, hidden spaces of buildings. Opossums do not dig their own burrows, but occupy abandoned burrows of other animals. They will seek some quite dark space that is dry. The dens are most often temporary but if a food source is nearby may be occupied for several weeks. A single opossum may have several dens that it will use periodically. Females with young tend to be the exception to this behavior and use the same den site for several weeks at a time. The dens usually are lined by grass or thin twigs mixed with dry leaves. In warmer months its construction is similar to that of a bird's nest. In the cooler months of fall and winter its construction resembles a hollow sphere and is similar in appearance to a mouse or squirrel nest. There have been numerous observations of opossums gathering denning materials. The materials are gathered using the mouth and forepaws, then passed to the rear under the abdomen to the hind feet, which the opossum uses to organize the gathered materials into a bundle. The bundle is then placed in a

Martha Stech of Ashland, Missouri, took this award-winning photograph entitled "Caught in the Act". Note the luxuriant, well-groomed coat of this fall/winter opossum raiding a bird feeder to eat the oil-rich sunflower seeds and corn.

loop of the tail and the prehensile tail used to drag the material to the opossum den. It should be remembered that *Didelphis virginiana* is somewhat nomadic, moving between old denning sites or constructing new ones as the opossum wanders through its territory.

Feeding

When foraging at night the opossum keeps its nose to the ground, relying primarily on smell and touch. Vision does not appear to play a large role in locating food during their night prowling although they can see distant objects. The opossum is **omnivorous**, consuming both animal and plant material. Its diet includes a variety of insects, earthworms, slugs, snails, crayfish, snakes and lizards, frogs, small rodents (primarily mice and rats), young rabbits, small birds, eggs, grasses, vegetables, fruits, berries, grains, human garbage, and **carrion** (dead animal material). Opossums seem to have a preference for sweet items such as various fruits and berries when available. The opossum is an **opportunistic feeder** and will eat whatever is available in its environment at a given time and its diet will change with the seasons. Olfaction (the sense of smell) of the opossum is keen and clearly important in prey and/or the location of food.

Although the types of food the opossum eats are highly varied, they must be abundant and closely spaced to support a significant population. If food resources become depleted in one area, the opossum simply will expand its territory or move to a new area.

The opossum has large, well-developed jaw muscles that make up much of the mass of the head in this species. The jaws are capable of generating incredible forces and are used in crushing the shells of snails and bones of small mammals and birds. Opossums have an unusual need for calcium in their diet and often consume the entire skeletal remains (particularly cottontail rabbits killed along roadways) of animals it encounters to satisfy this dietary requirement. If the exterior of an opossum skull is examined, a large crest will be found that courses down the midline on the top of the skull. This large crest serves as an attachment point for much of the jaw muscle mass and is indicative of just how well developed the jaw musculature is in this particular species.

An additional characteristic, as well as of most marsupials, is a projection called the angular process on the posterior margin of the lower jaw. This process curves inwards in marsupials but is directed straight back in eutherian mammals. The angular process functions as an attachment site for muscles of mastication. Interestingly, despite the large size of the jaw musculature, it has been clearly established that the opossum chews on one side of its mouth at a time. A large fascial area and a relatively small cranial cavity characterize the remainder of the opossum skull.

Scent Marking

Opossums mark or announce their presence in a region or territory with urine, droppings, and saliva and with secretions from glands located in different regions of the skin. Adult male opossums will mark structures in their territories by licking and rubbing the neck and head

The suprasternal gland patch is indicated by the yellow stained fur overlying the glands of this young male opossum captured and released in late January.

region against them. Olfactory cues from secretions of glands within the skin of neck and head region as well as within the saliva identify the presence of a specific male in a given area. Olfactory perception by female opossums is highly developed and can be used to identify individual males by such marked objects. Male opossums show definite aggressive responses when encountering such "signposts" of another male. These observations suggest that pheromones serve to keep other opossums informed of the presence of a specific male in a given area. Because home ranges of opossums are so overlapping, it is believed that such signposts may be used to attract females rather than repel competitive males in the same territory. Female opossums also have been observed marking structures by licking and rubbing the head and neck region against them. What influence such markings have on male behavior is unknown. Suprasternal glands, paracloacal glands, and glands within the footpads have been examined in the opossum. The suprasternal gland region of the opossum is located along the ventral midline between the neck and sternum. The distribution of glands in this area is easily recognized in adult males during the breeding season by a diamond shaped patch of fur stained either a bright yellow or a light amber-orange color. The stained fur is the result of secretions from the underlying glands. The coloration of fur over the suprasternal gland area does not occur until puberty and is thought to signal the onset of testosterone secretion. Coloration of a similar region of fur in the adult female opossum is faint or absent.

Both sexes do have, however, a prominent pair of paracloacal glands located along the lateral walls of the cloaca. Each pair consists of two parts: a central storage bladder and surrounding sudoriferous (scent) glands that drain into the former. A single long duct links the storage bladder to the cloaca. The orifices of these ducts are easily identified when handling opossums due the expression of stored secretory material. The substance secreted is a pasty, pea-green fluid that has a musky odor. The release of secretory material from the paracloacal gland complex appears to be, at least in part, under voluntary control and occurs primarily when opossums are handled. What role these glands play with regard to the scent marking of an opossums territory is unknown.

An interesting observation that several individuals have witnessed is that if captive adult female opossums are housed in outdoor pens during the breeding season, they will soon attract males to the area in which they are held. Whether the attraction of males to these females is due to a form of vocalization or to pheromones is unknown.

Vocalizations

Most witnessed vocalizations of opossums have occurred during aggressive encounters between adults and consist primarily of a hiss, growl, or screech dependent on the intensity of the encounter. An unusual sound, a metallic click, is also used during non-aggressive encounters and is most often witnessed when a male is signaling a female or vise-versa during the breeding season. Striking two large glass marbles together can create a very similar sound that imitates this vocalization. Pouch young opossums often make a somewhat similar vocalization (thought to be a distress call) to signal the mother when they are away from the safety of the pouch.

"Holed Up"

Hibernation is one of the ways many small and medium-sized mammals in north-temperate regions have solved the problem of low temperatures and winter scarcity of food. True hibernators, such as chipmunks and woodchucks, allow their body temperatures to drop to just above freezing. Their hearts beat very slowly and the rate of respiration is dramatically reduced. The decrease in overall metabolism is such that the consumption of stored fat accumulated during the late summer and fall is very slow. These true hibernators enter a deep sleep in their dens that may last for several weeks. **The opossum does not hibernate**, but may remain inactive (holed up) for short periods of time (several days) during severe winter weather. During prolonged cold spells they are lethargic and curl up in their dens to sleep. Opossums are not considered true hibernators because they awaken during the winter to feed and have only slight changes in body functions. They have little or no drop in body temperature. As the opossums stored energy reserves in the form of fat are not as extensive as in hibernating mammals; the opossum must forage for food on a regular basis, even during extreme weather conditions. It should be emphasized however, that the opossum does gain weight during the fall for the winter months. The stored fat is primarily subcutaneous and abdominal but may accumulate in other body regions such as the tail. Weight gain in opossums

A winter opossum out foraging for food during late December.

fed in captivity is often accompanied by an accumulation of fat in the tail, which may attain a considerable girth. It is the breakdown (catabolism) of fat, muscle, and perhaps other tissues that allow the opossum to "holed up" during severe weather conditions that may last for a considerable period of time. Opossums rarely leave their dens if the temperature is below -7°C (19°F). In addition to increasing fat stores, the opossums coat also changes dramatically in preparation for winter as is true of most mammals native to the United States. During the summer months the coat of the opossum may be quite sparse with a very thin undercoat. The summer pelage is then shed and replaced by a thicker coat in preparation for cooler weather. During the fall and winter months the coat is thick and luxuriant characterized by a heavy undercoat.

It was the coat of winter opossum that was at one time of commercial interest to the fur industry. Although the opossum is considered a furbearer by most state agencies and trapping for its fur regulated by an established yearly season, opossum fur is not highly sought after at the current time.

Many opossums lose the tips of their ears and tails to frostbite. This is why older opossums in the northern parts of their range often exhibit ragged ears and/or stubby or shortened tails. The wounds apparently heal readily as infection is rarely observed when animals suffering from the effects of frostbite are encountered. It is believed by most that it is the cold weather that is the limiting factor preventing the continued northward expansion of the opossum range. A leading cause of death at the northern edge of its range is starvation due to cold periods of long duration when the opossum cannot forage for food and/or actually freezing due to extreme cold.

Intelligence

The skull of the Virginia opossum is considered primitive (because it retains many of the cranio-facial features of early fossil therian mammals) and is characterized by a small brain case. The size of the opossum's brain case has been measured by filling the cranial cavity with dried beans and then counting the number of beans it took to fill the cavity. It was found that if the brain case of an opossum, a raccoon and a house cat were compared using this method, the opossum brain case held 25 dried beans; the cat brain case held 125 dried beans and that of a raccoon held 150 dried beans. Thus, the opossum has one of the smallest brain-to-body size ratios among mammals and it was generally assumed that the larger the brain size to total body ratio the more intelligent the animal is.

The opossum brain differs somewhat externally from a typical mammalian brain but in general the internal structure and pathways of its central nervous system show only minor differences from that of eutherian mammals. However, the external surface is smoother and has fewer folds and groves than the brains of eutherian mammals of similar size.

The opossum brain features a pair of large, elongated olfactory lobes, which is not surprising because of this animal's keen, well-developed sense of smell. The opossum brain like that of all other marsupials is characterized by one major distinction: **the complete absence**

of a corpus callosum. The corpus callosum is a large band of nerve fibers that functions to connect the cortical areas of the two cerebral hemispheres of the eutherian brain. It is the relative small brain size and the extremely shy and non-aggressive behavior of the opossum in comparison to other mammals that has contributed to the myth that the opossum is a very "stupid" animal. For example, when captured and held by hand, usually by the tail or the back of the neck, the opossum usually does not struggle or attack its captor. If held by the neck and the other hand used to support its back, the opossum simply gives up, relaxes and often clasps its forepaws together in a prayer-like pose. Such a method of handling another wild mammal of equivalent size such as a raccoon or fox would be difficult if not impossible and be met with aggression. It is this **non-aggressive behavior**, together with the knowledge of its relatively small brain size, that have been the major contributing factors in labeling the opossum as being stupid or of less mental capacity than other more aggressive animals. In addition, it must be remembered that the opossum is a nocturnal species. Many of the published observations and stories concerned with the behavior of the opossum were made during the daylight hours at a time when the opossum is normally asleep. If one considers the time of observation it is quite understandable that the opossum gained the reputation of being a bit sluggish in some of the early accounts.

In spite of their apparent primitiveness and small brain size, opossums have a remarkable capacity to find food and remember where it was found. When tested for their ability to remember, opossums scored better than rats, rabbits, dogs, and cats but did not score as well as humans. Opossums can remember the taste of noxious or toxic substances even a year after a single encounter.

Visual discrimination tests have shown that the opossum can learn to discriminate black versus white, different colors, patterns, and geometric forms. Additional studies designed to measure the opossum's ability to solve maze problems indicate that mature opossums were superior to most species (rats, cats) in maze learning tasks.

Defense

Opossums have no highly developed or specialized mechanisms for attack or aggressive offense. They are not fast runners but can climb trees and swim. However, defensive behavior is highly developed in the opossum. Intimidation (bluff) displays are commonly observed when an opossum is cornered and consist of the animal crouching down or sitting back on its haunches and opening the large mouth as much as possible to display its formidable fifty teeth. As this occurs the animal hisses, growls or screeches in that order and with increased intensity.

If caught and handled (picked up) the animal tends to defecate and release a pea-green secretion from the two paracloacal glands located on either side of the cloaca near the base of the tail. If faced with an extreme threat such as a dog attack, the animal may **feign death** or **"play possum"** for which this behavior is named. Playing possum is a passive defensive tactic used by the opossum as well as other species in the animal kingdom, such as the hog-nosed snake. Feigning death functions to turn off all potential signals that might trigger the predator to at-

A recently trapped male opossum exhibiting a typical bluff display. The opossum is crouched down and its mouth opened wide to expose its large canine teeth.

An example of a male opossum feigning death (playing possum). During this behavior the opossum's eyes and mouth remain open. The teeth are bared.

tack or continue its attack. Opossums are extremely tough animals and can withstand considerable abuse. Opossum skeletons that have been examined often reveal well-healed bone fractures which equivalent sized eutherian mammals never would have survived.

During feigned death the opossum often lies on its side with a ventral flexion of the body and tail (it curls up) with its eyes and mouth open and teeth bared. The tongue may hang out of the mouth and the animal continues to drool (salivate) considerably. The digits (toes) of the forepaws are closed and grasp anything in contact with them, even the other forepaw. The opossum appears to be in a catatonic state and this condition may occur for as little as a few minutes or last for several hours. Poking and shaking will not revive the animal from its catatonic state nor will it flinch from abuse while in this state. Younger animals are more apt to play possum than older ones. Studies using electrocardiograms (ECGs) to monitor heart activity and electroencephalograms (EEGs) to monitor brain activity have failed to show any significant differences between opossums feigning death and those in an awake, active state. Although the brain and heart are thought to be involved in the catatonic state observed, the role of their involvement is unknown at the present time. Some have suggested that the catatonic state of "playing possum" is analogous to fainting in humans.

Walking and Running

Opossums have what is described as a primitive plantigrade-quadrupedal type of movement when on the ground. This means that the plantar surface or soles of the forefeet and the hind feet of the opossum are placed flat on the ground. The tail is usually elevated somewhat trailing behind the opossum held at a position level with its back and is not dragged on the ground. The resulting, walking movement is best described as a waddle. When viewed from behind, the rear of the animal tends to roll or rock from one side to the other with each step.

When walking, the opossum has three feet on the ground at the same time for support. Forward progress is accomplished by advancing the opposite foot sequentially as follows: left forefoot, right hind foot, right forefoot, left hind foot. The faster the opossum moves forward, the quicker the rolling action resulting in an exaggerated waddling motion. Maximum running or waddling speed of the opossum is about 3.5 miles per hour. As the opossum's forward speed increases, only two feet are on the ground simultaneously. Balance while running is maintained by keeping diagonally opposed feet on the ground. For example, when the left forefoot and the right hind foot are on the ground, the right forefoot and left hind foot are raised and in motion. With an increase in speed the body rocks from side to side and the head bobs up and down. The tail is used for balance and shifts its position to the side where the hind foot is being raised. Thus, the tail shifts from side to side when running. Contrary to the popular belief that the opossum is an extremely slow moving creature, over a short distance they can be surprisingly quick to avoid capture or predators.

Swimming

The opossum is a strong but slow swimmer and will even swim underwater to escape predators. Two types of swimming patterns have been observed: one pattern uses the same limb movement as seen in terrestrial locomotion; the other involves the synchronous movement of the limbs on one side that alternate with those of the other side. The tail performs sculling movements in both types of swimming patterns. Maximum swimming speed is about 0.5 miles per hour and the opossum can cover distances of 150 yards or more with ease.

Grooming

Like all mammals in the wild, grooming skills of the opossum are extremely important as its life is dependent on this behavior. Opossums spend a great deal of time grooming. This is particularly true during the fall and winter months, as a matted or unkempt coat will result in the animal dying of exposure due to conditions of the weather. Opossums lick the forefeet to keep them clean and use the forepaws to cleanse the facial area in catlike fashion. They sit up on their hind legs like cats using the tail as a prop. The face is wiped in a circular pattern with the forepaws, which are constantly licked. The hind foot is used as a comb to groom the body. The four-clawed digits of the hind foot are held rigid and used to comb particulate matter and parasites (primarily ticks and fleas) from the fur. The forepaws are used to hold the tail during grooming which is licked carefully. During the breeding season female opossums pay particular attention to the pouch and lick this region extensively if pouch young are present to keep this area clean.

Social Behavior

The opossum is generally considered to be a somewhat nomadic, shy, solitary animal that may occupy a specific area or territory for a length of time (six months to a year) before moving on. The time and the size of the area occupied by an opossum are dependent primarily on the abundance of food and water. Individuals will defend the space occupied against other opossums at a given time. The social behavior between individuals is reported to be poorly developed or antisocial except between sexes during the breeding season. However, after mating, females are no longer receptive and will fight persistent males. At other times encounters between adults of either sex are said to be antagonistic. Aggressive behavior between females and males is similar and consists of displays of an open mouth with bared teeth, aggressive vocalizations of hisses and growls, and actual physical contact. The hindquarters are usually depressed, the forelimbs extended, and the head raised.

More recent studies of captive opossums suggest that these animals form stable, hierarchical social relationships with females usually being dominant. Most observed interactions among unrelated opossums kept in large outdoor enclosures were neutral with females often found nesting together. The extreme agonistic behavior observed was almost always between two males. Meetings of the opposite sex during the breeding season results in initial aggressive displays followed by courtship with the two opossums spending several days together. Such studies together with a growing body of evidence from field studies of unrestrained, wild

animals indicate that the social behavior of opossums may be much more complex than was once thought. Overlapping home ranges and a well-developed system of chemical (olfactory) communication suggests much higher encounter rates than previously thought. Environmental factors and availability of food clearly influence space use and the population density of this species. These factors also must influence opossum social structure, as it appears flexible enough to allow high population densities when food is abundant. Such social flexibility, though poorly understood, together with generalized den and food requirements, accounts at least in part, for the opossum's success in continuing to expand its overall range into a variety of environments.

Life Span

Although the opossum has a high mortality rate at all stages of its life cycle, mortality is particularly high during the first year. The death rate of young while still being carried in the pouch is high and ranges between ten and twenty percent. Of those that survive until after weaning, to go out on their own, fewer that ten percent live beyond the first year. A few tagged wild opossums have been shown to have a life span of about two or three years. Thus, the turnover rate of the opossum population is rapid and the indigenous population of a given region is heavily weighted toward the young of the year. The most important causes of death leading to their relatively short life spans are: human-caused deaths due to automobiles (road-kill victims) as well as hunting and trapping, diseases and parasites, exposure and starvation. It is estimated that between four million and eight million opossums are killed each year in the United States by automobiles. Although **predators** such as large owls, foxes, coyotes, and domestic animals (dogs and cats) may reduce the numbers of young animals somewhat, predation does not appear to be a significant cause of mortality in the adult population. The opossum is host to a multitude of external and internal parasites and these generally are debilitating and increase the susceptibility of the opossum to malnutrition and disease. Parasites and human activities apparently are the leading cause of death for most adult opossums.

Opossums, for their size, are one of the shortest-lived animals in the world. Those individuals that do live into their second year show many of the classic signs of advanced aging such as weight loss, lessened motor coordination, and formation of cataracts. Why this occurs so early in this species is unknown. Captive opossums have been recorded as living about twice as long as wild opossums with an occasional individual living up to ten years of age.

Chromosome Number

All cells of the opossum from conception to the adult have the same number of chromosomes and genetic makeup. The only exception being the sperm and egg cells which have half this number. In general, marsupials have low chromosome numbers in comparison to those of eutherian mammals and range from ten to thirty-two chromosomes dependent on the species. Marsupial chromosomes **are larger** than those of eutherian mammals and morphologically distinct sex chromosomes can be identified in marsupials. The largest chromosome pair in the

Virginia opossum measures 14 µm in length, which is about three times the size of the largest pair of human chromosomes. The correct number of diploid chromosomes for *Didelphis virginiana* is **twenty-two**.

The karyotype reported for the Virginia opossum is six pairs of subtelocentric and four pairs of telocentric autosomes, a small subtelocentric **X** chromosome, and a tiny **Y** chromosome.

Resistance to Snake Bite

During casual field studies on mammals indigenous to the Everglades region of Florida, a natural bite by a 160 cm eastern diamondback rattlesnake on an adult opossum was observed. The opossum displayed no apparent distress. After this initial documented observation, several field experiments were conducted manually causing snakes to inflict actual bites on captured opossums. Bites were inflicted using (1) timber rattlesnake (*Crotalus h. horridus*), (2) eastern diamondback rattlesnake (*Crotalus adamanteus*), (3) cottonmouth moccasin (*Agkistrodon p. piscivorus*), (4) Russell's viper (*Vipera russelli*), and (5) the common Asiatic cobra (*Naja n. kaouthia*). None of the opossums developed observable local reactions or systemic effects other than trauma attributable to fang penetration. Because the amount of venom injected by an actual snakebite is highly variable, another series of experiments was conducted in which a known volume of snake venom was injected directly into the opossum blood stream. These studies demonstrated that the Virginia opossum has a remarkable physiological tolerance to both snakebite and massive intravascular infusion of venom. Thus, the opossum appeared resistant to venomous snakebites (pit vipers), particularly from those snakes that share the same range and habitat with the opossum. If a copperhead, water moccasin, or rattlesnake bites the opossum, the reaction is only a small local swelling similar to that of a bee sting. It should also be pointed out that in some regions of the United States poisonous snakes represent an important part of the opossum's diet.

Scientists have now identified the protective factor in opossum blood serum using high-pressure liquid chromatography and named this small protein (a proteinase inhibitor) **lethal toxin-neutralizing factor (LTNF)**. The reason opossums are naturally resistant to the proteolytic effects of several venoms is that the proteinase inhibitors in their blood bind to and neutralize the venoms. The proteinase inhibitors are not antibodies but proteins that occur naturally in opossum serum. A series of experiments were conducted in which a predetermined lethal dose of toxins derived from animals, plants, and bacteria injected into mice were given LTNF. It was observed that LTNF neutralized the lethal effects of all snake venoms tested: western diamondback rattlesnake (*Crotalus atrox*), Thailand cobra (*Naja kaouthia*), Asian viper (*Daboia russelli*), Australian taipan (*Oxyuranus scutellatus*); is effective against scorpion (*Androcetonus australis*) and honeybee (*Apis mellifera*) venoms; plant-derived ricin (one of the most toxic plant-derived toxins from castor seeds) as well as botulinum toxin from the bacterium,

Clostridium botulinum. Recently, the active ingredient of LTNF (a ten amino acid peptide) has been sequenced and synthesized and tested on mice. The results proved that the synthetic form was effective in inhibiting the lethality of all the toxins tested. Lethality was inhibited even when the synthetic LTNF was administered two hours after toxin or venom injection. Most importantly, synthetic LTNF can now be made in abundance without depending upon the natural source, opossum serum. Thus, LTNF can be made in abundance and may prove to be a universal therapy against toxins from animals, plants, and bacteria to help save human lives.

Resistance to Rabies

Opossums **are rarely** found to be **rabid** and appear to be **resistant to many viral diseases** such as distemper, parvovirus, and feline hepatitis normally found in domestic animals (cats and dogs) as well as some wild mammals. The resistance to many of the viral diseases is thought to be due, at least in part, to the slightly lower body temperature of the opossum as compared to eutherian mammals.

Body Temperature

The body temperatures of marsupials are generally lower than those of eutherian mammals and are subject to considerable variation. The average body temperature of the Virginia opossum is about 35° C. The body temperature of the opossum fluctuates, being higher at night when they are active and lower during daylight hours when they are inactive. The opossum can control its body temperature at ambient temperatures (from 0° C to 37° C) and have been observed to lick their forepaws, hind feet and tail base to wet them with saliva and enhance cooling via evaporation during the hot summer months. Early pouch young cannot control their body temperatures and rely on the body temperature of the mother when in the pouch.

5. Reproductive Biology of the Male

Other than size, the male opossum is characterized by a larger head the snout of which appears more robust in comparison to the females' snout, which is more pointed and delicate. The two upper canines of the male also tend to be larger and are obvious on close inspection. During the breeding season, sternal glands within the skin secrete a yellow/orange colored substance that stains the fur overlying these scent glands. The stained fur appears as a diamond shaped patch located along the ventral midline between the neck and sternum. The testicles of the male are housed within a scrotal sac located between the hind legs. The testes remain descended throughout the year but can be retracted by a cremaster muscle as in eutherian mammals.

The reproductive system of the male opossum consists of a pair of testes and their excurrent ductal system, two accessory sex glands (the prostate and Cowper's glands), and a bifid penis. Under normal conditions, such as handling, the penis of the opossum is not visible or at least readily apparent. This is because in the non-erect state it is withdrawn into the body. The penis is located ventral to (beneath) the anal opening and is contained within a shallow space called the cloaca. Within the cloaca the free (bifid) end of the penis is surrounded by an invagination of skin called the preputial sac. During erection, the penis extends from the cloaca, is directed forward and the preputial sac stretched out so it disappears. The testes are contained within a visible, prominent **pre-penial scrotum**. In this particular arrangement the scrotal sac is positioned in front of the penis. The scrotum is pendulous with a narrow stalk that joins it to the ventral body wall. The scrotal skin of the opossum is thin and covered with a sparser and finer hair than the remainder of the body wall. Each testis is housed within an additional membranous sac called the tunica vaginalis. It is heavily pigmented and black in color. The actual testes of the opossum are cream white in color. The opossum has an elongated, carrot-shaped prostate that forms around the urethra. It consists of three separate regions that differ in color. Cowper's glands of the opossum consists of three pairs of glands. The accessory sex glands of the opossum, like those of the man and other mammals, function to provide a nutritive, fluid vehicle for the spermatozoa at mating.

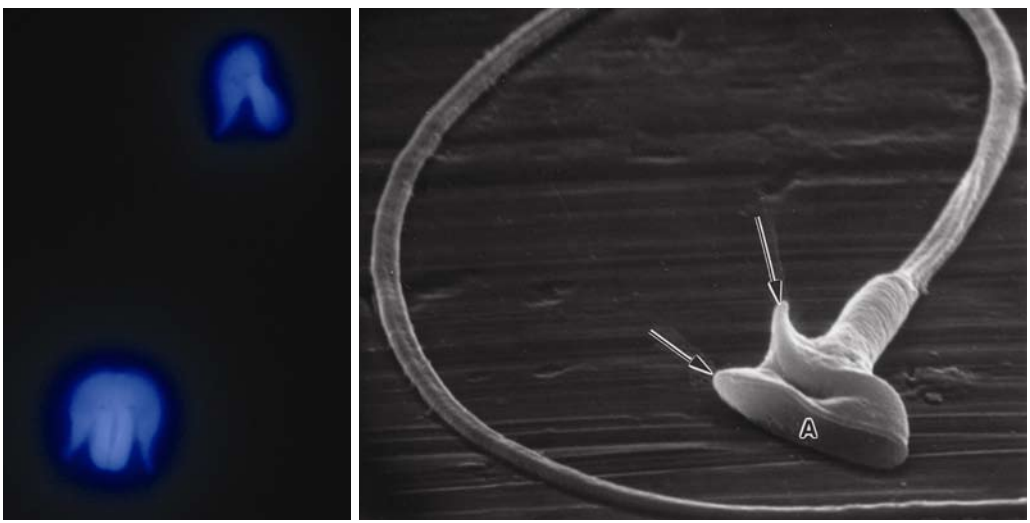
Low Sperm Numbers

Mammals generally produce many more spermatozoa than needed for fertilization. In cattle and sheep, for example, it is estimated that in excess of 500 million sperm are ejaculated into the female reproductive tract for the fertilization of just one or two eggs. Smaller mammals, such as rabbits or rats, may inseminate 150 million and 50 million sperm, respectively, to fertilize just a few eggs. In comparison relatively few spermatozoa (~3 million) are inseminated into the female reproductive tract by the male opossum. Why the opossum has a much lower sperm count when compared to the majority of eutherian mammals is unknown. However, in spite of their low numbers, opossum spermatozoa are remarkably efficient and may fertilize fifteen to thirty ova or more dependent on how many are released by the ovary.

The success of the opossum's spermatozoa in the female reproductive tract is thought to be due to two unusual phenomena: sperm pairing and temporary sperm storage in oviductal crypts of the female. The phenomenon of sperm pairing is reported to occur in other marsupials native to Central and South America but does not occur in marsupials native to the Australasian region.

Sperm Pairing

Like other mammals, the testes of the opossum produce sperm cells (spermatozoa). Once formed the sperm cells enter the male excurrent ductal system and are stored in the distal end of a region of the ductal system known as the epididymis. During their transit through this portion of the male reproductive system, the sperm undergo physiological maturity, ie, they become motile and capable of fertilization. Unlike eutherian mammals, several structural

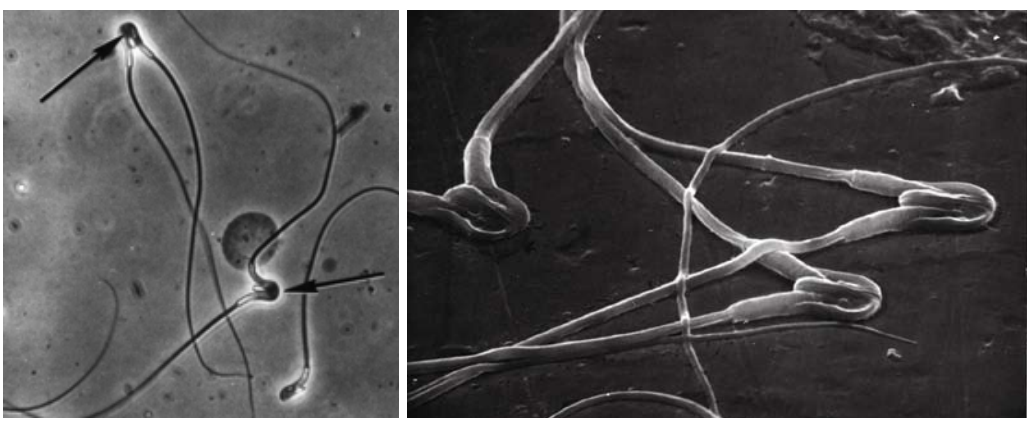


The head (nucleus) of an immature spermatozoon or sperm cell is shown in the upper left illustration. Heads of paired mature spermatozoa are shown at the bottom of this figure. These spermatozoa were treated with DAPI which binds to DNA and shows a blue fluorescence when examine with UV light. An illustration of an immature spermatozoon as seen with the scanning electron microscope (right) shows the shape of the head (arrows) which contains the DNA, the position of acrosome (A), and the tail of an immature spermatozoon. (Krause and Cutts, Arch. Histol. Jpn. 42: 1979).

changes can be observed as the sperm move through the opossum epididymis. In the first part or proximal portion of the epididymis, the spermatozoa are single and characterized by V-shaped heads (nuclei) positioned at right angles to the tails.

The nuclei contain the DNA of the male germ cells. One nuclear arm is larger than the other arm and the larger arm is covered on one surface by a thin bag-like structure called an **acrosome**. The acrosome contains several different enzymes that are essential for fertilization to take place as these enzymes digest a hole in membranes surrounding opossum ova (eggs) thereby allowing sperm to penetrate the eggs. Initially, a large cytoplasmic droplet envelops the V-shaped nuclear area resulting in a funnel-shaped head characteristic of the immature spermatozoon. Unlike most other mammals, sperm from the central region of the opossum epididymis show two dramatic major morphologic changes: the **cytoplasmic droplet disappears** and the **nucleus undergoes a 90° rotation.**

As a result of these changes the two nuclear arms come to lie on a plane parallel to the long axis of the tail. Following the 90° rotation of the heads, two spermatozoa **pair**. The formation



Mature spermatozoa (left) taken from the distal epididymis and photographed through a light microscope. Two pairs of paired spermatozoa (arrows) are shown as well as a mature unpaired spermatozoon. Note that the arms of the sperm head have rotated 90° and now lie parallel to the sperm tail. The photograph to the right illustrates three pairs of spermatozoa as seen in the scanning electron microscope. The acrosome of each sperm head lies adjacent to that of the opposing sperm of the pair. (Krause and Cutts, Arch. Histol. Jpn. 42: 1979).

of each sperm pair involves the close association of the large arms of the nuclear heads, acrosome to acrosome. Both unpaired and paired sperm are found in the mid region of the epididymis and all show a marked increase in motility as compared to sperm observed in the proximal region. The majority of sperm are paired (~96%) in the distal region of the epididymis.

Although some motile sperm remain in the uterus of the female three hours after mating, the majority are found in the oviducts. The majority of motile, viable sperm are confined to the oviducts twelve hours postcoitus and are remarkable in their ability to remain in the oviduct as viable cells for a considerable period of time. It is believed that the spermatozoa are maintained and stored temporarily as viable cells within a special microenvironment of crypts (small cavities) within the oviduct. Though low in number, the overall effectiveness of opossum sperm is thought to depend on sperm pairing and their concentration in the oviduct where fertilization takes place. Sperm pairing is thought to prevent the acrosome of each sperm forming a pair from coming into contact with secretions within the female reproductive tract until the time of fertilization. Sperm pairing involves the precise alignment of the cell membrane overlying the acrosome enabling paired sperm to behave as a single unit with coordinated tail beat patterns that greatly increases straight-line velocity compared with unpaired sperm. The sperm heads are glued together by specific cell adhesion molecules found only in the region of the cell membrane overlying the acrosome, which interact only with identical adhesion molecules within the cell membrane of other sperm cells. Pairing improves swimming efficiency and speed to gain access to the oviduct as quickly as possible. The action of a swimming sperm pair is reminiscent of watching a seagull in flight.

Following the separation of the two sperm forming a pair in the oviduct, the acrosome is activated and becomes swollen and fills with membranous vesicles. Thus, opossum sperm pair and cooperate to reach the site of fertilization. The phenomenon of sperm pairing may exist to protect the low number of sperm produced by the opossum without compromising fertility. Because of the pairing phenomenon, opossum sperm and those of other species from the Americas are unique when compared to Australasian marsupials or eutherian and prototherian mammals. In the latter groups of mammals sperm pairing does not occur.

6. Reproductive Biology of the Female

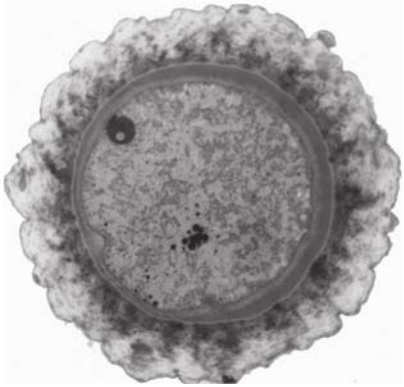
The reproductive system of the female opossum consists of two ovaries, two oviducts, and two completely separate uteri that terminate as two separate narrow cervices. The opossum cervices are short, neck-like structures of the distal uteri that connect to a vaginal-cul-de-sac. When viewed from the interior the cervices appear as prominent papillae protruding into the lumen of the vaginal-cul-de-sac. The vaginal apparatus of the female reproductive system consists of two distinct lateral vaginal canals, which extend from the vaginal-cul-de-sac to the urogenital sinus. Thus, unlike eutherian mammals with two uteri and a single vagina the opossum retains two vaginae. Early in embryologic development all therian species have paired vaginae which later fuse to from a single structure. These paired structures do not fuse to form a single vagina in the case of the opossum because unlike eutherian mammals the tubes (ureters) that carry urine from the kidneys to the bladder lie between them and prevent them from fusing together during development. **Neither of the vaginae functions as a birth canal**. Instead, a new opening forms in the connective tissue that lies between the vaginae. This **special pseudovaginal (birth) canal** develops at the time of delivery and then disappears; hence it is a transitory structure in the opossum. A deep, well-defined pouch also characterizes the female opossum, which functions as "living incubator" in which to maintain her young during the first seventy-five to eighty days after their birth.

Breeding Season

The Virginia opossum is a seasonal breeder, with the reproductive cycle beginning shortly after the winter solstice and lasting until June through most of its range. The female opossum is **polyestrous** with each cycle lasting about twenty-eight days. Variations of the cycle do occur and are thought to be due to a seasonal shift in the cycle length and may be due to dietary deficiency as well. Females are receptive for one-two days. The opossum usually produces two litters of young per year in the United States with an occasional third litter occurring in southern California and in southern Texas. In general, the Virginia opossum breeds less often and has larger litters in the north whereas in the south more litters of smaller size are a common occurrence. However, the net production of young for both regions is about the same. First matings usually occur in January or February in most of the United States but may be as late as March in regions of southern Canada or as early as mid December in Florida or Louisiana. The interval between litters is about 120 days. An average of about twenty-three young are born, with as may as fifty or more being reported. Of these those that are able to locate a teat and attach to it survive, the rest quickly perish. It is thought that female opossums have only two years of reproductive activity.

Superovulation

Ova (eggs) are discharged from both opossum ovaries at the same time, reach the uteri at the same time, and initially appear structurally identical whether fertilized or unfertilized.



A single unfertilized ovum (egg) flushed from the uterus of an opossum with physiologic saline. The egg was preserved in fixative, cut through its center, and photographed through a microscope. A thin homogenous membrane (the zona pellucida) surrounds the ovum, which in turn is enveloped by a thick layer of mucoid material. (Krause, Adv. Anat. Embryol. Cell Biol. 143: 1998).

The number of ovarian follicles that ovulate varies considerably with about sixteen being the average. However, as many as sixty have been recorded. Mature opossum oocytes or eggs **lack a corona radiata** and are surrounded **only** by a thin, homogenous appearing **zona pellucida** that measures between 2.4 μM and 3.3 μM in thickness at the time of ovulation. The zona pellucida of the opossum consists of proteins and glycosaminoglycans. This situation is in contrast to most, if not all, eutherian mammals including man where a layer of follicular cells called the corona radiata lies on the external surface of the zona pellucida and completely surrounds the ovum. Like other marsupial species, if fertilization of the ova occurs, the Virginia opossum completes its gestation period within the time frame of a single luteal phase. The luteal phase is less than 60% of the estrus cycle in the opossum and is followed by a follicular phase leading to the next estrus and ovulation.

Fertilization

The fertile estrus period of the opossum is thought to be about twelve hours in duration but may be longer. Following ovulation and mating, the paired sperm separate in the oviduct. With the separation of the sperm pair, the acrosome of each sperm swells and is filled with small membranous vacuoles. Just prior to fertilization the acrosomal surface of a sperm comes to lie flat on the zona pellucida surrounding the ovum. The hydrolytic enzymes (acrosin, arylsulfatase, hyaluronidase, N-acetylhexosaminidase) contained within the acrosome are then released and digest a relatively large, uneven hole in the zona pellucida. The acrosomal surface of the sperm head then fuses with the cell membrane (oolemma) of the ovum. **Fertilization is monospermic**. Granules called cortical granules, concentrated in the peripheral margin of the opossum ovum, disappear at fertilization. The release of the cortical granules is referred to as the cortical reaction and prevents other sperm from penetrating the egg. There is an absence of egg and sperm membranes around the decondensing sperm head soon after its incorporation into the ovum. This pattern of gamete interaction more closely resembles that of non-mammalian vertebrates and invertebrates than eutherian mammals.

The second polar body, containing excess genetic material resulting from previous reduction divisions (meiosis) by the egg, is shed by the oocyte following the incorporation of the single spermatozoon.

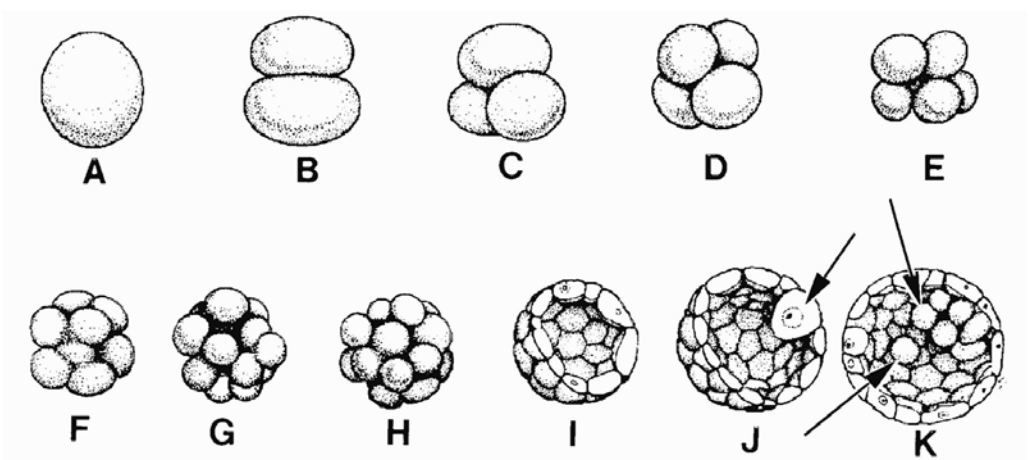
Immediately following cortical granule release by fertilized ova, the oocytes are covered by a thick layer of oviductal mucus produced by non-ciliated secretory cells within the epithelial lining of the oviduct. Oocytes together with their oviductal mucoid covering may reach a diameter of 0.75 mm. The fertilized oocytes now referred to as zygotes reach the uterus between twelve and twenty-four hours after ovulation.

7. Formation of the Embryo

Cleavage

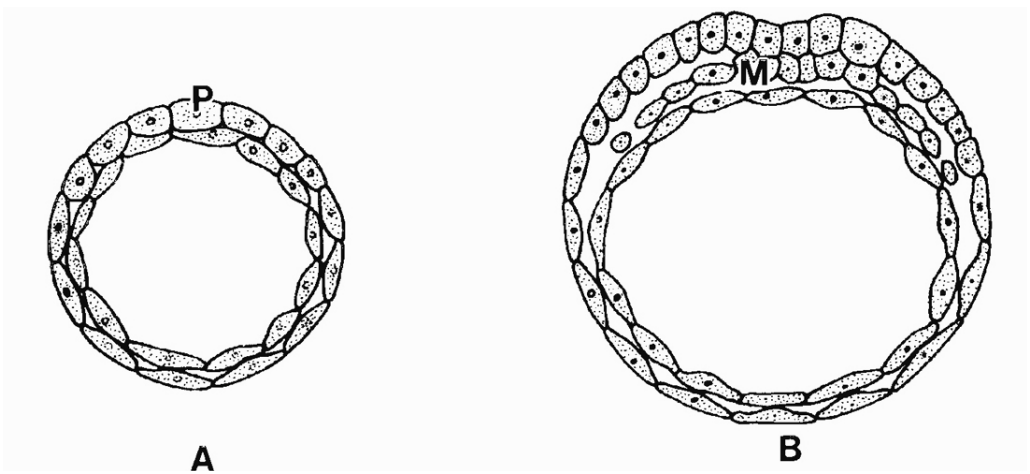
The short gestation period of the opossum as well as most other marsupial species can be subdivided into five basic categories: cleavage, unilaminar blastocysts, bilaminar blastocysts, trilaminar blastocysts, and early organogenesis.

The first **cleavage** of the fertilized ovum occurs on the second day after ovulation. The second, third, and fourth cleavages occur during the third day of gestation. The resulting blastomeres (cells) formed during the early cleavage stages become arranged around a presumptive space or blastocoele and lie separately along the inner surface of the zona pellucida. The blastomeres establish contact with one another as a result of increased cell number due to mitotic activity (cell division) as well as the spreading and flattening of individual blastomeres. A **unilaminar blastocyst,** that measures about 0.11 mm in diameter and usually consisting of thirty-two cells uniform in appearance, is formed by the fourth day of gestation. The unilaminar blastocyst stage lasts between twelve and twenty-four hours in the opossum. As the unilaminar blastocyst undergoes expansion, there is a decrease in the width of the surrounding zona pellucida, the mucoid layer, and a surrounding shell membrane. When the unilaminar blastocyst measures about 0.34 mm in diameter and consists of fifty to sixty cells, enlarged cells called **"mother endodermal cells"** appear between the cells at one hemisphere of the blastocyst.



A series of drawings illustrating the early embryonic development (cleavage) of the opossum immediately following fertilization: A one-cell stage, B two-cell stage, C three-cell stage, D four-cell stage, E six-cell stage, F eight-cell stage, G twelve-cell stage, H sixteen-cell stage, I thirty-two-cell unilaminar blastocyst, J first appearance of endodermal mother cells (arrow), and K the spread of endodermal mother cells (arrows) along the blastocyst interior to create a bilaminar blastocyst. (Krause, Adv. Anat. Embryol. Cell Biol. 143: 1998).

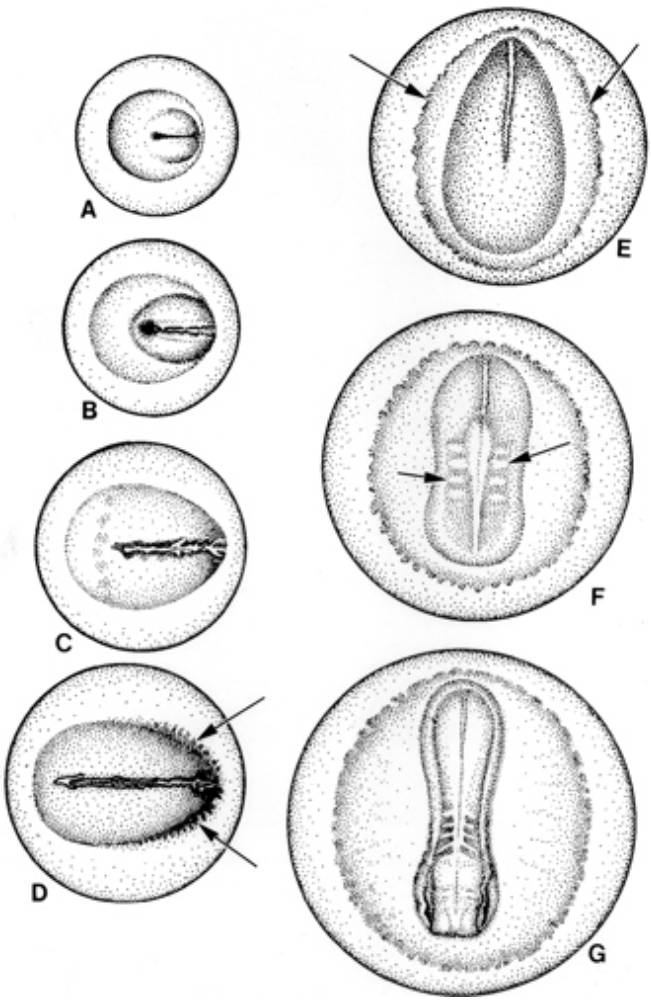
These endodermal cells then migrate into the interior space of the unilaminar blastocyst and establish a single layer of cells that line the interior surface thereby transforming it into a bilaminar blastocyst. By about the sixth prenatal day the transformation of a unilaminar blastocyst to a **bilaminar blastocyst** is complete and now measures about 0.75 mm in diameter. Both the mucoid layer and the zona pellucida are lost (disappear) as the bilaminar blastocyst is established. It is by this means that the definitive endoderm (the inner most layer of cells) is established in the forming opossum embryo. It is from the endoderm that the respiratory and digestive systems will eventually develop. The cells forming that region of the blastocyst where the endodermal cells originated and migrated from now appear taller and more crowded together as compared to adjacent cells. These cells, referred to as **protodermal cells**, identify the position where the future opossum embryo will form. The forming embryonic area of the opossum unlike eutherian mammals occupies a superficial position within the wall of the blastocyst and is not covered by trophoblastic cells. Likewise, a morula stage does not form nor is an inner cell mass observed in the opossum as in eutherian mammals. Mesodermal cells begin to appear between the ectodermal and endodermal layers of the six-day opossum blastocyst and a **trilaminar blastocyst** measuring 1.4 mm in diameter is present by the seventh day of gestation.



The drawing labeled A illustrates a region through the center of an opossum bilaminar blastocyst. The enlarged cells in the region labeled P form an area known as the medullary plate and is the region within the blastocyst wall in which the opossum embryo will develop. A single layer of endodermal cells lines the blastocyst interior. The drawing labeled B illustrates a region through the center of a trilaminar blastocyst. Mesodermal cells labeled M form a layer of cells between the inner lying endodermal cells and the outer lying ectodermal cells in the region of the embryo called the medullary plate. The mesodermal cells arose from cells forming the medullary plate and migrated into the region between the two other cell layers. The mesodermal cells are found only in the region of the medullary plate and do not migrate completely around the circumference of the blastocyst. (Krause, Adv. Anat. Embryol. Cell Biol.143: 1998).

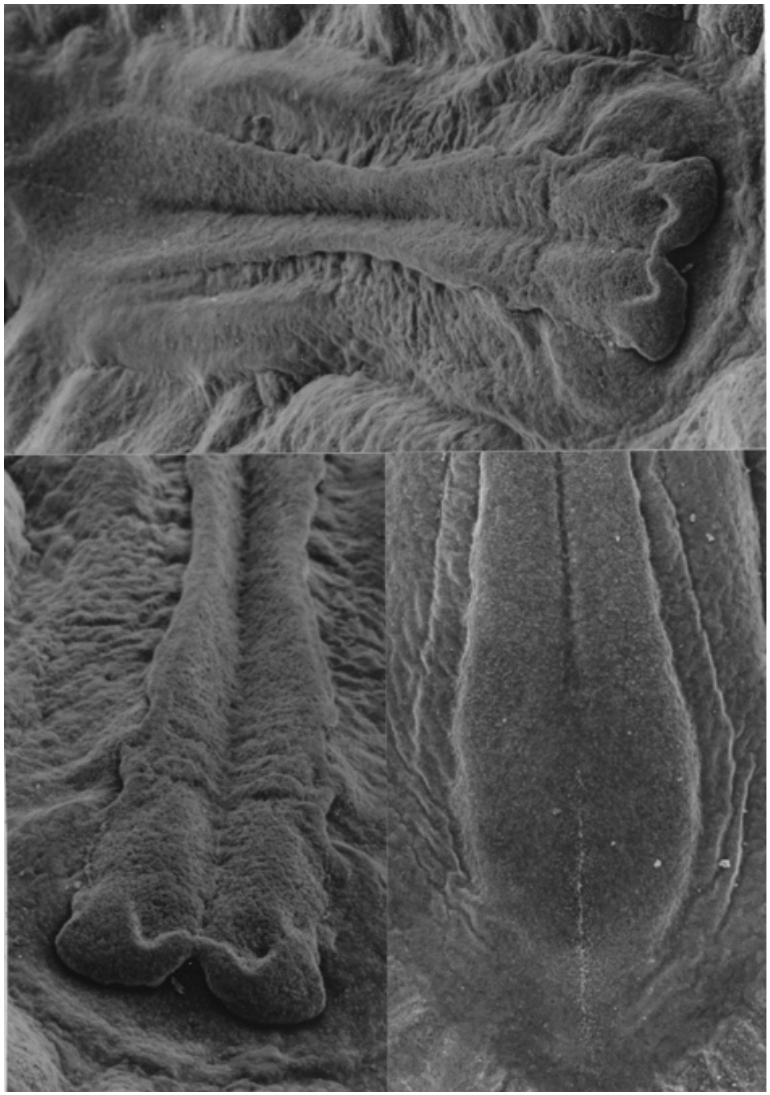
Initial Embryo Formation

The mesodermal layer expands and extends beyond the forming embryo to lie between the extra embryonic endoderm and ectoderm by the eighth day of gestation.



Drawings of a series opossum embryos representing embryonic day five to embryonic day nine illustrate the spread of mesoderm and the early development of the embryo: A the primitive steak with mesodermal crescents, B Hensen's node, C the primitive groove (mesodermal cells occur only beneath the medullary plate of the forming embryo at this stage of development), D and E mesodermal cells are beginning to extend beyond the medullary plate of the embryo (arrows), F initial formation of somites (arrows), and G the first appearance of coelomic rudiments. (Krause, Adv. Anat. Embryol. Cell Biol.143: 1998).

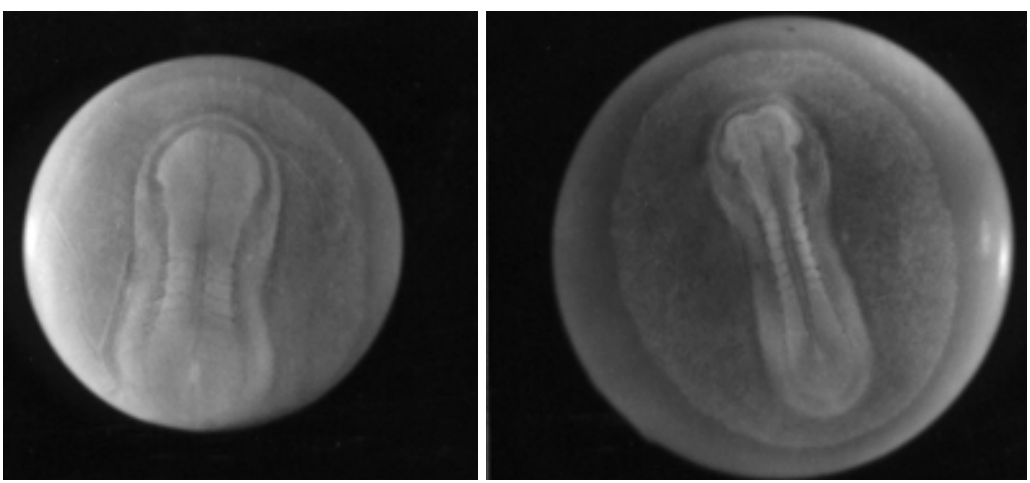
It is the mesodermal layer within the region of the embryo that will eventually give rise to the musculoskeletal and cardiovascular systems of the developing opossum. The outer ectodermal layer will develop into the brain, spinal cord, and skin of the opossum embryo.



Scanning electron micrographs depicting the surface features of an opossum embryo growing within the wall of a nine-day blastocyst. The outer ectodermal cells of the forming embryo at this point in time are transforming into the central nervous system. In the upper figure the developing brain is seen at the right, the spinal cord (which appears as deep groove) courses horizontally along the length of the embryo. The illustration to lower left is a photograph taken at a different angle. The forming brain (near the bottom) is elevated in comparison to the remainder of the embryo and the neural groove that will become the spinal cord are clearly shown. The figure at the lower right illustrates the tail region of the forming opossum embryo.

The extra embryonic region of the trilaminar opossum blastocyst may show either two or three cell layers. The one-third of the extra embryonic blastocyst located on the side opposite the forming embryo is never invaded by mesoderm and represents a persisting portion of the original bilaminar blastocyst. This region of the embryonic vesicle consists of only two layers (ectoderm and endoderm) and will form what is known as the non-vascular portion of the definitive yolk-sac placenta. The vascular portion of the yolk-sac placenta will develop from the extraembryonic region of blastocyst wall that contains mesoderm, ie, all three-germ layers.

Prior to and during the ninth day of gestation, the developing blastocysts are **spherical in shape** and **float freely** within secretions of the uterine cavity. The opossum embryos at this stage of development obtain their nutrition from secretions produced by cells lining the uterine cavity that are rich in protein, particularly albumins and pre-albumins. A shell membrane surrounds each blastocyst during the first nine days of the twelve and a half day gestation period. The surrounding shell membrane is porous and allows the diffusion of nutritive materials from the uterine cavity to the embryo. Cells (trophectoderm) forming much of the exterior surface of the embryonic sphere (blastocyst) absorb the nutrients. Thus, the shell membrane of the opossum is thought to act primarily as a physical barrier that functions to separate maternal and fetal tissues.

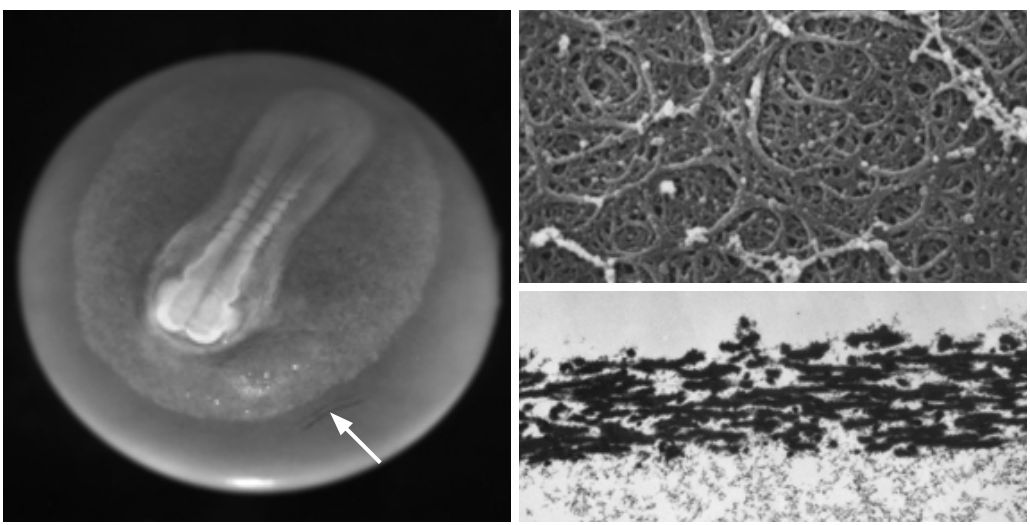


A photograph of an eight-day blastocyst floating in tissue culture medium (left) demonstrates that the opossum embryo forms in the wall of the embryonic sphere. The mesoderm of a nine-day blastocyst (right) has spread a considerable distance beyond the forming embryo (in the center) and appears as a textured material within the wall of the embryonic sphere. (Krause, Adv. Anat. Embryol. Cell Biol. 143: 1998).

The Shell Membrane

When the opossum zygote enters the uterus it measures between 0.4 and 0.5 mm in diameter and is surrounded by a zona pellucida, a thick mucoid layer and an outer, limiting shell membrane. The **shell membrane** of the opossum is transparent during life but looks like a mat of closely interwoven fibers when examined with the electron microscope. The shell membrane is made up of a disulfide-rich structural protein called **ovokeratin**. Structurally, the shell membrane of the opossum appears very similar to that associated with bird eggs. Unlike birds, however, the shell membrane of the opossum never becomes a calcified structure. The ovokeratin protein is thought to be the secretory product of non-ciliated secretory cells forming uterine glands located near the orifice of the oviduct. The physical separation of fetal and maternal tissues by the shell membrane is thought to allow the opossum embryos to attain a critical mass and/or produce factors so that they will not be destroyed (absorbed) by uterine lining cells that would otherwise recognize them as foreign tissues.

The shell membrane of the opossum acts as a porous physical barrier separating maternal and embryonic tissues for the first nine days of the twelve and a half day gestation period.

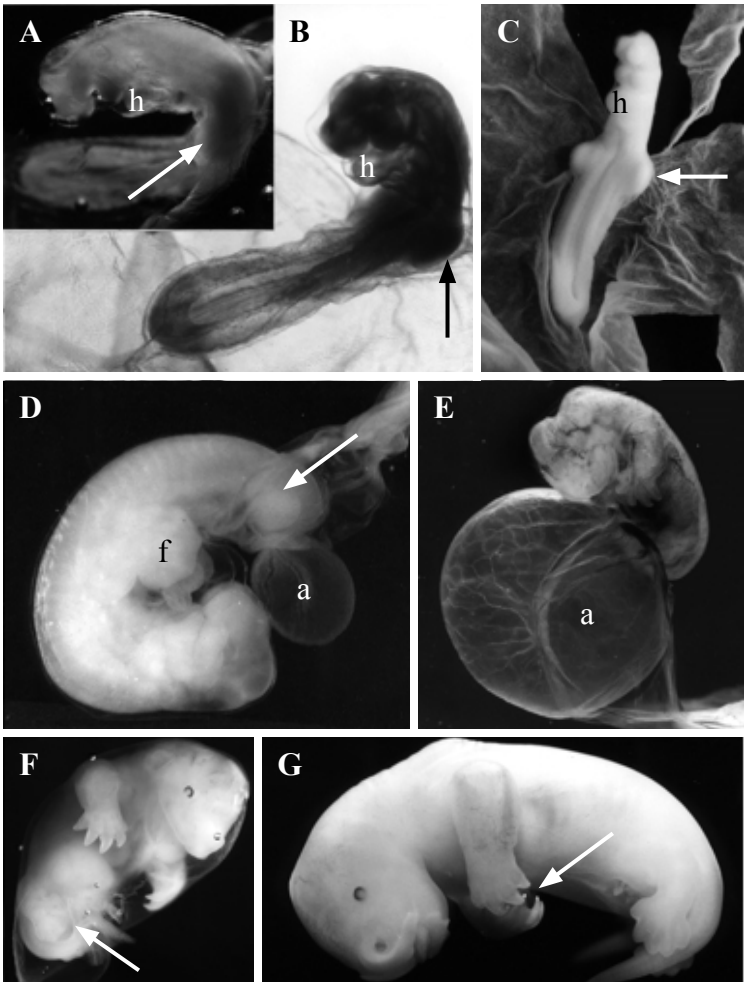


A nine-day opossum blastocyst showing the forming embryo and surrounding extra embryonic mesoderm recognized by its rough texture. This blastocyst was photographed directly while floating in tissue culture. The entire blastocyst is surrounded by a transparent shell membrane, which has the toughness of cellophane or saran wrap. A small wrinkle in the shell membrane is shown at the arrow. If studied with the scanning electron microscope (upper right) the features of the external surface of the shell membrane can be seen in detail. The shell membrane consists of a mat of interwoven ovokeratin fibers that vary in diameter. When viewed in section with the transmission electron microscope (lower right), the ovokeratin fibers appear dense and homogeneous without apparent substructure. (Krause and Cutts, Anat. Rec. 207: 1983).

Initial Organogenesis

Early organogenesis occurs during the **last three days** of the twelve and a half day gestation period. **During this three-day period, organogenesis of the forming embryo proceeds at an astonishing rate, resulting in a viable fetus capable of survival in the external environment and of independent migration to the pouch**. The forelimbs are present as limb buds early during the tenth prenatal day, the cervical flexure has occurred in the forming head region, and a developing heart is clearly visible. In contrast to the cranial or head region, the caudal (tail) half of the opossum torso is elongate and smooth in appearance and does not exhibit any external evidence suggestive of hindlimb development. Late in day ten the buds of the forming hindlimbs are visible for the first time. The snout, mouth and tongue become well defined during prenatal day eleven and by prenatal day twelve the digits of the forepaws possess deciduous claws.

In contrast, the hindlimbs are paddle-like in structure and show only the initial stages of digit formation at the time of birth.

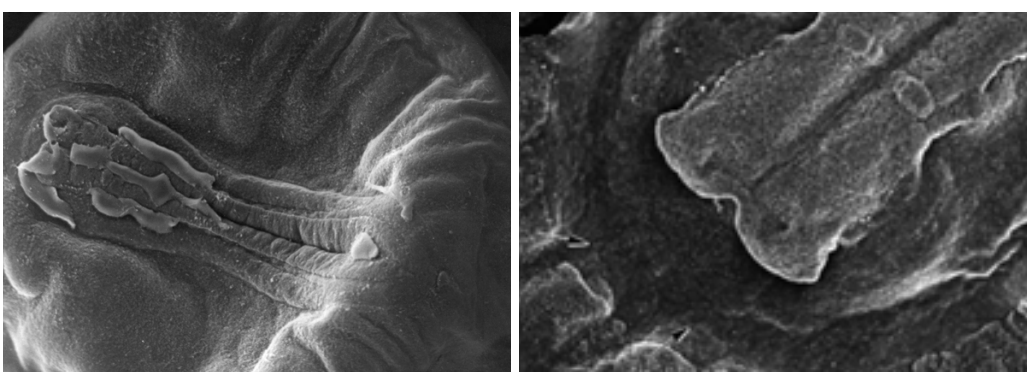


An explosive amount of growth occurs in the opossum embryo between embryonic day nine and embryonic day twelve of the twelve and a half day gestation period. Figures A, B, and C show opossum embryos representative of development that has occurred early in the tenth embryonic day. In these embryos the developing heart (h) and buds of the forelimbs (arrows) are clearly visible. Figure D is representative of an opossum embryo late in embryonic day ten. The head and torso can be recognized, the forelimb (f) appears as a paddle with forming digits whereas the hind limb appears only as a bud (arrow) and is in the initial stages of formation. The tiny sac-like structure is the allantois (a). Figure E represents an eleven-day opossum embryo. Note the continued development of the head and the establishment of the paw and digits on the forelimb. The allantois (a) has expanded in size equal to that of the embryo. Figure F illustrates another eleven-day opossum embryo that more clearly illustrates features of the developing head. Note the large, open mouth and the well-developed tongue. The pigment layer of retina in the developing eye is also clearly visible. Compare the structural features of the forelimb with those of the hind limb (arrow). Figure G is an opossum from the twelfth day of gestation just hours prior to birth. Note that deciduous claws (arrow) tip the digits of the forepaws. The retina of the eye remains visible through a transparent skin. Nostrils and the oral shield around the mouth are clearly visible. The hind limb continues to have a paddle-like configuration but now shows the initial formation of digits. (Krause, Adv. Anat. Embryol. Cell Biol. 143: 1998).

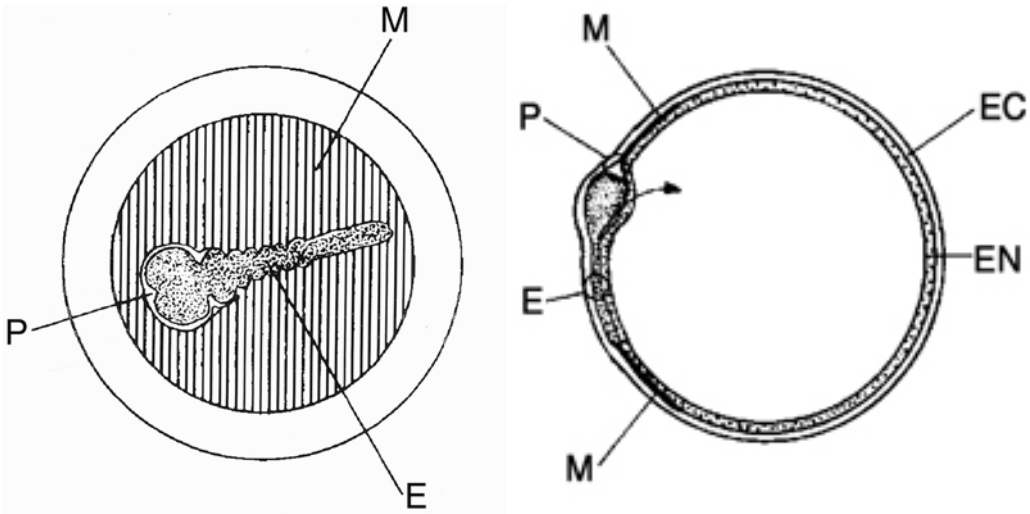
Fetal Membranes

The shell membrane disappears near the end of ninth prenatal day and as a result of the disappearance of this physical barrier, a **noninvasive yolk sac (choriovitelline) placenta** is established that persists throughout pregnancy. The region of the embryonic sphere (blastocyst) beyond the forming embryo that is to become the yolk sac placenta forms prior to the loss of the shell membrane. With the establishment of blood vessels within the extraembryonic mesoderm, a distinct blood vessel called the **sinus terminalis** forms at the most distal extent of the vascularized mesodermal layer.

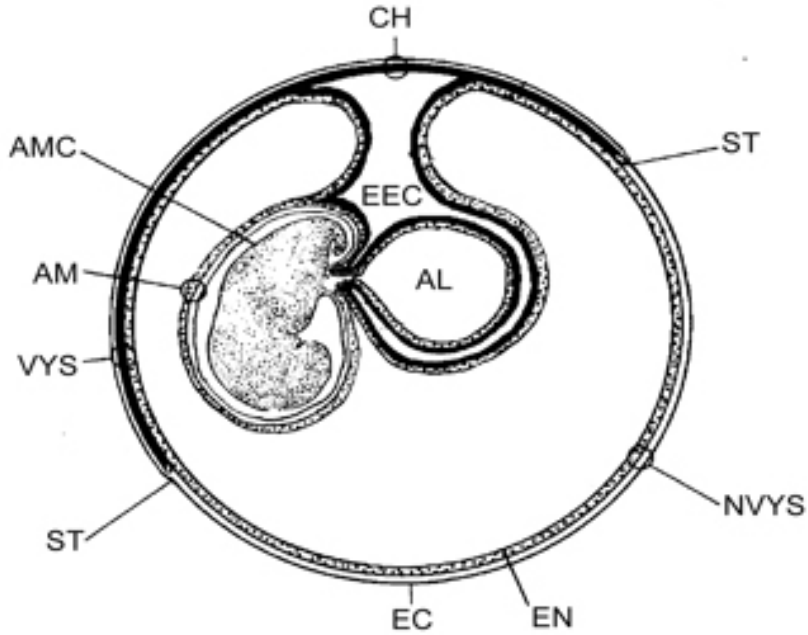
The sinus terminalis is a large collecting blood vessel that forms at the edge of the mesodermal layer and courses around the equator of the embryonic sphere. The sinus terminalis clearly identifies the point where the remainder of the wall of the embryonic sphere consists only of endoderm and ectoderm (trophectoderm) and remains similar in structure to the original bilaminar blastocyst. Likewise, the extraembryonic mesoderm does not invade another region of the embryonic sphere called the **proamnion** located around the head of the forming embryo. As the opossum embryo elongates, the head region flexes and extends with the surrounding proamnion to enter the central cavity of the original embryonic sphere. As a result of the embryo extending into the central cavity of the embryonic sphere, that portion of the amnion, which surrounds the cranial half of the embryo, consists of only ectodermal and endodermal layers. The remainder of the opossum **amnion** develops as a result of folding and consists of ectoderm and avascular somatic mesoderm.



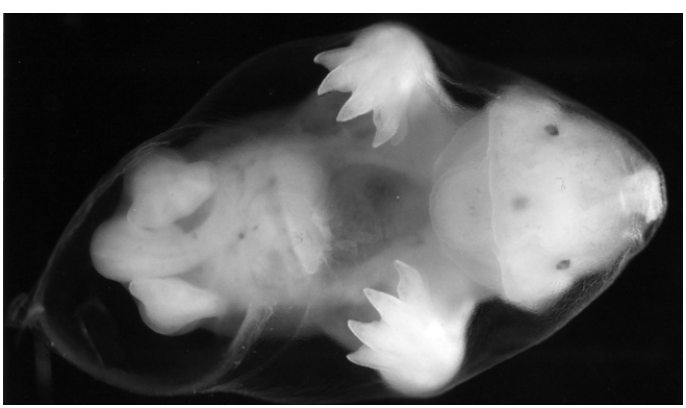
Following removal of the shell membrane, a scanning electron micrograph (left) demonstrates that the nine-day opossum embryo is continuous with, and part of the embryonic sphere forming the wall of the blastocyst. A crescent-shaped region of the blastocyst wall called the proamnion (arrows) surrounds the forming brain and head area of the nine-day opossum embryo (right). As development continues the head region of embryo flexes inward toward the central cavity of the blastocyst. Eventually, the entire embryo enters the central cavity of the blastocyst and in doing so becomes enshrouded by a thin, transparent fetal membrane known as the amnion. (Krause, Adv. Anat. Embryol. Cell Biol. 143: 1998).



A diagrammatic representation of a nine-day opossum blastocyst and embryo. The left figure illustrates the position of the opossum embryo (E), the extent of the extra-embryonic mesoderm (M), and the position of the proamnion (P) when examining a blastocyst as seen from above. When viewed in section, as if the blastocyst was cut in half and viewed along its cut surface as shown in the right figure, the position of the various cell layers (M mesoderm, EC ectoderm, EN endoderm), the proamnion and the embryo forming in the wall of the blastocyst are illustrated. The arrow indicates the direction the embryo will follow as it enters the cavity of the blastocyst. In doing so, cells of the proamnion proliferate and as the embryo enters the central cavity pulls with it and becomes enshrouded within the thin amniotic sac. This action can be visualized if one imagines pushing a finger (the opossum embryo) into a partially inflated balloon (the proamnion region of the blastocyst wall). The rubber of the balloon stretched around the finger would be equivalent to the amniotic sac. The end result is that two membranous sacs surround the forming opossum embryo. The amnion which is closest to the embryo and forms largely from the proamnion and the yolk sac placenta which is derived from the remaining blastocyst wall following the entry of the opossum into the central cavity of the blastocyst. (Krause and Cutts, Acta Anat.123: 1985).



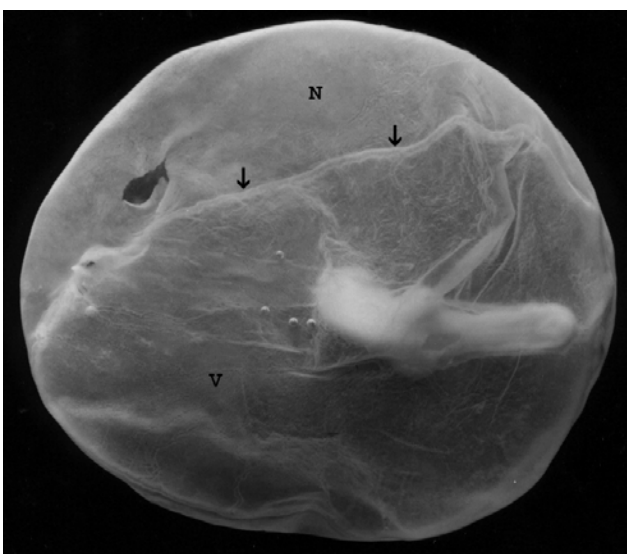
As the developing opossum embryo enters the interior of the blastocyst, the fetal membranes rapidly differentiate and expand. The amnion (AM) closely enshrouds the embryo separated from it by narrow space called the amnionic cavity (AMC) filled with amnionic fluid. The original blastocyst wall differentiates into three regions: a small chorion (CH), a vascular yolk sac placenta (VYS), and a non-vascular yolk sac placenta (NVYS). The two major regions of the yolk sac placenta are separated by a large collecting blood vessel, the sinus terminalis (ST). The third fetal membrane, the allantois (AL), is depicted developing in the extraembryonic coelom (EEC). The endoderm (EN) and ectoderm (EC) also are shown. (Krause and Cutts, Acta Anat. 123:1985).



An eleven-day opossum embryo enshrouded by the amniotic sac. Amniotic fluid lies between this fetal membrane and the opossum embryo. The surrounding yolk sac placenta has been removed. Note the position of the heart in the chest cavity of this partially transparent embryo. (Krause and Cutts, Anat. Anz. 161: 1986).

The result of the forming opossum embryo extending into the central cavity of the embryonic sphere (the original blastocyst) is that two fetal membranes come to surround the embryo. The *amnion,* which forms an enveloping sac closest to the embryo and is derived largely from the proamnion and the early *yolk sac placenta,* which forms from the surrounding blastocyst wall following the entry of the opossum into the central cavity of the blastocyst.

After these events have occurred, the developing yolk sac placenta of the opossum can be subdivided into two major regions: **a vascular yolk sac placenta** (trilaminar omphalopleure) formed of trophectoderm, vascularized mesoderm, and endoderm and a **non-vascular yolk sac placenta** (bilaminar omphalopleure) formed only by trophectoderm and endoderm. The non-vascular region represents that region of the original embryonic sphere never invaded by mesoderm and the sinus terminalis separates the non-vascular region from the vascular region of the yolk sac placenta.



Following the entrance of the opossum embryo into the blastocyst interior and its acquisition of a surrounding amniotic sac, the remaining and surrounding blastocyst wall becomes transformed into a structure known as the yolk sac placenta. Thus, by this stage the opossum embryo is surrounded by two fetal membranes: an inner amniotic sac and an outer yolk sac placenta. The latter consists of two regions separated by a large collecting blood vessel called the sinus terminalis (arrows). In one region, the vascular yolk sac placenta (V), small blood vessels develop in the mesodermal layer. This vascularized region establishes an intimate relationship with the uterine epithelium but does not fuse with or invade the latter. It is this region of the yolk sac placenta that is involved in respiration and absorbs and transmits nutrients from the uterus to the embryo. The remaining region, the non-vascular yolk sac placenta (N), lacks mesoderm and as a result does not become vascularized. It may be involved in the absorption of nutrients to a limited degree. (Krause and Cutts, Acta Anat. 123: 1985).

As the surrounding shell membrane breaks down during the tenth prenatal day, the yolk sac placenta expands rapidly but only the vascular region establishes contact with the uterine lining epithelium. The vascularized region of the yolk sac placenta continues to expand and by the eleventh prenatal day covers the elaborate folds and crypts of the uterine lining. The sinus

terminalis continues to clearly define the boundaries of the vascular yolk sac placenta. Once established, the intimate relationship between the uterine lining and the vascularized region of the yolk sac placenta remains unchanged until birth. Although closely associated with the uterine epithelium, cells of the trophectoderm (which form the outer layer of the yolk sac placenta) **never attach directly to or invade the uterine lining** as occurs in most eutherian mammals. Thus, despite numerous earlier accounts referring to eutherian mammals as "the placental mammals", **marsupials also have a placenta called a yolk sac placenta or a choriovitelline type of placenta**. The opossum yolk sac placenta is different from the yolk sac of birds, reptiles, and the monotremes in that it never contains a true yolk substance.

It is important to understand that the cells making up the uterine lining epithelium during the last three days of gestation also differ markedly in structure from cells forming the lining epithelium during the first nine days of the gestation period. The uterine lining through the ninth day of gestation is very glandular and consists of a thick pseudostratified columnar lining epithelium with scattered ciliated cells. Scattered ciliated cells and tall secretory cells also form the uterine glands. The secretory cells have structural features that indicate that it is this cell type which is responsible for synthesizing materials and secreting them into the uterine cavity to be used by the developing opossum embryo. At this time (the first nine days of the twelve and a half day gestation period) the yolk sac placenta has yet to form and the embryonic spheres are floating free within the uterine secretions. From prenatal day ten until birth, cells forming the uterine lining epithelium become simple columnar in structure, lack cilia, and are filled with secretory vacuoles containing lipid droplets and proteins. The number of uterine glands present decreases. These observations suggest that the nutritive role of the glands in the uterus to be taken over or at least supplemented by cells forming the uterine lining epithelium during the last three days of gestation in the opossum. Thus, uterine epithelial cells and cells of the trophectoderm are active in the transport of nutritive materials. Uterine lining epithelial cells transport these materials into the uterine cavity where they are absorbed by cells of the trophectoderm. They then enter the vasculature of the yolk sac placenta and are transported to the developing embryo by umbilical vessels. The transport of materials reaches its peak during the last three days of the gestation period at the time when the body of the embryo is established and an explosive amount of growth takes place. Both the bilaminar and trilaminar regions of the yolk sac placenta are thought to be involved in the uptake of uterine secretions (histotrophes). In addition, the trilaminar portion is believed to be important for respiration.

Both the opossum embryo and a forming third fetal membrane (the allantois) remain free within a pocket of the yolk sac placenta. The **allantois** develops as a ventral outgrowth from the forming hindgut region of the opossum embryo during the middle of the tenth prenatal day and extends into the extraembryonic coelom. With continued development the allantois expands away from the embryo proper and accumulates a yellow/amber colored fluid to form a balloon-like vesicle, the **allantoic sac**. The allantois continues to expand and reaches its maximum size during the twelfth prenatal day. The enlargement of the allantois is due to the accumulation of materials produced by the embryonic (mesonephric) kidneys, which begin

to function during the latter part of the tenth prenatal day. The allantois never establishes a firm relationship with either the chorion or the yolk sac placenta, which occurs in many other mammalian species.

The allantois of an eleven day opossum embryo appears as a large balloon-like vesicle that contains a yellow/amber colored fluid. In the opossum this structure functions as a storage vesicle during the last three days of gestation and holds materials produced by the embryonic kidneys. It does not become part of the placenta in the opossum. The amnion and surrounding yolk sac placenta have been removed. (Krause and Cutts, Anat. Rec. 211: 1985).

8. Birth

At birth, the young opossums take a different route to the urogenital sinus than the sperm took after mating which lead to fertilization. Following mating, sperm of the opossum pass through two separate vaginal canals, two separate cervices, and two separate uteri to the upper regions of two separate oviducts where fertilization takes place. At birth, the young opossums do not pass through the vaginal canals but instead pass from the uteri to a centrally located birth canal called the **median** or **pseudo-vaginal canal**. The pseudo-vaginal canal forms in a loose connective tissue that lies between the vaginal culs-de-sac and the anterior end of the urogenital sinus at the time of birth. The newly formed passageway is simply a split or separation in the connective tissue at this location and after the birth process may contain fragments of fetal membranes and scattered blood clots. This cleft within the connective tissue disappears soon after birth and reforms with the birth of each new litter. What factors control the formation and reabsorption of the pseudo-vaginal canal in the opossum are unknown.

A scanning electron micrograph of the deciduous claws from a twelve-day opossum embryo. These claws are essential for grasping the mother's abdominal fur as the newborn opossum crawls, without help by the mother, from the birth canal to the safety of the pouch. Following nipple attachment, these claws are shed and the permanent set of nails form during the protracted postnatal period. (Krause and Cutts, Anat. Anz. 161: 1986).

Journey to the Pouch

The newborn opossums climb **unaided by the mother** from the opening of the birth canal to the pouch using their well-developed forelimbs and clawed digits.

During the birth process, the female usually sits on her haunches, curls over somewhat and approximates the opening of the birth canal to an area beneath the pouch thereby shortening the distance the newborn opossums must travel to locate and enter the pouch. The tiny forepaws of these newborn animals are capable of grasping, and with their clawed digits grasp the mother's fur and wriggle their way to the pouch. The newborn opossum exhibits a swimming motion (overhand stroke) where the head and neck are flexed to one side followed by the forward movement of the opposite foreleg. The head and neck are then flexed to the opposite side while the deciduous claws of the forepaw clasp the abdominal fur of the mother. As the forepaw is pulled back toward the newborn opossum it is pushed forward. In a short while (the journey from the birth canal to the pouch usually takes between two and four minutes), the newborn opossum enters the relatively large space of the pouch, tangled with large, coarse curly hairs among which are usually thirteen tiny teats.

Guiding Cues

Despite the embryonic nature of the brain and spinal cord of the opossum at birth, the newborn has acquired enough neuromuscular control to permit it to crawl from the birth canal to the pouch unaided by the mother. Recent scientific evidence has shown that the neuromuscular system controlling head and forelimb movement as well as body orientation is coordinated with the sense of **smell** and **touch** and the **ability to sense gravity**. Newborn opossums always orient and crawl away from gravity (this activity is referred to as being negatively geotropic) and as a result the opossums travel upward toward the pouch as the mother sits on her haunches. A band of about twenty sensory hair cells appear in the forming utricle of the inner ear mechanism about twenty-four hours prior to birth.

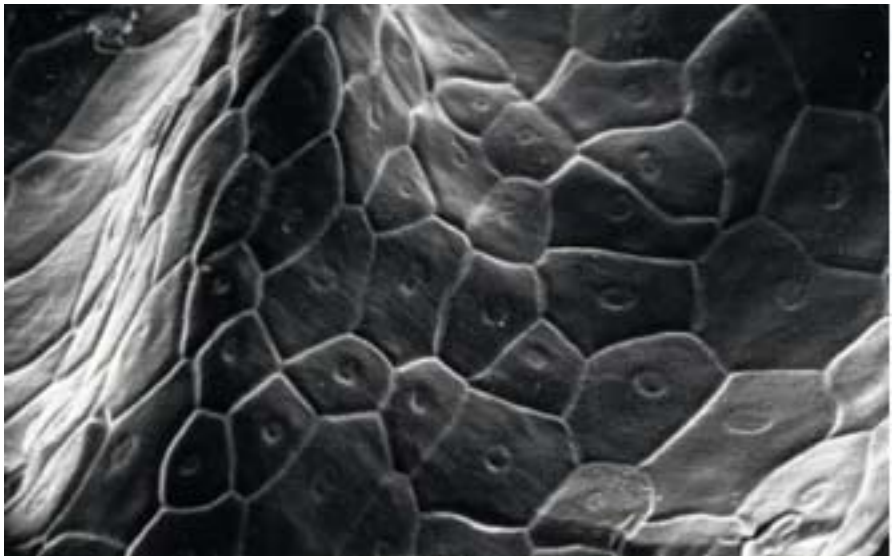
These sensory hair cells give the newborn opossum the capacity to sense the direction of gravity. Likewise, olfactory bipolar nerve cells appear in the opossum nasal cavity just prior to birth and are located immediately interior to the openings of the external nares (nostrils). Nerve processes (axons) from these sensory nerve cells have been traced coursing directly into the developing opossum brain just prior to birth. Thus, the opossum also is born with a keen sense of smell, a sense that it uses to guide itself to the safety of the pouch. Prior to the birth of her litter, the mother opossum assumes a position where she sits on her haunches. She thoroughly licks and cleanses the vulva area, grooms the abdominal fur between the birth canal and the pouch, as well as teat area within the pouch. The instinctive licking behavior of the mother is thought not only to cleanse the migratory pathway used by the newborn young to gain access to the pouch area and nipple attachment, but also serves to provide olfactory cues via the saliva which aid in guiding the young in which direction to crawl. How newborn opossums are able to recognize such cues without prior experience is unknown. Once the pouch is located and entered, the head of the newborn opossum is moved in wide arcs and

A scanning electron micrograph of the snout of an opossum just prior to birth shows several structural features essential for the opossums survival: large open external nares (nostrils), a large mouth containing a large well developed tongue, and an oral shield with its characteristic scalloped appearance. Just interior to the orifices of the external nares lies a patch of olfactory epithelium that provides the newborn with a sense of smell used to guide the young to the pouch. The snout and oral shield contain nerve endings sensitive to touch and temperature that aid in the location of teats (nipples) as the newborn moves its head in wide arcs once within the pouch. Once touched by the snout, the teat is immediately sucked into the mouth with the aid of the large tongue. (Krause and Cutts, Anat. Anz. 161: 1986).

when the sensitive snout touches a teat it is immediately sucked into the mouth using its large well-developed tongue. Therefore, tactile cues of the snout also are important in the final step for the location and attachment to a teat.

Thus, the most essential structures needed for survival immediately after birth are the most advanced in their development and include: a functional utricle, well developed forelimbs with opposable digits and deciduous claws, large open nostrils and a region of olfactory epithelium in the developing snout with connections to the brain, a well innervated snout sensitive to touch and temperature, and a large open mouth with a well developed tongue. The remaining organs are embryonic in appearance and continue their develop when the young are within the safety of the pouch.

A thin layer of tightly adherent cells called the **periderm** covers the entire opossum embryo immediately prior to birth. The cells of the periderm expand to cover the developing eyes, external ears (pinna) and ear canals (external auditory meatus) and contribute to the collar-like structure around the mouth called the oral shield.



The surface features of individual cells forming the periderm are clearly illustrated by this scanning electron micrograph. The periderm covers the entire external surface of the newborn opossum and is thought to prevent dehydration and bacterial invasion during the first weeks of postnatal life in the pouch. Individual cells appear as irregularly shaped paving stones or tiles. The circular structure within each cell is called the nucleus and contains each cell's DNA. (Krause et al., J. Anat. 125: 1978).

As a result of the growth of cells forming the periderm, the opossum, at birth, is sealed within this protective layer of cells that are thought to prevent dehydration as well as form the initial barrier against bacterial invasion until the immune system becomes active. The only openings to the external environment are the two large nostrils and the opening at the apex of the snout for the mouth.

9. Postnatal Life

Once a teat has been secured, the presumptive lips begin to fuse beginning at the lateral angles of the mouth and proceed over the following two-three days until only a small circular orifice remains at the apex of the mouth to permit the entrance of the teat into the mouth. Cells of the periderm continue to expand and aid in forming a tight seal around the teat of the mother. The enlargement of that part of the teat which lies within the mouth of the newborn opossum results in its becoming a bulbous structure that acts to permanently anchor the young to the mother. Although closely apposed, the tissues of the mother's teat and that which lines the mouth of the young opossum never fuse during this period. As a result, the young opossum will remain permanently attached to the same teat for about the first sixty days of postnatal life. This period of pouch life is known as the **fixation period**. If the young are removed (pulled off) from the teat during this period, the forming mouths are torn and the young are unable re-attach themselves back on the teats because the definitive lips have yet to form. The end result is that the young will die.

Opossum neonates as well as young of other marsupials are able to suckle; however, the mechanics of taking milk from the mother differs from that used by suckling eutherian mammals.

In eutherains, the nursing young compress the teat between the tongue and forming hard palate (roof of the mouth) beginning at the base of the teat and extending the compression toward its tip, thereby stripping out the contained milk. As pressure at the base of the teat is released, it refills with milk due to the contraction of specialized cells (myoepithelial cells) within the mammary tissue, a phenomenon known as milk letdown. In the opossum, the teat lies within a shallow groove on the tongue surface and the mouth is sealed due the fusion of lips around the entrance point of the teat at the tip of the snout. As the opossum sucks, the entire tongue is lowered through the contraction of muscle cells that extend perpendicularly from the dorsal surface to the base of the tongue. As the surface of the tongue is lowered, a negative pressure is created within the mouth and milk is drawn from the teat. The ducts within the teats refill by the mechanism of milk letdown as in eutherian mammals once the pressure is relieved. Thus, young opossums must suckle to obtain milk from the mother, as do most other mammals.

The **epiglottis** of the pouch young opossum is **intranarial** and projects into the posterior nares at the back of the nasal cavity. Because of its position and tubular shape, milk can pass around both sides of the epiglottis to enter the esophagus without interrupting breathing, allowing the young opossum to breathe and nurse simultaneously. Air is taken in exclusively through the nose and after weaning the epiglottal elongation regresses to the adult form.

Opossum Milk

Opossum milk and that of other marsupials differs substantially from that produced by eutherian mammals. The reproductive strategy of marsupial (metatherian) mammals is based primarily on the lactation phase rather than intrauterine development of the young, which is the primary reproductive investment made by eutherian mammals. It should be understood

A newborn opossum photographed prior to its attachment to a teat. Note the large mouth, the nostrils, and the forelimbs the digits of which are tipped with deciduous claws. Compare the advanced development of the forelimb with the immature appearance of the immotile hind limb. The pigmented portion of the eye (the retina) can be seen beneath the transparent periderm and skin.

that the emphasis on lactation by marsupials, in contrast to intrauterine development, is an alternative reproductive strategy and not a more primitive form of mammalian reproduction. In eutherian mammals only minor changes in milk composition occur during the entire lactation period. In contrast, major differences are found in the composition of marsupial milk at different phases of lactation. In the early stages of milk production (lactation), opossum milk is dilute and daily production is low. In later stages of lactation opossum milk is concentrated, shows an increase in the amount of protein, and production is high. Water is the major component of opossum milk at all stages of lactation. However, other differences between marsupial and eutherian milk have been found. In the Virginia opossum for example, total solids, hexose, protein and lipid increase progressively during the first weeks of lactation. Carbohydrate and lipid levels then fall later in the lactation period whereas protein is the main constituent of milk solids at all stages of lactation. There are no consistent changes in the concentration of potassium, sodium, or magnesium during the lactation period in the opossum. In contrast

to these ions, calcium ion concentration increases during the first six weeks of lactation, maintains a concentration of about 100 mM/l until about ten weeks postpartum, which then declines to about 65 mM/l at the end of lactation.

The total dependence by the young of the opossum on milk during the first sixty days of lactation suggests that other substances, in addition to nutrients, may be acquired via this route. Immunoglobulins are known to be transferred from the mother opossum to her young solely through the colostrum and milk. Whether or not other maternally derived factors (hormones and/or peptides) also are transferred to the offspring from the mother through the milk is unknown at present; however, a protein known as parathyroid hormone-related peptide has been identified in the milk of the opossum.

Appearance of Pouch Young Opossums

Opossums are about the size of a honeybee at birth and light pink in color. The newborn opossum weighs about 0.16 grams (0.006 of an ounce) and measures about 10 mm in length.

The animals are born naked, blind, and deaf. The most obvious external morphological feature of the early pouch young opossums is their overall embryonic appearance. A large mouth and nostrils characterize the head. The forelimbs and paws are well developed and functional which is in contrast to the hindlimbs, which are only in the paddle stage of development and immotile. Deciduous claws tip the toes of the forepaws. By the end of the second week in the pouch, further development in addition to overall size becomes apparent. The external ears are now visible as swellings on the sides of the head. The deciduous claws have been shed from the digits of the forepaws and the hindlimbs are rapidly developing. The eyes remain tightly closed and the mouth tightly sealed around the teat. The upper and lower lips become well defined by the seventh week of postnatal life, but remain fused to one another. The external ears are visible by this time and a light, downy hair that is more prominent along the spine covers the skin. By the end of the tenth postnatal week the young opossums are quite well developed. The eyes are beginning to open but the upper and lower lips remain fused at the corners. The young animals can let go of the teat, are freely mobile, and leave and return to the pouch. The young opossums are fully furred except for the ventral (belly) surface. The hindlimbs and forelimbs now show a similar degree of development.

At the end of the eleventh postnatal week, the young opossums are completely furred and the coarse guard hairs are a prominent feature. The eyes are fully opened and the lips are completely separated. Near the end of the lactation period, the young opossums may be left in a nest or denning area while the mother forages for food. This latter part of the lactation period is often referred to as the **nest phase of lactation**.

The young opossums are not completely **weaned** until about 96-108 days after birth. Some young, particularly those of the second litter, may continue denning with the mother or other littermates for three to four months before becoming completely independent and solitary. Sexual maturity is attained by about six to eight months.

Organogenesis

A major feature that distinguishes marsupial from eutherian mammals is the short period of intrauterine development and the immaturity of their young followed by a protracted period of development within a pouch. The opossum has a remarkably short gestation period that lasts only twelve and a half days. **This is the shortest gestation period of any mammal**. Consequently, opossum young are in a very immature state of development at the time of birth. Major body systems such as the digestive, urogenital, respiratory, and endocrine systems are only in the initial stages of development at the time of birth, with the majority of development and growth taking place during the postnatal period while the young are within the protection of the pouch. Therefore, most of organogenesis and all of fetal development occurs when the young are in the pouch. In general, the various organs of the opossum show the same general pattern of development typical of other mammals. However, some subtle modifications do occur early in development, which presumably compensate for the short period of gestation, and increase the chances of survival in the new external environment of the pouch. The brain and organs involved with special sensory reception (sight, hearing, and smell) of the opossum, as with the majority of organ systems, develop almost entirely in the postnatal period. However, essential sub-components of the various organs and systems do develop precociously that allow the very immature opossum to survive the initial events after birth. For example, specific regions of the brain are functional at birth to coordinate movement of the forelimbs with information provided by special sensory receptors. Approximately 91% of brain development occurs **after** the opossums' migration and attachment to a teat within the pouch.

Acquisition of Sight

Ocular development in the opossum essentially follows the same basic pattern as in man and other eutherian mammals. Because of the short gestation period, it is not until around six weeks after birth that most components of the adult eye are identifiable, although in an immature form. These include the corneal layer, the iris, ciliary processes, retinal pigment epithelium/tapetum, and laminated retina with immature photoreceptors. It is not until the opossums are about nine to ten weeks old that the lids begin to open and at this time the structural development of the iris and ciliary body is complete. There is an apparent central-to-peripheral gradient in maturation with regard to the retina, which remains immature peripherally even at thirteen weeks after birth. It is assumed that the young opossums can see lights and shapes with the opening of the lids but it may very well be that full visual acuity is not achieved until much later in development nearer the time of weaning.

Acquisition of Hearing

The ear of the opossum like other mammalian forms consists of three basic subdivisions: an external ear, middle ear, and inner ear. The external ear consists of the auricle (pinnae) and the ear canal (external auditory meatus) that conducts sound waves to the eardrum of the middle ear. The external auditory meatus develops from the first pharyngeal groove and

bounding arches. The pinnae begins to form around the orifice of the external auditory meatus during the eleventh prenatal day; however, just prior to birth the periderm covers the forming pinnae and the meatus becomes filled with peridermal cells.

As a result of the growth of the peridermal layer of cells, the head of the newborn opossum appears smooth and the only openings present are those of the nostrils and mouth. Two weeks after birth the pinnae appear as swellings of skin on both sides of the head. It is not until about the end of the sixth week that the pinnae become visible as independent flaps of skin. The external auditory meatus is open by the end of the seventh week after birth but continues to contain regions filled by peridermal cells. The auricles continue to enlarge until about ninety days into the postnatal period when they acquire most of the adult configuration and size.

The middle ear cavity contains the auditory ossicles, a chain of three bones (malleus, incus, and stapes) that unite the eardrum of the middle ear to the inner ear. The three ossicles consist of cartilage at birth. The cartilage of the ossicles is slowly replaced by bone and this process is not entirely complete even at seventy-eight days into the postnatal period. The formed eardrum lies somewhat horizontally to the external auditory meatus at this time, but as the meatus continues to grow it gradually becomes more erect and assumes the adult position.

The inner ear consists of two major subdivisions. One is referred to as the vestibular labyrinth and consists of the three semicircular canals and two larger chambers, the utricle and saccule. These structures are important for sensing motion, angular movement of the head, position in space and balance. They function primarily in coordination and regulating locomotion and equilibrium. The second major subdivision is called the cochlea and functions in the reception of sounds from the external environment (hearing).

About twenty sensory hair cells appear within the developing utricle of the opossum just prior to birth. These cells are thought to be responsible for the negative geotropic behavior, the instinct of newborn opossums to crawl upward in the opposite direction of gravity, and contribute in guiding the newborn in the direction of the pouch. The remainder of the vestibular portion of the inner ear does not become functional until about the sixth week of postnatal life when righting (vestibular) reflexes are observed. Likewise, the structure of the hearing mechanism (organ of Corti) within the cochlea of the inner ear is not fully established until this time. About fifty days after birth is the earliest time at which acoustic reflexes can be observed.

Acquisition of Olfaction (Smell)

In the adult opossum the olfactory bulbs of the brain are remarkably large and measure about 12 mm in length. These structures make up a major portion of the opossum brain. The large olfactory bulbs are indicative of the large surface area occupied by the olfactory epithelium in the nasal cavity of the snout and the importance of this sense in the opossum. The opossum has an extremely keen sense of smell. Mature appearing olfactory nerve cells are present in opossum olfactory epithelium prior to birth, but are restricted in distribution to just inside the orifices of the external nares along the roof of the developing nasal cavity. The olfactory nerve cells have processes that can be traced to the developing brain of the newborn opossum.

These olfactory nerve cells decrease in number after birth and eventually disappear from this region of the nasal cavity by the end of the second postnatal week. Following teat attachment the snout begins to elongate and restructuring occurs within the nasal cavity. The olfactory epithelium then re-appears later in development within the more protected regions of the nasal cavity and with time covers extensive regions on the turbinates, nasal septum, and roof of the nasal cavity. It is quite significant that mature olfactory nerve cells whose axons can be traced into the developing brain can be found immediately interior to the openings of the nostrils prior to birth in the opossum. Such a strategic position for functioning nerve cells in the newborn opossum strongly suggests that olfactory cues are used to guide the sightless, deaf newborn from the birth canal to the sanctuary of the pouch for teat location, attachment, and survival.

Acquisition of Locomotion

Although the newborn opossum is able to crawl unassisted from the birth canal to the pouch using its precocious forelimbs, opossums are unable to walk until much later in the postnatal period. It is not until about seventy days into the postnatal period that the structural development of the hindlimb finally "catches up" with the development of the forelimb. If the young opossums are examined in the pouch two weeks after birth the limbs do show signs of spontaneous movement that resemble a walking pattern but it is not until about four weeks of age that opossums can support their own weight on their forelimbs for the first time. Opossums are able to support their own weight and step for the first time, but not walk, by about the end of the sixth postnatal week. At the end of the seventh week opossums can stand and support their weight on all four limbs but are still unable to walk. Opossums begin to walk and run effectively between seventy-six and eighty-four days after birth.

Maternal Behavior

During the first few weeks (about sixty days) of postnatal life young opossums are permanently attached to the same teat of the mother due to the mechanical swelling of the teat within the mouth cavity and the development of the mouth around the teat effectively sealing it inside. During this period of time maternal care is limited to keeping the pouch area clean and maintaining some control over thermoregulation by relaxing and contracting the pouch musculature thereby opening and closing the pouch to the environment. The latter activity is important as the early pouch young opossums cannot regulate their own body temperatures and rely on the mother to maintain temperature. When the young opossums reach an age of about nine weeks their brains become sufficiently developed so that the young can at this point begin to control and regulate their own body temperatures. Other than these activities, early maternal care appears to be closely correlated with the normal self-grooming activities of the mother. The female opossum's behavior becomes more oriented toward the care of the young as they begin to emerge from the pouch (about seventy days after they were born). As the young begin to leave and enter the pouch, the mother becomes more attentive towards them and begins a clicking vocalization to aid the young in recognizing her. Likewise, the young make clicking or barking sounds with their lips and mouths to remain in contact with the mother. It is during the later days of pouch life, when the young are no longer permanently anchored to the same

teat and begin exploration within and outside the pouch that the mortality rate of the young rises. During this period the young opossums stay in very close proximity to the mother. Females often express an interest in the young of others when they are encountered and have been observed sniffing and licking them.

The physical and behavioral development of the young at **weaning** is rapid and occurs between ninety-five and one hundred and eleven days after birth. At this time the young may not follow the mother when she leaves the nest but appear to become more independent of both maternal and sibling influences. Young react with fear if they encounter an unknown adult or other animals. During such encounters they have been observed to hiss, growl, and quickly retreat. This time is a critical period in the life cycle of the opossum, as the young must have the muscular strength and physical coordination necessary to find food and avoid and/or escape predators as they go off on their own. The end result is a solitary, independent creature.

10. Relationship to Man

Economic Importance

The opossum is classified as a furbearer by most state agencies dealing with wildlife. However, hunters or trappers do not harvest the opossum in large numbers as its pelt has little value on today's market. In the past opossum fur was used primarily for trim on less expensive coats and hats.

Opossums are very beneficial as scavengers and carrion feeders and function to keep the environment clean. In addition, they are important in rodent and insect control as well as eating snails and slugs. In this regard they are often referred to as nature's "sanitary engineers."

Because opossums will consume any or all forms of vegetable and animal matter they are valuable **indicators of** the overall health of the **environment**. They also perform a valuable function in the spread of various seeds (persimmons and other fruits) in the environment as these pass undigested through their digestive tract.

Scientific Importance

The Virginia opossum is of scientific interest from an evolutionary point of view because it is thought to have retained some features similar to those associated with the original stock of both South American and Australasian metatherian radiations. The opossum as well as most members of the family Didelphidae are considered among the most "primitive" or "unspecialized" of the living metatherians and their generalized morphology also may have retained features that resemble those of the very first therian mammals. Of these, the opossum has received the most scientific attention because historically it was the first Didelphid found and because as a common mammal over much of North America was easily obtained. In contrast, the Australasian forms continued to evolve after the separation of Australia from Gondwanaland and their morphology changed into the variety of forms recognized today as they adapted to occupy a wide range of ecological niches.

The developing opossum is of particular scientific interest in the medical community because of its very short gestation period (about twelve and a half days) and is a species in which "premature birth" is the normal condition. Indeed, most organ development occurs during the postnatal period when the young are within the pouch and can be examined and observed directly. It is thought that by studying the developing organs of the young from this species, which normally are born at such an immature state, a greater understanding might be achieved concerning the development and care of human infants born prematurely.

The short gestation period and simple placental system of the opossum make it an attractive model for studying the initial differentiation and development of organs in tissue culture. Opossum fetuses gathered at ten days gestation have been grown in tissue culture using roller tubes until term. They are one of only a few species in which near term fetuses have been grown in tissue culture successfully. The reason for the success using this species is directly

related to the opossums' embryology. At ten days gestation the yolk sac placenta only lightly adheres to the endometrial lining of the uterus and can teased away easily and transferred undamaged to a culture vessel. Because damage to the fetal membranes can be avoided, the opossums can be maintained in culture medium for the remainder of the gestation period (two and a half days) until term. As the last three days of gestation is that period when the majority of initial organogenesis occurs in the opossum, opossum fetuses grown in culture holds promise as a biomedical tool in which to study chemicals and other factors (teratogenic agents) that may cause birth defects. In addition, the pouch young opossum has proven to be an extremely valuable biomedical model in which to study spinal cord regeneration following injury.

Threat to Human Health or Property

Although opossums often live in close proximity to human habitation, they seldom if ever are the cause of serious problems for farmers, ranchers, or suburban homeowners. Opossums are often blamed for raiding trash containers, but more often than not, they are secondary in such raids and are foraging in what remains following the previous activity of domestic animals (dogs and cats) or raccoons. When pets are routinely fed outside and any excess food remains, if discovered by an opossum, it will adapt that location as a feeding area. This activity can be discouraged by simply removing the food source and when this is done the opossum will move on to a new area in which to feed as is its habit.

The only potential threat opossums may pose to pets or domestic livestock is with regard to horses. Opossums should be considered a threat to the health of horses and other equines particularly if opossums den or feed where horses are being housed and/or have access to the horses' food/water source that can be contaminated. A protozoan known as *Sarcocystis neurona* is thought to be a major contributing factor of a neurologic disease in horses known as **equine protozoal myeloencephalitis** or **EPM**. Opossums are the definitive hosts, and horses and other mammals are the aberrant hosts for this protozoan. If sporocysts (the larval form or infectious agent in the next host) from the intestinal tract (via feces) of infected opossums are ingested by horses in contaminated food or water, they are at high risk for contracting this disease. Studies that examined the blood serum for antibodies against *Sarcocystis neurona* of horses from the Rocky Mountain states demonstrated that these horses have a lower seroprevalence for this protozoan organism than horses from the eastern regions of the United States. This data corresponds to areas of opossum population density.

Tips on Temporary Care of Orphans

Quite often during the summer months dead female opossums with a litter of young in her pouch are found along a roadway after being stuck by a motor vehicle the previous evening. The question that arises is: does one leave the young with the mother and let them die slowly of exposure and starvation or should an effort be made in an attempt to rescue the young opossums to be released back into the wild at a later date? It should also be kept in mind that, in general, **it is illegal** in most states to care for opossums or other wildlife unless you are licensed to do so by obtaining a Wildlife Rehabilitation Permit. Opossums **should not be**


A sleeping litter of young opossums rescued following the death of their mother killed on a roadway. They have found some comfort by snuggling tight to the fur of a teddy bear shown at the left. The two young opossums shown at the right are about seventy-five days old. They are exploring their temporary teddy bear mother. The opossum to the left yawns after waking to take part in this activity.


Orphaned pouch young opossums can be made most comfortable by temporarily rearing them in a confined space such as a large pocket or an old sock. Wildlife rescuers in Australia have used similar but larger "artificial pouches" to raise orphaned kangaroo joeys. The opossum shown in the left photo was asleep in a pocket but poked its head out to temporarily explore the surroundings when this photograph was taken. A young opossum peaks out of a sock in the right photo to survey the surroundings while its three littermates remain within the comfortable confinement of the sock.


A litter of older opossums (left) photographed in a den or nest made from straw in the bottom of a barrel. Right photograph, dinner time.


After eating food provided, if given the opportunity young opossums will explore a yard or field and instinctively begin to hunt. They eagerly explore using their keen sense of smell and hearing to locate beetles, moths, and grubs. The very young opossum at the left is actively "bugging" in pursuit of prey. The opossum to the right has secured its favorite prey, a June beetle.


Guess whose coming to dinner (left). The opossum (right photograph) was rescued as a pouch young animal (Joey) following the death of her mother along a roadway. Because of a leg injury she was not released back into the wild and has become an affectionate member of this household.

captured in the wild and the young removed from the mother in an attempt to raise the young just for the sake of raising opossums. Likewise, if a den of young opossums is accidentally discovered in the wild, these animals **should be left alone** and allowed to make their own way in the world.

The following comments are directed to those compassionate individuals who have discovered a dead opossum whose young are still alive and wish to do something in an attempt to save the young. If possible get the young to a **wildlife rehabilitation organization** or a **licensed wildlife rehabilitator** with experience in raising opossums. Unfortunately, more often than not, it has been our experience that if a humane society or various state or federal agencies are contacted they will simply euthanize the animals, as they have neither the time nor money to invest in opossums, which are considered common and rather ordinary. Indeed, many regard the opossum as vermin.

When the young are removed from the mother there are two immediate concerns that need to be addressed prior to taking them to an experienced individual or organization: **dehydration** and the **cold**. A **warm Pedialyte solution** diluted with water (one part Pedialyte to two parts water) should be offered slowly with an eyedropper as soon as possible. Young pouch opossums cannot regulate their own body temperature and are therefore dependent on the body temperature of the mother when they are in the protection of the pouch. The animals should be wrapped in smooth cloth (old t-shirt or sweatshirt type of cloth) or a fabric that is ravel-free.

They then need to be placed in a box with a heating source (heating pad set on low or an incubator of some type). The temperature should be maintained at about 95º F with about 70% humidity if an incubator is used. Opossum young prefer a confined (small) space that mimics the pouch. If kept for a longer period of time a large old sock or a pocket of some type can be used and is often a comfortable, preferred sleeping place for young opossums. If a sock is used the young should be stimulated to eliminate wastes after feeding by gently rubbing the cloacal area with a moist warm cloth. A similar trick is often used when raising young orphaned dogs and cats to keep the bedding area as clean as possible.

If the animals need to be maintained for a length of time while a wildlife rehabilitator is located the following tips are offered that have been used successfully in maintaining young opossums prior to their release back into the wild.

If the young are quite small (four to five inches) they can be fed with **milk replacer for puppies** and should be **provided by an eyedropper**. Slightly larger animals can be fed with same formula to which a whipped banana has been added and the mixture given by eyedropper. If the eyes of the opossum young are completely open they can be offered water and food in a jar lid or some type of small shallow container. Moistened lite dog chow or kitten chow should also be given as a food source. The diet of slightly older young should be supplemented with mealworms, crickets, or June bugs as well as chopped fruit and vegetable matter. High protein diets of red meats, hearts, or liver **should not be offered** to the pouch young as this type of diet may result in metabolic bone disease of the young. Metabolic bone disease has a crippling effect on limb development.

If the young can be maintained until they reach a good body size, the juvenile stage (about 19 inches in length or 120 days old), they have an excellent chance of surviving when released back into the wild. Unlike many wild mammals of similar age, the young opossum seems to rely more on instinctive rather than learned behaviors in order to survive. How many behaviors the young opossum learns from its mother is unknown at present. What has been observed is that when even quite young and having been maintained in captivity, they will instinctively hunt insects and grubs they either smell or hear in a lawn and eat them. A favorite insect in the Midwest is the June bug.

If young juveniles of good size are released near a water source and if they can avoid predators their chances of survival are very good as they seem to have an uncanny knack for finding food. It should be remembered that opossums are omnivorous and will eat almost any plant or animal material they find. Therefore, they should be **released near water** and **away from predators** if possible, particularly away from areas frequented by cats and dogs. It should be understood that if opossums are kept for a length of time and handled daily to maturity, opossums will not acquire a fear of man, become affectionate, and develop a pattern of behavior similar to other domesticated mammals.

Toilet training

Both young opossums (those old enough to explore outside the pouch) and adults have interesting toilet habits and most are easily trained. If they are kept within a confined area, a large covered pen for example, observe the toilet area **they have selected** to use. Once chosen, even an entire litter of opossums will usually continue to use this selected area. The caregiver can then simply place a cat litter box or papers in this area to aid in keeping the housing facility clean and sanitary. This simple observation can make the maintenance of these animals much easier than for most wild mammals. Unfortunately, there are occasional opossums (usually adult females) that prefer to use a water source as their toilet area, which they foul. Movement of the water source has little effect as they actively seek it out and continue to use the water as the preferred toilet area.

Myths

When sleeping, opossums hang upside down by their tails in trees. Opossums are terrestrial animals and spend the vast majority of their time on the ground and not in trees. They climb up into trees primarily to escape predators or are in search of food. They do not sleep in the branches of trees like birds. Instead, they make dens in a variety of places on the ground and curl up to sleep within the protection of the den, as do many other mammals. The tail is prehensile and used as an aid to help with climbing and with balance during walking and running. It is also used to carry bundles of denning material. An adult opossum rarely if ever hangs solely by its tail and, if it did, could do so for only a limited amount of time due to its own body weight.

Opossums breed through the nose. The ridiculous notion that the male opossum copulates with the female through her nose is thought to have occurred during colonial times as a result of assumptions made by early European settlers. The idea for such a notion arose from the observation that penis of the male is bifid (forked) and the behavior that the female opossum grooms her pouch immediately prior to the birth of a litter. It was assumed that when the female placed her snout into the pouch (while grooming) she sneezed the young into the pouch where they then developed.

Opossums will attack pets and livestock. The opossum is a very shy, non-aggressive animal that usually avoids contact with other species unless they are smaller and represent potential prey. The opossum will not attack typical pets such as dogs or cats and certainly not larger livestock. Conflict may arise, however, if pets are fed outside and excess food is left in the feeding area. If the opossum discovers food it will enter such an area to feed on what is available. On occasion, the pets being fed (usually dogs) will surprise the feeding opossum and a conflict will arise. Even then the opossum will try to escape to avoid confrontation and is usually discovered cornered trying to defend itself.

Opossums are the dumbest of all mammals. Despite its small brain size relative to body mass, the opossum when tested scientifically performs as well as rats and other species on various tests that measure intelligence. Its shy, non-aggressive demeanor and ease of handling when

captured have been interpreted by many as this species having a diminished mental capacity. Nothing could be further from the truth as recent testing with regard to the opossum's intelligence has proven.

Opossums are dirty little animals and like rats, spread disease. The opossum like most other animals in the wild are well groomed. Indeed, their very survival depends on being clean and well groomed. If not, their protective fur coat will become matted and dirty and without such a protective layer the animal will eventually die of exposure. The opossum appears quite resistant to some viral diseases such as rabies, distemper, forms of feline hepatitis, and other diseases that plague domestic pets. However, the opossum is heavily parasitized by a wide variety of organisms, which are thought to contribute directly to its short life span.

Opossums are closely related to monkeys because they have similarly shaped hind feet. The opossum is classified as a marsupial; monkeys are classified as primates. The similarities of the hindfeet arose independently in these two widely different species. The opposable digit on the hindfoot of each species evidently provided a specific advantage for each animal in its environment.

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Additional information and references specific to the Virginia opossum *(Didelphis virginiana)* can be found in a comprehensive bibliography entitled:

Krause, WJ (2001) Information Resources on the North American opossum *(Didelphis virginiana)*. A Bibliography on its Natural History and use in Biomedical Research. AWIC Resource Series Number 9. National Agricultural Library, USDA, Beltsville, Maryland.

The bibliography on the Virginia opossum, *Didelphis virginiana* Kerr, is also available as an electronic publication located in the National Agricultural Library at the Animal Welfare Information Center web site: **http://www.nal.usda.gov/awic/pubs/opossum.htm**