

## The Nine-Banded Armadillo: A Model for Leprosy and Other Biomedical Research<sup>1, 2</sup>

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The nine-banded armadillo (*Dasypus novemcinctus* Linn.), a primitive mammal, has unique potential for use in many areas of medical and biological research. Among characteristics which make this animal a useful research model are: (a) a low body temperature (32°–35° C), (b) regular production of litters of monozygous quadruplet young, (c) possible weak immune responses, (d) susceptibility to some human diseases, (e) long life span, (f) ability to build up an oxygen debt, (g) a complexly structured carapace which appears to mutate readily and (h) a delayed implantation period of the blastocyst of about 14 to 16 weeks duration.

It was because of this first factor, the low body temperature, that I first became interested in the armadillo as an animal model in leprosy studies since it was known that *Mycobacterium leprae* multiplies best in man in the cooler regions of the body. Once this first spark of interest was generated, it was not difficult to perceive the advantages inherent in characteristics (b) through (e). This animal has now proved its usefulness for this purpose since a nine-banded armadillo has developed a lepromatoid form of infection after inoculation with human leprosy bacillus inoculum (<sup>30</sup>).

In addition to low body temperature, the fact that quadruplet young are produced regularly from a single fertilized ovum may make this animal extremely valuable for studying the genetic basis for susceptibility to leprosy. This feature may also greatly simplify the development of a strain of animals naturally susceptible to leprosy since one or two animals of a quadruplet

set can be used for inoculation, and the others reserved for future breeding if positive results are obtained.

The long life span of the armadillo (estimated at 12 to 15 years) is advantageous for the study of diseases which develop slowly. Also, preliminary results indicate that the immune responses of this animal may be weak (<sup>58</sup>).

In addition to apparent susceptibility to leprosy, the armadillo is susceptible to other diseases of man, including relapsing fever, exanthematic typhus, murine typhus, trichinosis, and schistosomiasis (*vide infra*). Thus the potential of this animal for use in disease transmission and chemotherapy studies could be significant.

### DESCRIPTION

**Taxonomy and evolution.** The nine-banded armadillo, *Dasypus novemcinctus*, Linn., is a mammal of the Subclass Eutheria, Superorder Edentata, Order Xenarthra (which includes sloths, anteaters and armadillos) and Family Dasypodidae. The Family Dasypodidae comprises the armadillos of which there are six subfamilies. The nine-banded armadillo is a member of the Subfamily Dasypodinae, genus *Dasypus*. All members of the order Xenarthra occur exclusively in the Western Hemisphere. There are 20 species of armadillos found in Central America, South America and the Caribbean. The only species native to the United States is the nine-banded armadillo. The armadillo is a primitive mammal, little changed over eons of time. Its presumed ancestral relationships are diagrammed in Fig. 1.

**Range of habitat.** The nine-banded armadillo has a vast natural range including northern Argentina, all countries east of the Andes, Ecuador, Central America, Mexico and southern United States. This animal migrated from Mexico to the Rio Grande Valley area of Texas at least 150 years ago

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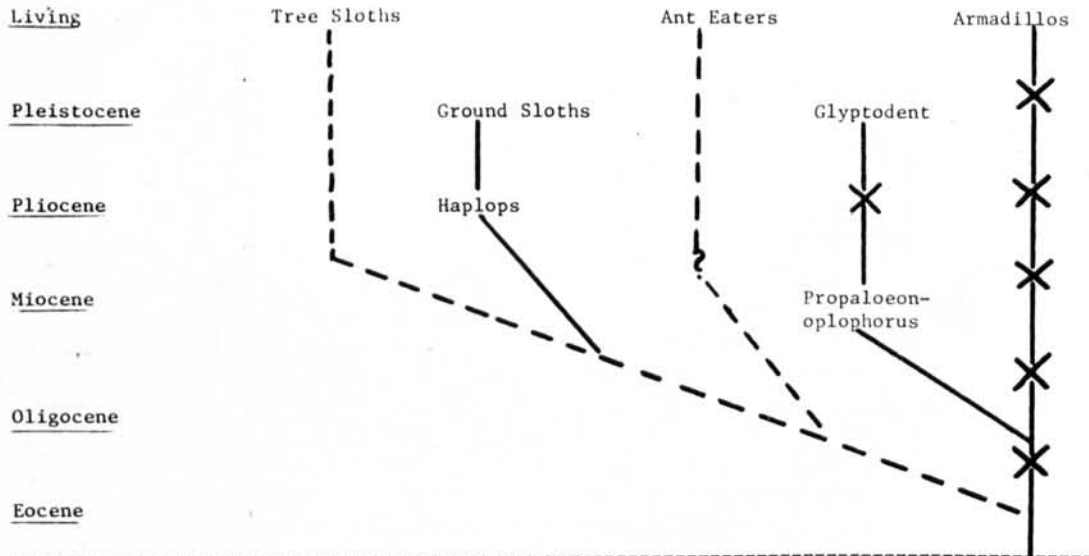


Fig. 1. The evolutionary line of Xenarthra (62).

(<sup>3</sup>), and has been migrating northward and eastward since that time (<sup>60, 62</sup>). It was found in Central Texas in the Austin-Edwards Plateau area by the early part of the 20th century (<sup>4</sup>). The armadillo does not hibernate, and so must seek food during the winter as well as during the warmer summer months. This factor probably limits its range, which in the United States now extends northward into Oklahoma and Arkansas, and eastward to Georgia, the Carolinas, and northern Florida (<sup>73</sup>, and personal observations). Colonies which have inhabited central Florida for many years were introduced into that area by man (<sup>42, 56, 57, 62</sup>).

**Feeding habits.** The nine-banded armadillo is nocturnal, and in its natural habitat is an insectivore. Talmage and Buchanan (<sup>62</sup>) report that typically, 75% of the armadillo diet consists of insects while another 15% consists of animal material including amphibians, reptiles, mammals, birds, and eggs. Vegetable matter and dirt comprise the remaining 10% of the diet.

In captivity armadillos thrive on a diet of dog or cat chow supplemented with vitamins and minerals. They also relish eggs, liver, chicken, and other types of meat, and some fruits such as bananas and melon.

Adaptation of wild armadillos to the laboratory diet is often difficult. Individual animals sometimes appear unable to locate

or recognize the food presented to them. We have overcome this problem, in most cases, by combining the food and water supply in one dish, making a very soupy mixture. The animals then gain nutrients when they drink and learn in this way to eat out of a food pan.

**Physical description.** An adult armadillo (Fig. 2) weighs from three to five kilograms. Armadillos have short but powerful legs, well adapted to burrowing. There are sparse tufts of coarse hair on the underside of the body. The upper portions of the body are protected by a leathery carapace. The carapace is divided into three main sections. The head and shoulders are covered with a large scapular shield. A large dorsal section covers the upper hips and pelvic area and extends onto the tail. There is a fold of skin between the head and shoulder sections, and also between the dorsal and tail sections. The central section of the carapace consists of nine movable bands connected by folds of skin. Each band is composed of 50 to 75 scutes which superficially resemble fish scales. Anomalies in scute and band patterns occur fairly often and are presumably inherited. These anomalies consist most often of doubling of scutes or interruption of regular scute patterns on specific bands of the carapace. Sometimes an additional band, or the occurrence of a partial band, is found.

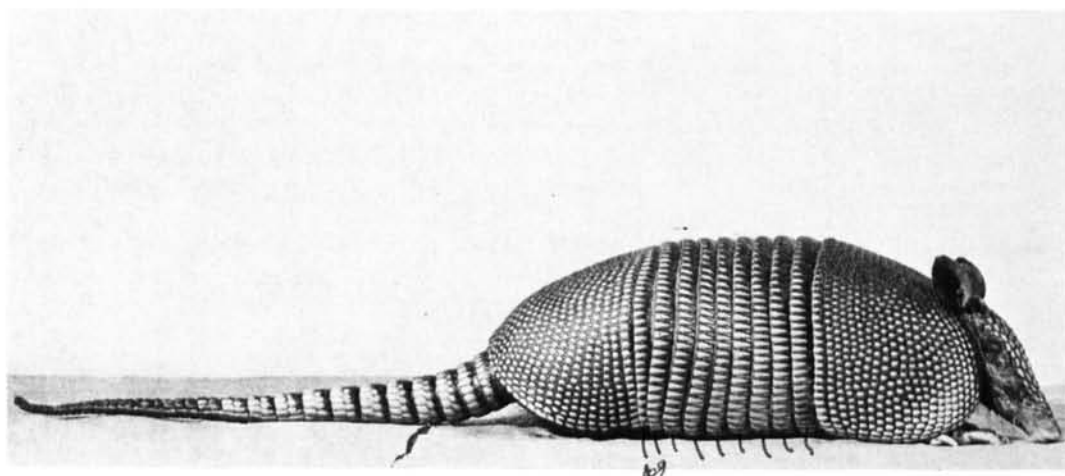


Fig. 2. The nine-banded armadillo, *Dasypus novemcinctus* Linn.

There are sixteen rudimentary molars in the back portion of each jaw with poorly developed enamel. Since they do not attempt to bite, armadillos are relatively easy to handle.

The female has a urogenital cleft serving as the vagina and terminal urethra, while the male has testes located within the abdominal cavity. The young are born at a fairly advanced stage of development with their eyes fully opened. They are ambulatory and have soft but fully developed carapaces, which become harder and more leathery with age. The newborn weigh from 50 to 150 gm and measure 25 to 30 cm in length from nose to tip of the tail. Since the animals are monozygous, each litter is always of the same sex. The young remain in seclusion for a period of from four to six weeks after which they accompany the mother in foraging for food.

**Embryology.** The embryonic development of the young has been studied in detail by Newman and Patterson (44) and by Hamlett (22, 23). Enders (18) has also studied the period of nidation, and some work is being carried on at the present time on steroid patterns during pregnancy (8, 19, 31).

The female armadillo regularly produces monozygous quadruplet young, developed from a single fertilized ovum. Parturition occurs once each year in the spring. Mating occurs during the preceding summer with a period of delayed implantation lasting for approximately 14 to 16 weeks. During this

time the blastocyst remains free and quiescent in the uterus, unattached to the uterine wall but bathed in uterine fluids. When implantation of the blastocyst occurs following this period of delay, the embryo develops rapidly and the primordia of the four individuals are formed. Four buds are developed from the floor of the embryonic vesicle after attachment of the vesicle to the uterine wall. Two primary buds are first developed at opposite ends of the ellipsoidal vesicle. From each of these primary buds, a secondary bud then develops. From these primary and secondary buds, primary streaks are formed from which the four fetuses develop. Each fetus develops its own amniotic sac and placental attachment. These four placental attachments form one large lobed disc but with no circulatory connections between the placentae of the individuals (2).

**Morphology and biochemistry.** *Adrenal glands.* The adrenal in the armadillo fetus is very large, as in man and a few other primates. Postnatal involution of the fetal zone in this gland is similar to that in man (41). Steroid synthesis by armadillo adrenals has been studied (6, 7). Weights of newborn armadillo adrenals have been compared within monozygous groups of young, and between unrelated individuals (58, 59). Adrenal weights per 100 gm of body weight varied from 0.20 to 0.81 gm with an average of 0.3 gm/100 gm body weight for 65 individuals. As with other

measurements made in this same study, comparative adrenal weights varied within monozygous sets but they varied even more between unrelated individuals.

**Spleen.** The armadillo spleen has been studied by a few workers who have reported that the relative weights of the spleens of edentates are in the same range as for most mammals. Storrs<sup>(58)</sup> found spleen weights in newborn young to vary between 0.13 and 0.64 gm per 100 gm of body weight. The average obtained on 65 animals was 0.33 gm/100 gm.

Hayes<sup>(26)</sup> studied the ellipsoid sheath in the spleen of the nine-banded armadillo using both light and electron microscopy. He described a high-type endothelium in the sheathed capillaries. The reticular cells comprising the sheath proper contain various sized vacuoles and inclusion bodies suggestive of their potentially phagocytic nature. He also noted, in both light and electron microscopy studies, that within the wall of the venous sinuses patent openings are apparent between the lining cells. This could provide for an additional means of exposure of both plasma and cellular elements to the adjacent phagocytic cells of the sheath proper.

**Blood.** Several studies on armadillo blood have been made which provide a few basic parameters. In most cases, however, the populations studied were so small that these values are not necessarily representative of the average values which would be obtained on larger populations. Lewis and Doyle<sup>(35)</sup> found that cellular elements differed only slightly from human blood. Erythrocytes were smaller, platelets more variable in size, and megakaryocytes (bone marrow) contained one multilobed nucleus. Total plasma protein and fibrinogen were higher than human levels but serum electrophoretic patterns were similar. Adult and fetal hemoglobin was found to migrate at the same rate as human A hemoglobin on starch gel electrophoresis at pH 8.9; slightly slower on cellulose acetate at pH 8.9 and agar gel at pH 6.0<sup>(17)</sup>. The hemoglobin appears to be unique in that it is relatively insoluble in the oxygenated state but quite soluble when reduced. It has also been reported that the armadillo red cell contains high potassium and low

sodium concentrations in the same range found for man and in contrast to the dog, cat, and certain sheep.

Some basic blood serum and plasma values have been determined by Strozier *et al*<sup>(61)</sup> and Peters and Storrs<sup>(49)</sup>. The former, using a total of ten animals, analyzed serum levels of calcium, inorganic phosphorus, carbon dioxide, glucose, blood urea nitrogen, uric acid, cholesterol, total protein, total bilirubin and alkaline phosphatase. Peters and Storrs analyzed the plasma of four animals for amino acid levels, l-asparaginase activity, and histaminase activity. In the latter study, l-methylhistidine was found to be present in significant amounts in armadillo plasma whereas it was not detected in human plasma. Histaminase activity was also very high in the armadillo plasma.

**Brain.** The morphology and the morphogenetic development of the armadillo cerebellum has been described and compared with that of other animals<sup>(32)</sup>. The pattern of neocortical projections of the mesencephalon has also been studied<sup>(24)</sup> and was found to closely parallel that described for certain other mammals. The neocortical projections to the pons and medulla have also been examined by Harting and Martin<sup>(25)</sup>.

Analysis of brain homogenates of 65 newborn young yielded values for amino acid and norepinephrine levels<sup>(58, 59)</sup>. No epinephrine was detected while norepinephrine levels averaged 0.074  $\mu\text{g/gm}$ .

**Physiology. Body temperature.** The body temperature (rectal) of the nine-banded armadillo ranges from 30° C to 36° C when the ambient temperature is close to 25° C<sup>(10, 28, 72)</sup>. Johansen<sup>(28)</sup>, in a detailed study, found the core temperatures of thirteen armadillos to range from a low of 34° C to 35° C early in the morning to a high of 35° C to 36° C around midnight. The ambient temperature was held constant at 25° C. These temperatures are higher than those reported by Wislocki and Enders<sup>(72)</sup> and by Burns and Waldrip<sup>(10)</sup>. The latter authors found body temperature differences between male and female armadillos. The rectal temperature for fifteen males averaged 33.4° C (range 31.0 to 35.0° C) while for seven females the tem-



perature averaged 31.3° C (range 30.0 to 33.0° C). These temperatures were taken between 0800 and 1200 hours during the months of October through January at an ambient temperature of 23° C.

Johansen compared rectal and skin temperatures and oxygen consumption for armadillos exposed to different ambient temperatures ranging from -10° C to 40° C. As ambient temperature became lower, skin temperature decreased while oxygen consumption and rectal temperature increased. This temperature increase amounted to about 3.5° C when ambient temperature was decreased stepwise from 30° to -10° C. This overcompensation of the armadillo to a cold stress indicates that the central nervous thermostatic control is relatively primitive.

At high ambient temperatures both the skin and rectal temperatures increased, and oxygen consumption increased to a level of 400 ml/kg/hr and then leveled off. The rectal temperature increased to as high as 38° C at an ambient temperature of 40° C.

Thus it appears that the core temperature can be as low as 30° C in some animals; however, either an increase or decrease in ambient temperature can result in increased core temperature because of the relatively primitive regulatory mechanism.

**Respiration.** The armadillo is capable of building up an oxygen debt during periods of intense activity. This probably occurs in nature when the animal burrows into the ground quickly as an escape mechanism.

Scholander *et al* (<sup>55</sup>) have suppressed breathing in the armadillo for periods of up to 10 minutes. During this period of arrested breathing, bradycardia developed quickly, but the body temperature showed little change. The oxygen content of the arterial blood decreased rapidly; 80% was used up in the first two minutes, leaving only 20% for the remaining eight minutes. Despite rapid activity during this period of apnea, little lactic acid entered the blood, indicating perhaps a reduced flow of blood to the muscles.

Recovery periods of more than two hours were observed with increases above the resting values in heart beat, oxygen con-

sumption and in lactic acid content of the blood.

Reduced oxygen demand under stress situations may lend some credence to the commonly advanced claim that armadillos have been observed to cross streams by walking under water.

**Parasites and Diseases.** Although studies on parasites of *Dasypus novemcinctus* are far from complete, armadillos appear to be infested with relatively few parasites compared to the opossum, raccoon, or skunk. However, they harbor *Trypanosoma cruzi*, and several other parasites of importance.

Since the armadillo has a dorsal covering or carapace which also protects the head and tail, the animal has few ectoparasites. The hair on the underbelly is also very sparse, discouraging invasion by fleas, lice, and ticks. A few species of ticks have been reported on the nine-banded armadillo including *Amblyomma cyemense* (<sup>20</sup>), *A. concolor* (<sup>20</sup>), and *A. crassum* (<sup>20</sup>), and *A. parvum* (<sup>5</sup>).

Fleas found, although rarely, on the nine-banded armadillo include *Tunga traversosi* (<sup>51</sup>), *Juxtapulex echidnophagoides* (<sup>71</sup>), *Edhidnophaga gallenacea* (<sup>27</sup>), *Polygenis roberti* (<sup>21</sup>), and *Polygenis occidentalis* (<sup>21</sup>).

Relapsing fever spirochetes were found in two of thirty-two armadillos in Panama which may indicate that these animals are sometimes bitten by ticks of the genus *Orethodoros* (<sup>62</sup>).

Two armadillos inoculated with a human strain of the spirochete causing relapsing fever became infected (<sup>62</sup>), as did nine-banded armadillos inoculated with exanthematic typhus rickettsiae (strain VB) (<sup>36</sup>). They also seem to be susceptible to murine typhus infection (<sup>70</sup>). Despite the above findings, armadillos in the United States have never been found to naturally harbor spirochetes or rickettsia type parasites.

*Dasypus novemcinctus* has long been known to be a carrier of *Trypanosoma cruzi* (<sup>14</sup>) and is also known to be a host for *Treatoma dimidista* (<sup>47</sup>). About 40-60% of a group of animals examined in Brazil were found to be infected. In addition, nine-banded armadillos in Argentina (<sup>40</sup>), Venezuela (<sup>63</sup>), Panama (<sup>16</sup>), and Mexico

(<sup>9</sup>) have been found to be infected. The nine-banded armadillo in Texas is also known to carry *Trypanosoma cruzi* (<sup>46, 52</sup>).

A few helminths have been reported in the nine-banded armadillo including nematodes and flukes, one species of tapeworm, and Acanthocephalans.

Flukes parasitizing the armadillo include *Dictyonogryptus dictyonogryptus* (<sup>53, 66</sup>). *Brachylaemus virginianus* has been found in Texas armadillos (<sup>15</sup>). It is interesting to note that the six-banded armadillo, *Euphractus sexcinctus*, when inoculated with *Schistosoma mansoni* in the laboratory, became infected (<sup>50</sup>).

Cestodes found in the armadillo include *Oochoristica surinamensis* which was present in a few South American animals, and an *Oochoristica* species found in a nine-banded armadillo in Texas (<sup>62</sup>).

One species of Acanthocephala, namely *Travassosia carinii*, is found only in armadillos. Cases have been described in Brazil (<sup>33, 65</sup>) and Bolivia (<sup>40</sup>). Immature *Hamanniella* have been recorded by Chandler (<sup>15</sup>). Also, encysted forms of *Oncicola* species have been reported in *Dasypus*. *Oncicola canis* is found in armadillos in Texas. Chandler (<sup>15</sup>) believes the armadillo may represent a dead-end in the life cycle of this parasite.

*Trichinella* have never been found to occur naturally in *Dasypus novemcinctus* although here again an experimental case of trichinosis was developed in the hairy armadillo *ChaetophRACTUS villosus* (<sup>45</sup>).

Armadillos are hosts for several Strongyloidea. These include: *Macielia macieli*, *M. flagellata*, *Delicata ransomi*, *D. uncinata*, *D. cameroni*, *Pulchrostrongylus complexus*, *Dayspostrongylus filamentosus*, *Moennigia moennigi*, *Pintonema intrusa*, *P. pulchra*, *P. pseudopulchra*; and *P. pinto* (<sup>64, 67, 68, 69</sup>).

Several *Aspidodera* have been isolated from Dasypodidae, and they appear to be specific for this group. *Aspidodera anisrupta*, *A. vasi*, and *A. binansata* have been described from *D. novemcinctus* in South America (<sup>12</sup>). *A. fasciata* has been reported from the nine-banded armadillo in Texas (<sup>15</sup>). *Cruzia mazza* has been described from *Dasypus novemcinctus* (<sup>29</sup>), while *Lauroi travassosi* (<sup>13</sup>) and *Heterakis fasciata* (<sup>54</sup>) have also been found.

Three spiruroid larvae have been reported from the nine-banded armadillo, with the armadillo serving as a transport host. These include larvae of *Physatoptera* from Trinidad (<sup>11</sup>), and larvae of *Physocephalus* and *Ascarops* species (<sup>15</sup>).

Finally, the filaroid *Dipetatonema anti-clava* has been reported from *Dasypus novemcinctus* (<sup>34</sup>).

### THE ARMADILLO AS A MODEL FOR RESEARCH

There is much interest in the armadillo for use in various fields of biomedical research. Several specific areas of interest are discussed below. Since December 1968, the author has had a grant to develop the armadillo as a laboratory animal. There are now over 200 animals in this colony, including adults, young born and raised in captivity, and young born in the wild and raised by hand in captivity. The broad program goals are to learn how to breed and rear these animals in the laboratory on a nutritionally-balanced diet. As a direct result of this program, a study of the armadillo as an animal model for leprosy research was begun.

**Leprosy.** We, in collaboration with the USPHS Hospital, Carville, Louisiana, are now completing the second year of the program to study the armadillo as a potential animal model for leprosy research. A total of 76 armadillos have been inoculated with *Mycobacterium leprae* (July 1971).

Biopsy materials have been obtained from the following sources: untreated patients from Dr. Chapman Binford, Leonard Wood Memorial and Dr. Waldemar Kirchheimer, Carville, a patient with apparent DDS-resistant bacilli from Dr. Waldemar Kirchheimer of USPHS Hospital at Carville, La., mouse foot-pad from Dr. Louis Levy of the USPHS Hospital, San Francisco, Calif., and from an armadillo with lepromatoid infection. Details of preparation are given in a paper by Kirchheimer and Storrs (<sup>30</sup>).

Most armadillos were inoculated intradermally on the abdomen, ears, or between the bands. Several armadillos were inoculated in the foot-pad and several received intravenous injections of inoculum.

On February 10, 1970, four animals were

inoculated intradermally from biopsy material supplied by Dr. Chapman Binford. This biopsy material was the S45 strain sent from Surinam by Dr. S.J. Bueno de Mesquita. Each armadillo was inoculated intradermally in the right and left abdomen and both ears with 0.1 ml of inoculum at each site. This inoculum, prepared at the USPHS Hospital in Carville, had a count of  $8.9 \pm 0.4 \times 10^7$  bacilli/ml with a MI of 3%. In May of 1971 one of the inoculated animals (number 8) showed large palpable granulomas at inoculation sites on the abdomen and ears. The armadillo died on July 16, 1971. This animal had developed lepromatoid infection. Complete evidence for this appears in another publication (<sup>30</sup>).

Mycobacteria obtained from armadillo number 8 have been used for the inoculation of other armadillos, the goal being the development of a susceptible strain of animals. To achieve this, one or two young from several monozygous litters have been inoculated, and the others reserved for future experimentation. If any of the inoculated animals develop lepromatous leprosy, the genetically identical but uninoculated members of the sets will be mated to attempt to establish a susceptible line. The success of this phase of the program will depend on obtaining both male and female sets which are susceptible.

**Organ transplantation and immunochemistry studies.** Preliminary work in the field of tissue transplants has been carried out by Anderson and Benirschke (<sup>1</sup>). In these studies, skin grafts were made between quadruplet pairs. These transplants were compared with homografts and also with grafts between unrelated individuals. Indications of some histocompatibility differences between monozygous individuals were found. However, these differences were not as great as those between unrelated individuals. Storrs (<sup>58</sup>), in work on histocompatibility differences between monozygous armadillos, was unable to find differences in immune response to kidney tissue between these individuals on the basis of antibodies developed in the rabbit and then used in immunoelectrophoresis assay. These results indicate that the armadillo may be an important tool in the

study of the mechanism of the immune response and for use in studies on immunosuppressant drugs.

The advantages of using quadruplet sets of monozygous armadillos in studies on organ transplantation and immunochemistry are self evident since rejection processes should be minimized. Since numerous sets of monozygous animals are now available at the Gulf South Research Institute colony, these studies could be undertaken on a scale not previously possible.

It is of interest to note also that armadillos have proved to be susceptible to several diseases of man. In addition to leprosy, these include: relapsing fever, exanthematic typhus (strain VB), murine typhus, trichinosis, and schistosomiasis. One wonders how many other diseases of man could be reproduced in the armadillo.

**Teratogenesis and mutagenesis.** Since the thalidomide disaster of 1962, much effort has been spent in the development of assay methods for the study of potentially teratogenic agents when administered to the mother during pregnancy. The effects of certain agents on the human embryo are well known. Progestational hormones produce virilizing effects on the fetus, and defects produced by rubella virus contracted by the mother during the first trimester of pregnancy have been observed frequently. However, the effects of the drug thalidomide on the human embryo were entirely unexpected and tragic. This drug does not produce teratogenic effects in most experimental animals, and this is the main factor which led to human use of the drug.

Thalidomide, however, has been shown to produce congenital malformations in armadillo young when administered to the pregnant female (<sup>37, 38</sup>). Thus the armadillo shows promise as an animal for use in assays studying potential teratogenic agents, with the additional unique advantage of having four replicate young to study for possible effects.

In addition to use in the study of teratogenic agents, the armadillo has potential use in mutagenic studies. Anomalies in scute and band patterns on the armadillo carapace occur fairly often, and these anomalies may be inherited. (<sup>43</sup>).

During a research program conducted by the author (<sup>58</sup>), one set of quintuplet armadillos was born. In addition to the uniqueness of this event, a common tag was also present in the form of a partial tenth band on the carapace. This band was located between the scapular shield and the first movable band and was present in all five individuals, although not in the mother. The mode of attachment of the band varied somewhat, indicating developmental differences between the offspring. In three cases the band showed attachment to the first movable band, while in two cases attachment was to the scapular shield. In this latter pair of individuals, the band arrangements were mirror images. This seems to be an indication that these individuals were developed from an additional division of a primary or secondary bud and that this division occurred late in the developmental sequence.

The study of scute and band inheritance patterns would be of interest in studies of potential mutagenic agents, since these anomalies occur fairly often and they may result from genes which undergo mutation fairly readily.

**Studies on somatic variability.** Although all evidence indicates that the armadillo young are monozygous individuals (<sup>10, 22, 23, 44</sup>), there also are evidences of differences between the newborn young. These could be attributed to somatic differences in development.

Storrs (<sup>58, 59</sup>) measured various physical and biochemical parameters and related the differences found between monozygous individuals to those found between unrelated groups.

Physical measurements made on the newborn young included numbers of scutes and weights of various organs such as adrenals, brain, heart, kidney, liver and spleen. Coefficients of variation within quadruplet sets showed wide variations with two as high as 30%. Coefficients of variation between unrelated sets in general were higher, as would be expected. Several biochemical parameters were also measured including epinephrine and norepinephrine levels in brain and adrenals, and brain amino acid levels. Here coefficients

of variation between monozygous individuals were sometimes very high, especially epinephrine and norepinephrine levels. Again the variation between unrelated sets was, in general, greater than between monozygous animals with some notable exceptions.

**Replication of experiments.** The goal of workers using animals in many areas of research is to have as little variation between individual animals as possible. With the armadillo, replication of experiments using genetically identical individuals will be possible. Areas where these animals could be used to great advantage are in chronic toxicology studies, including pharmacological agents, pesticides, alcohol and others; in many types of assay procedures where replicates are desired; in nutritional studies; and in learning and aging experiments where individuals within a group can be treated differently.

**Embryology.** The armadillo shows several embryological similarities to man in that the young are produced in a simplex uterus and the placenta is of the hemochorial type similar in the maternal fetal border to that of man. However, the end product is usually a set of monozygous quadruplets, while in man twinning occurs infrequently and production of quadruplets rarely. Since twinning sometimes ends disastrously (production of Siamese twins) and there is a general feeling that multiple births may be on the increase due to the use of hormonal drugs, a study of the biochemical and cellular factors which regularly lead to multiple births in the armadillo should be initiated. Presumably the end result would be the development of drugs that could prevent or induce twinning. The nine-banded armadillo is the only mammal known to regularly produce monozygous quadruplets; therefore it would be a unique model for such studies. A companion animal for such investigation is the seven-banded armadillo (*Dasypus hybridus*) which reportedly produces monozygous litters of eight to twelve. It is not clear whether these result from two to three sets of identical quadruplets, or arise from a single ovum.

**Drug metabolism.** Since it has been shown that leprosy can apparently be



transferred to the armadillo, the metabolism of antileprosy drugs in this animal becomes important. Preliminary work in our laboratories has shown that the armadillo can acetylate DDS to MADDS and hydrolyze MADDS to DDS.

It is important to determine whether the armadillo is a slow acetylator, a fast acetylator, or whether both phenotypes occur as in man (<sup>48</sup>). In the latter event, studies on the susceptibility of armadillos to leprosy should be coupled to a study on the inheritance of acetylating power in order to determine if the two properties are linked genetically or are inherited independently. In the latter event, it might be possible to secure susceptible armadillo strains with both high and low acetylating capacity, and thereby resolve the question of the importance of this factor in DDS therapy.

Studies are also needed on the absorption and excretion patterns and on plasma levels of DDS, DADDS, B-663 and other antileprosy drugs in the armadillo in order to develop dosage regimens that will control the disease in this animal.

It has been shown that thalidomide produces terata in the armadillo. One of many reasons for this might be slow metabolism of the drug. Contrary to this hypothesis, we have found that it takes very large doses of phencyclidine HCl (4 mg/kg) to quiet an armadillo in order to obtain biopsy specimens. Does this indicate rapid metabolism of the drug, poor transport, or weak affinity of the binding sites of this primitive mammal for the drug? Only in depth study of the interactions of drugs with this important model for the study of human diseases can provide the answer.

### SUMMARY

The taxonomy, range, diet, morphology, biochemistry, physiology and known diseases of the nine-banded armadillo are described briefly. Fields in which the armadillo may be a potentially valuable research model include transmission of leprosy and other communicable diseases, studies on birth defects and mutations, organ transplantation, immunology, studies on somatic variability, toxicology, and drug metabolism.

### RESUMEN

Se describen brevemente la taxonomía, rango, dieta, morfología, bioquímica, fisiología y enfermedades conocidas del armadillo de nueve bandas. Los campos en los cuales el armadillo puede ser potencialmente importante como modelo experimental incluyen la transmisión de lepra y otras enfermedades infecciosas, estudios sobre defectos congénitos y mutaciones, trasplantes de órganos, inmunología, estudios sobre variabilidad somática, toxicología y metabolismo de drogas.

### RÉSUMÉ

On décrit ici brièvement la taxonomie, la répartition, le régime alimentaire, la morphologie, la biochimie, la physiologie, et la pathologie connue de l'armadillo à neuf raies. Les domaines dans lesquels l'armadillo pourrait se révéler un modèle éventuellement utile pour la recherche, incluent la transmission de la lèpre et d'autres maladies transmissibles, les études sur les malformations congénitales et les mutations, le transplantation d'organes, l'immunologie, les études portant sur la variabilité somatique, la toxicologie, et le métabolisme des médicaments.

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### REFERENCES

1. ANDERSON, J. M., and BENIRSCHKE, K. Tissue transplantation in the nine-banded armadillo, *Dasypus novemcinctus*. Ann. N.Y. Acad. Sci. 99 (1962) 399-414.
2. ANDERSON, J. M., and BENIRSCHKE, K. Fetal circulations in the placenta of *Dasypus novemcinctus* Linn. and their significance in tissue transplantation. Transplantation 1 (1963) 306-310.
3. AUDOBON, V. G., and BACHMAN. Quadrapeds in North America, III. 1854. V. G. Audobon, New York.
4. BAILEY, V. Biological survey of Texas.

- North American Fauna. **25** (1905) 222 pgs.
5. DEBEAUREPARE ARAGAO, H. Nota sobre os Ixodídeos da República Argentina. Mem. Inst. Os. Cruz. **33** (1938) 319-327.
  6. BLOCH, E. The metabolism of  $7\text{-}^3\text{H}$ -pregnenolone and  $4\text{-}^{14}\text{C}$ -progesterone by adrenal homogenates of fetal guinea pigs and other mammalian fetuses. Steroids **13** (1969) 589-603.
  7. BLOCK, E., and BENIRSCHKE, K. *In vitro* steroid synthesis by fetal, newborn and adult armadillo adrenals and by fetal armadillo testes. Endocrinology **76** (1965) 43-51.
  8. BRINCK-JOHNSON, T. Homonal steroids in the armadillo *Dasypus novemcinctus*: H. Oestrone and 17 B-oestradiol in pregnancy and their *in vitro* formation by preparation of placentae, early and late in development. Acta. Endocrinol. **63** (1970) 696-704.
  9. BRUMPT, E., MAZZOTTI, L. and BRUMPT, L. C. Enquetes epidemiologiques sur la maladie C. Chagas au Mexique (1). Réduvidés vecteurs. Animaux réservoirs de virus, cas humains. Ann. Parasit. Hum. et Comp. Paris **17** (1939) 229-312.
  10. BURNS, T. A., and WALDRIP, E. B. Body temperature and electrocardiographic data for the nine-banded armadillo (*Dasypus novemcinctus*). J. Mamm. **52** (1971) 472-473.
  11. CAMERON, T. W. M. Studies on the endoparasitic fauna of Trinidad mammals. VI. Parasites of edentates. Canadian J. Res. Sect. D. Zool. Sci. **17** (1939) 249-264.
  12. CAVALCANTI PROENÇA, M. Revisão do genero *Aspidodera* Railliet and Henry, 1912 (Nematoda: Subuluroidea). Mem. Inst. Os. Cruz. **32** (1937) 427-438.
  13. CAVALCANTI PROENÇA, M. Sobre um novo typo de Heterakinae Railliet and Henry 1912 (Nematoda: Subuluroidea). Livro Jub. Prof. Lauro Travassos (1938) 419-420.
  14. CHAGAS, C. Sobre um Trypanosoma de tatu (*Tatusia novemcinctus*). Brazil-Med. **26** (1912) 305-306.
  15. CHANDLER, A. C. Helminths of armadillos, *Dasypus novemcinctus* in eastern Texas. J. Parasit. **32** (1946) 237-241.
  16. CLARK, H. C., and DUNN, L. H. Experimental studies on Chagas' disease in Panama. Am. J. Trop. Med. **12** (1932) 49-77.
  17. EBAUGH, F. G., and M. A. BENSON. Armadillo hemoglobin characteristics and red cell survival. J. Cell. and Comp. Physiol. **64** (1964) 183-192.
  18. ENDERS, A. C. The structure of the armadillo blastocyst. J. Anat. **96** (1962) 39-48.
  19. ENDES, A. C., and SCHILAFKE, S. Cytological aspects of trophoblast-uterine interaction in early implantation. Amer. J. Anat. **125** (1969) 1-29.
  20. FIASSON, R. Notes sur les parasites animaux du Haut-Apure (Venezuela). Rev. Sci. Med. Pharmaceut. Vet. Afr. Franc. **2** (1943) 125-151.
  21. GUIMARÃES, L. R. Notes sobre Siphonaptera e rediscricão de *Polygenis occidentalis* (Almeida Cunha, 1914). Arg. Zool. S. Paulo **2** (1941) 215-250.
  22. HAMLETT, G. W. D. Delayed implantation in the mammals, and its supposed relationship to polyembryony. Anat. Rec. **44** (1929) 251.
  23. HAMLETT, G. W. D. Polyembryony in the armadillo genetic or physiological? Quart. Rev. Biol. **8** (1933) 348-358.
  24. HARTING, J. K., and MARTIN, G. F. Neocortical projections to the mesencephalon of the armadillo, *Dasypus novemcinctus*. Brain Res. **17** (1970) 447-462.
  25. HARTING, J. K., and MARTIN, G. F. Neocortical projections to the pons and medulla of the nine-banded armadillo (*Dasypus novemcinctus*). J. Comp. Neurol. **138** (1970) 483-494.
  26. HAYES, T. G. Structure of the ellipsoid sheath in the spleen of the armadillo (*Dasypus novemcinctus*). A light and electron microscopic study. J. Morphol. **132** (1970) 207-224.
  27. HIGHTOWER, B. G., LEHMAN, V. W., and EADS, R. B. Ectoparasites from mammals and birds on a quail preserve. J. Mamm. **34** (1953) 268-271.
  28. JOHANSEN, K. Temperature regulation in the nine-banded armadillo (*Dasypus novemcinctus mexicanus*). Physiol. Zool. **34** (1961) 126-144.
  29. KHALIL, M., and VOGELSANG, E. G. On some nematode parasites from South American animals. Zentralbl. Bakt. I Abt. Orig. **123** (1932) 477-485.
  30. KIRCHHEIMER, W. F., and STORRS, E. E. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. Internat. J. Leprosy. **39** (1971) 692-701.
  31. LABHSETWAR, A. P., and ENDERS, A. C. Progesterone in the corpus luteum and placenta of the armadillo, *Dasypus novemcinctus*. J. Reprod. Fert. **16** (1968) 381-387.

32. LARSELL, O. The comparative anatomy and histology of the cerebellum from monotremes through apes. The University of Minnesota Press: Minneapolis, Minnesota, U.S.A. 1970.
33. LENT, H., and TEXEIRA DE FREITAS, J. F. Pesquisas helmintológicas realizadas no estado do Pará. VI. Acanthocephala. Mem. Inst. Os. Crus. **33** (1938) 455-459.
34. LENT, H., and TEXEIRA, DE FREITAS, J. F. Contribuição do conhecimento dos filários de Dasypodídeos. Revista Brasileira de Biologia (1942) 275-280.
35. LEWIS, J. H., and DOYLE, A. P. Coagulation, protein and cellular studies of armadillo blood. Comp. Biochem. Physiol. **12** (1964) 61-66.
36. MAGALHÃES, O. DE and ROCHA, A. Contribuição para o conhecimento do tifo exantemático neotrópico no Brasil. Mem. Inst. Os. Cruz. **40** (1944) 1-8.
37. MARIN-PADILLA, M., and BENIRSCHKE, K. Thalidomide induced alterations in the blastocyst and placenta of the armadillo, *Dasypus novemcinctus mexicanus*, including a choriocarcinoma. Am. J. Pathol. **43** (1963) 999-1016.
38. MARIN-PADILLA, M., and BENIRSCHKE, K. Thalidomide injury to the myocardium of armadillo embryos. J. Embryol. exp. Morph. **13** (1965) 235-241.
39. MAZZA, S., and ROMANO, C. Ulceras y edema cutáneo del tatú naturalmente infectado por *S. cruzi*. 9. Reunion Soc. Argent. Patol. Reg. **1** (1936) 526.
40. MEYER, A. Acanthocephala. Bronn's Kl. u. Ordn. Tier-Reichs. Vermes Askhelminthen **4** (Abt. II, Buch 2 (b) lief 2) (1933) 333-582.
41. MOSER, H. G., and BENIRSCHKE, K. Fetal zone of the adrenal gland in the nine-banded armadillo, *Dasypus novemcinctus*. Anat. Rec. **143** (1962) 47-59.
42. NEWMAN, C. C. Florida's armored invasion. Florida Wildlife **3** (1949) 3-5.
43. NEWMAN, H. H. Heredity and organic symmetry in armadillo quadruplets. Biol. Bull. **29** (1915) 173-209.
44. NEWMAN, H. H., and PATTERSON, J. T. The development of the 9-banded armadillo from primitive streak to birth, with especial reference to the question of specific polyembryony. J. Morph. **21** (1910) 359-437.
45. NIÑO, F. L. Triquinosis experimental en el "peludo". 9. Reunión Soc. Argent. Patol. Reg. **2** (1937) 630.
46. PACKHANIAN, A. Reservoir hosts of Chagas' disease in the state of Texas. Am. J. Trop. Med. **22** (1942) 623-631.
47. PETANA, W. B. American trypanosomiasis in British Honduras: IV. Laboratory observations on *Triatoma dimidiata* (Hemiptera, Reduviidae) and its efficiency as a vector of Chagas' disease in British Honduras. Ann. Trop. Med. Parasitol. **61** (1967) 413-416.
48. PETERS, J. H. U.S.-Japan Cooperative Medical Science Program. Proceedings of first workshop on the pharmacology of antileprotic drugs. Internat. J. Leprosy **38** (1970) 331-335.
49. PETERS, J. H., and STORRS, E. E. Amino acid levels and L-asparaginase and histaminase activities in plasma of the armadillo, *Dasypus novemcinctus* Linn. Lab. Animal Sci. In press.
50. PINTO, C. Um ano de combate as doenças parasitárias que atacam os rodoviários da estrada Rio-Bahia, 1942a 1943. Mem. Inst. Os. Crus. **40** (1944) 209-340.
51. PINTO, C., and DREYFUS, A. *Tunga traversosi* n. sp. parasita de *Tatusia novemcinctus* do Brasil. Bol. Biol., S. Paulo **9** (1927) 174-178.
52. PIPPEN, W. F. The biology of vector capability of *Triatoma sanguisuga texana* Usinger and *Triatoma gerstaeckeri* (Stal) compared with *Rhodnius prolixus* (Stal) (Hemiptera: Triatominae). J. Med. Entomol. **7** (1970) 30-45.
53. RIBEIRO, D. J. Contribuição para o conhecimento da fauna helmintológica de Minas gerais. "Eurytrema minensis" n. sp., parasito de "*D. novemcinctus*" L. Rev. Bras. Biol. **1** (1941) 235-237.
54. SCHNEIDER, A. F. Monographic des nematoden. (1866) Reimer Publishers, Berlin.
55. SCHOLANDER, P. F., IRVING, L., and GRINNELL, S. W. Respiration of the armadillo with possible implications as to its burrowing. J. Cell. Comp. Physiol. **21** (1943) 53-63.
56. SHERMAN, H. B. List of the recent wild land mammals of Florida. Proc. Fla. Acad. Sci. **1** (1936) 102-128.
57. SHERMAN, H. B. The armadillo in Florida. Florida Entomologist **26** (1943) 54-59.
58. STORRS, E. E. Individuality in monozygotic quadruplets of the armadillo, *Dasypus novemcinctus*, Linn. Dissertation, University of Texas (1967).
59. STORRS, E. E., and WILLIAMS, R. J. A study of monozygous quadruplet armadillos in relation to mammalian inheritance. Proc. Natl. Acad. Sci. **60** (1968) 910-914.

60. STRECKER, J. K. The extension of the range of the nine-banded armadillo, *J. Mamm.* **7** (1926) 206-210.
61. STROZIER, L. M., BLAIR, C. B., and EVANS, B. H. Armadillos: basic profiles. 1. Serum chemistry values. *Lab. Animal Sci.* **21** (1971) 399-400.
62. TALMAGE, R. V., and BUCHANEN, C. D. The armadillo (*Dasypus novemcinctus*). A review of its natural history, ecology, anatomy and reproductive physiology. The Rice Institute Pamphlet Monograph in Biology. Volume XLI, Number 2 (1954) 1-135.
63. TORREALBA, J. F. El primer caso de enfermedad de Chagas diagnosticado en Zeraza por empistaji debido al edema monocular, conjuntivitis esquitotripanosica 1 "Signo de Romaña." *Gac. Med. Caracas* **44** (1937) 321-323.
64. TRAVASSOS, L. P. Trichostrongylideos brasileiras. *Brazil-Med.* **29** (1915) 388-389.
65. TRAVASSOS, L. P. Gigantorhynchidae brasileiras. *Cong.-Med. Paulista.* **2** (1917) 181.
66. TRAVASSOS, L. P. Contribuição para a sistematica dos Dicrocoelinae Looss, 1899. *Arch. Escola Super. Agric. e Med. Vet., Rio de Janeiro* **3** (1919) 7-24.
67. TRAVASSOS, L. P. Contribuições para o conhecimento da fauna helmintologica brasileira. XIII. Ensaio monografico da familia Trichostrongylidae Leiper (1909). *Mem. Inst. Os. Cruz.* **13** (1921) 5-135.
68. TRAVASSOS, L. P. Alguns novos generos e especies de Trichostrongylidae. *Rev. Med. Cirurg. Brasil* **43** (1935) 345-361.
69. TRAVASSOS, L. P. Revisão da familia Trichostrongylidae Leiper 1912. Monographic Inst. Os. Cruz. (1937).
70. VARELA, G. and MAZZOTTI, L. Das mami-feros *D. novemcinctus* and *Neotoma fus-cipes* canescens susceptibiles al tifo murino. *Rev. Inst. Salubr. and Enferm. trop. Mex.* **2** (1947) 125-127.
71. WAGNER, J. Aphanipteren—Material aus der sammlung des Zoologischen Museum der Berliner Universitat *Mitteil. Zool. Mus. Berlin* **18** (1933) 338-362.
72. WISLOCKI, G. B., and ENDERS, R. K. Body temperature of sloths, anteaters and armadillos. *J. Mamm.* **16** (1935) 328-329.
73. WOLFE, J. L. Armadillo distribution in Alabama and northwest Florida. *Quart. J. Fla. Acad. Sci.* **31** (1969) 209-212.