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Growing Points in Leprosy Research

(1) The Armadillo as an Experimental Model for the Study of Human Leprosy

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The armadillo was selected as an animal model for the study of leprosy because of its low body temperature, availability, presumed weak immunological competence, longevity, and the fact that it regularly produces monozygous quadruplets. Most of these advantages have been realized. At present, 37 armadillos have developed symptoms of leprosy of which 13 have been killed and the disease confirmed by histopathological examination and other tests. These animals have yielded a total of 1255 g of lepromas which contain an estimated 20 g of leprosy bacilli.

The armadillo has great potential for studies on the chemotherapy, immunology and epidemiology of leprosy. It has immediate value for the production of large numbers of leprosy bacilli which could be used for studies on the biochemistry and metabolism of *Myco. leprae, in vitro* cultivation experiments, and preparation of a sufficient supply of standardized lepromin to meet the world's needs.

The nine-banded armadillo (*Dasypus novemcintus,* Linn.), is one of approximately 20 species of armadillos comprising several genera. It belongs to the Edentata, a primitive mammalian order which also includes sloths and anteaters. Edentata are found exclusively in the Western Hemisphere in Central and South America, with the exception of the nine-banded armadillo which ranges from central Argentina to the southern United States. Although little is known about the characteristices of many of the edenates, it ia known that they possess low body temperatures.

The nine-banded armadillo is the first unaltered animal model to develop disseminated leprosy following inoculation with *Mycobacterium leprae* isolated from human tissues (Storrs, 1971; Kirchheimer and Storrs, 1971; Kirchheimer, Storrs and Binford, 1972; Storrs, 1973; Storrs *et al.*, 1973; Storrs, in press; Storrs *et al.*, in press). Leprosy in the armadillo is characterized by a high incidence of susceptibility (at least 40%), bacterial counts as high as 10^{10}/g of tissue and a high degree of pathological involvement. Invasion of the central nervous system and lung by leprosy bacilli have been observed in some armadillos.

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Because of the magnified form in which leprosy occurs in this species, the armadillo promises to be an excellent model for studies on the chemotherapy, epidemiology and immunology of leprosy and other mycobacterial diseases. Of immediate importance is the finding that large amounts of infected tissue containing massive numbers of leprosy bacilli can be harvested from infected animals. These can be used in investigations concerned with the biochemistry and metabolism of *Myco. leprae.* A sufficient supply of standardized lepromin could now be prepared from armadillo lepromas to meet the world's needs. In addition to being a model for the study of disease, the armadillo could serve as a source of leprosy bacilli for cultivation studies.

The armadillo is a unique model for the study of leprosy because of a number of practical and scientific considerations. These include availability, low body temperature, suspected weak immunological competence, longevity and the fact that it regularly produces monozygous quadruplets.

Availability

Availability of animals is a very important although mundane consideration in selecting potential models for biomedical research. The nine-banded armadillo is abundant in the southern United States, Mexico, Central America and large areas of South America. These animals have increased remarkably with respect to population and range in the southern United States during the past 50 years. Approximately 80% of the animals brought in from the wild adapt to captivity; the remainder which do not adapt are released. The time allowed for adaptation to captivity prior to inoculation is 3 to 6 months. Armadillo young born in captivity are obtained by bringing in pregnant animals in late autumn; parturition occurs in the spring.

The direct cost of maintaining armadillos is about U.S. $0.37 per day per animal, which includes food, bedding and labour.

Adult armadillos weigh from 3 to 55 kg and are strong but not aggressive and do not attempt to bite. Two technicians can restrain an armadillo for routine technical procedures without anaesthesia and with no mechanical protection other than gloves.

Shipment of animals to locations where armadillos are not native poses no problem. In February of 1972 we received shipments of seven-banded armadillos (*Dasypus hybridus*) and hairy armadillos (*Chaetophractus villosus*) from Argentina. These animals became dehydrated *en route* and a few of them did not survive. However, the majority of animals recovered and all of the survivors are still alive.

While armadillos are not as available, inexpensive to maintain, or easy to handle as rats and mice, they compare very favourably with members of the dog, cat, and monkey families in these respects.

Body Temperature

Most leprologists believe that *Myco. leprae* grows best at low temperatures, since leprosy occurs primarily in the cooler regions of the human body such as the skin, nose, ears, digits and testes (Binford, 1956; Brand, 1959). This was the primary reason for selecting the armadillo as a model for the study of leprosy.

The body temperature (rectal) of the nine-banded armadillo ranges from 30° to 36°C when the ambient temperature is close to 25°C. Johansen (1961) in a detailed study, found the core temperatures of 13 armadillos to range from a low of 34° to 35°C early in the morning to a high of 35° 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 (1935) and by Burns and Waldrip (1971). 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 temperature averaged 31.3°C (range 30.0 to 33.0°C). These temperatures were taken between 0800 and 1200 h during the months of October to 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° 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 thermal 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/h and then levelled 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 may be as low as 30°C in some animals; however, either an increase or decrease in ambient temperature can result in a change in core temperature because of the relatively primitive regulatory mechanism.

Immunological Competence

In reviewing the scientific literature on armadillos, it was observed that these animals had been successfully inoculated with a number of diseases which can infect man including relapsing fever, exanthematic typhus, murine typhus, trichinosis and schistosomiasis (Storrs, 1971). They are also carriers of Chagas disease (Chagas, 1912) and have been reported to be susceptible to African sleeping sickness (Coyle, 1972). Thus, armadillos can be infected with a variety of organisms including spirochaetes, ricettsia, trepanosomes and schistosomes. Because of this, it was suspected that the armadillo might have weak immunological competence.

The papers describing this work were, for the most part, published in South American journals. This older work was summarized in a review by Talmage and Buchanan in 1954 in a Rice Institute Pamphlet Monograph in Biology (Talmage and Buchanan, 1954), but the authors did not draw any deductions from the information they had collected, and in all probability, this pamphlet was not widely distributed.

It now appears possible that the armadillo may be generally susceptible to diseases caused by organisms classified with the Actinomycetales, since in addition to leprosy it has become infected with *Norcardia brasiliensis* (Gezuele, 1972) and *Myco. ulcerans* (Walsh *et al.*, 1973), the causative agent of Buruli ulcer.

Some direct evidence has recently been obtained which suggests that the armadillo might have an immune deficiency which is temperature-related.

It is generally accepted that resistance to leprosy is a function of cell-mediated immunity (CMI). Since it was suspected that the low body temperature of the armadillo was somehow responsible for susceptibility, it was decided by Purtilo *et al.* (1973) to investigate the effect of temperature on the immune performance of this species. The ability of lymphocytes to transform in the presence of certain mitogenic substances is believed to be a reflection of the immune competence of an individual. With this rationale, these workers investigated the ability of armadillo lymphocytes to transform to phytohaemagglutinin (PHA) and lepromin at both normal human temperature (37° C) and temperatures below this (33° and 28° C) in a range comparable to normal armadillo temperature. They found that lymphocyte transformation was depressed by 66% at 33° C and 81% at 28° C when compared to transformation at 37° C, suggesting that there is an immune dysfunction of the lymphocytes of the armadillo at normal temperatures. Interestingly, the results of a parallel experiment using human lymphocytes also suggested that reduced temperatures had a depressive effect on lymphocyte transformation.

Other investigations by Purtilo and his group showed that from a histological standpoint the tissues of the lympho-reticular system (thymus, lymph nodes, tonsils, etc.) appeared to possess the elements necessary for immune competence. This finding provided additional evidence that the immune deficiency is one of function rather than a result of an anatomical defect.

Thus low body temperature may render the armadillo highly susceptible to leprosy for two reasons: (a) it provides an optimum temperature for growth of *Myco. leprae,* and (b) this temperature may depress the activity of the CMI system of the armadillo. It is emphasized that these are hypotheses which will have to be verified by in-depth studies.

Longevity

It has been speculated that leprosy may require 3 to 5 years to develop in man following inoculation by *Myco. leprae* (Skinsnes, 1964). Thus, the use of a long-lived animal model in which leprosy develops over a prolonged period of time is advantageous. Also, a long-lived model would be preferable for studies on chemotherapy, since leprosy is a chronic disease which requires treatment with drugs over a period of many years. Its chronicity and the limitations of available chemotherapy are illustrated by the finding that of 22 patients at the U.S. Public Health Service at Carville, Louisiana, U.S.A., in whom Promin treatment was initiated in 1941, 10 still had active leprosy 30 years later (Faget *et al.*, 1966). Therefore, in order to assess adequately the efficacy of new drugs, the animal model employed should have a long life span and develop lepromatous leprosy. The armadillo satisfies both of these requirements since its life span is estimated to be at least 12 to 15 years, and it develops severe disseminated disease 10 to 40 months after inoculation with *Myco. leprae.*

This estimate of life span of at least 12 to 15 years is based on the facts that the armadillo requires two years to reach sexual maturity, and the period of gestation totals nine months, which includes a three-month period of delayed blastocyst implantation. Thus, it matures less rapidly than dogs or cats, which have life spans of about 15 years.

Armadillos which were captured as full grown adults in 1968 were still alive in the Gulf South Research Institute colony in late 1973; hence these animals must be at least 7 years old. Three of the first 4 animals to be inoculated with leprosy bacilli (not viable in the mouse footpad) in December of 1968 are still alive, indicating that they must be at least 6 years old since these were also adults, at least 2 years old, when captured.

Monozygous Quadruplets

The nine-banded armadillo regularly produces monozygous quadruplets derived from a single fertilized ovum. The monozygosity of armadillo quadruplets has been well established by the embryological investigations of Newman and Patterson (1910), Hamlett (1927) and Enders (1966). In our laboratories we have never observed a mixed set of young; they are always all males or females. The seven-banded armadillo, which is very closely related to the nine-banded armadillo, regularly produces 8 to 16 monozygous young (Benirschke, 1968).

It must be emphasized that although the monozygous young are in all probability identical genetically, they are not necessarily completely identical immunologically, biochemically, or morphologically. Anderson and Benirschke (1962) found that sygenic grafts of skin between monozygous quadruplets were ultimately rejected although at much slower rates than allografts. Storrs and Williams (1968) made biochemical and morphological measurements on 16 sets of monozygous quadruplets and concluded that there were significant differences within sets. However, the differences within sets were usually smaller than the differences between sets. All of the measurements were made at birth or on animals delivered by Caesarean section just before birth to minimize environmental influences. They speculated that the differences observed within sets might arise from cytoplasmic inheritance. There is a greater opportunity of this occurring in the armadillo than in man because of the unique embryological development of the armadillo which is illustrated in Fig. 1.

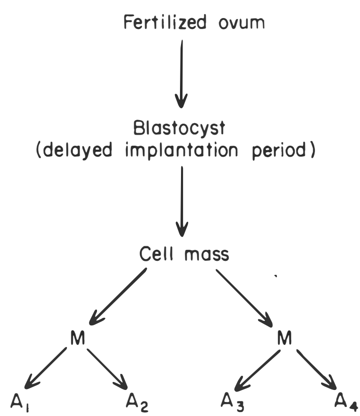

Fig. 1

Cell division of the fertilized ovum takes place and a blastocyst is formed which remains quiescent for a period of 14 to 16 weeks. After implantation, the cell mass develops 2 buds. These subsequently redivide to yield 4 cell masses which develop into the armadillo embryos.

Although these monozygous quadruplets may not be entirely identical somatically they are nevertheless very similar, and can be used to test the hypothesis that susceptibility to leprosy is inherited.

Therefore, 11 sets of monozygous quadruplets, one year old, were inoculated with leprosy bacilli according to the schedule shown in Table 1. All conditions were kept constant except for the number of animals inoculated. In 3 cases all members of the sets were inoculated, and in 8 cases only 2 of the 4 animals were inoculated in order to preserve the genomes should the inoculated members develop leprosy.

TABLE 1

Inoculation schedule for monozygous quadruplet armadillos

Monozygous set no.	Sex	Inoculated	Uninoculated
1	♀	2	2
2	♀	2	2
3	♀	2	2
4	♀	2	2
5	♂	2	2
6	♂	2	2
7	♂	2	2
8	♂	2	2
9	♂	4	0
10	♂	4	0
11	♂	4	0

Six months after inoculation all 4 members of set number 9 were positive for acid and fast bacilli at the sites of inoculation (abdomen) while all of the other animals were negative. The probability of this happening by chance is 1 in 20,475. However, the disease did not develop uniformly in all 4 animals. At the time of writing, 2 of these animals have developed small nodules and the other 2 rather large nodules.

The diagram showing when divisions of the cell masses take place indicates that there are 2 sets of twins within each group of 4; A1 and A2 may be more similar to each other than to the other group of twins (A3 and A4).

It is assumed that differences in immunological competence within sets will be least apparent when resistance to infection afforded by the genome is low. In these cases, the differences in resistance between individuals may not be great enough to prevent all the animals from developing leprosy. However, when resistance provided by the genome is high, the time required for leprosy to develop in animals within sets could vary, since small differences in immunological competence between individuals could be magnified. with some being just below the borderline of infection and others just above it, However, since this work with monozygous young is only in the early stages of development, evidence for this hypothesis will have to await further results.

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