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The nutritional myopathy of the quokka as a model for research in muscular dystrophy

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Abstract

Muscular paralysis in the captive quokka was investigated because it seriously handicapped laboratory research. *Inter alia* the inherent ability of muscle to regenerate completely was conclusively demonstrated for the first time in this work.

Since then the regenerative capacity of muscle has been well established in many animals and in man. In all of these regeneration occurs in a well-defined sequence when the cause of myonecrosis is removed or corrected.

Principles of myopathology which govern the muscle lesions in those myopathies in which muscle fibre necrosis is the primary event have been formulated from these studies and are now widely accepted.

Field studies including vitamin E status at various times of the year and other biological observations are also described.

Introduction

The quokka *(Setonix brachyurus)* in its natural habitat is prone to a number of nutritional problems which are well-described in the literature (See Hodgkin and Sheard 1959). This deterioration is due to the long summer drought which, combined with a high reproduction rate and an absence of predators, causes the quokka population on Rottnest to be at the limit sustainable by the food supply.

One of the more dramatic effects of this poor nutritional state is the development of muscular paralysis. This myopathy is caused by vitamin E *(α-tocopherol)* deficiency. On Rottnest the myopathy is usually subclinical with the disorder becoming overt in captivity (Kakulas 1982). Quokkas maintained in cages on the mainland are subject to the effect of an "enclosure size factor" which aggravates the deficiency and which is remarkably specific (Kakulas 1963). Thus the vitamin E requirement of quokkas in small cages is much higher than that of animals housed in pens or larger enclosures. The basis of this phenomenon is at present unknown, but a similar effect has been observed in other conditions e.g. in dogs with vitamin D deficiency (Mellanby 1919).

Many of the biological aspects of the quokka myopathy are described in "Man Marsupials and Muscle" (Kakulas 1982). This work contains a scientific record of the investigation of the myopathy, an account of the pathology of the muscle disorder and its reproduction in the laboratory. The results of a field survey of the vitamin E status of quokkas on many parts of the island revealed high levels in winter and spring and very low levels toward the end of summer.

One of the most noteworthy features of these investigations was the observation of full regeneration of muscle leading to total restoration of architecture. This discovery was made in paralyzed quokkas which were treated with vitamin E (Kakulas 1961). The phenomenon of muscle regeration has relevance to many, as yet incurable human diseases, especially the progressive muscular dystrophies. The quokka studies have shown that full regeneration is a possibility, in the future, and thus serves as a powerful stimulus to further medical research.

Additionally by using the quokka myopathy as a model for the duplication and study of acute and chronic muscle disorders the principles which govern the reaction of muscle to injury have been greatly

extended. These new concepts are now part of the general body of knowledge of the subject and include the standard teaching with regard to the pathogenesis of muscular dystrophy. In brief, necrosis and regeneration occur in a cyclical fashion which eventually leads to "end-stage muscle disease" through the exhaustion of regeneration and with fat and fibrous tissue replacing lost muscle fibres.

It, *inter alia*, was also shown that selenium was ineffective in preventing or treating paralysis (Kakulas 1963). This finding suggests that vitamin E is the sole anti-oxidant available to the quokka. This might explain the ease with which quokkas develop a deficiency of the vitamin. As a practical outcome of the investigation a dietary régime was developed, which maintains the animals in a healthy condition with the vitamin E requirement being between 35-70 mg per kg diet.

History of muscle paralysis in the quokka

The first reference to nutritional muscular paralysis in a macropod marsupial is that of Goss (1940) who reported the development of paralysis of the hind limbs of four Australian tree kangaroos. The animals, which were on exhibition during the World's Fair in New York had been fed large quantities of fish oil to make them sleek and presentable with the result that they developed muscular dystrophy. Three died and one recovered when fish oils were withdrawn and large doses of vitamin E were given.

The development of muscle paralysis in captive quokkas on the local scene became a problem only when for special reasons they were housed in small pens or cages. The difficulty greatly increased when large numbers of animals were kept in cages during the study of trace element metabolism by Barker (1960). The disease was observed on several occasions and affected both quokkas and tammar wallabies (*Macropus eugenii*) captive in the yards of the Zoology Department. The principle manifestations of the disease were, wasting of skeletal muscles of the hind limbs, paralysis of the hind limbs and eventual death. At about the same time the disease appeared in domesticated quokkas, held by Kaldor in the Department of Physiology for the study of iron metabolism.

Robinson (1960), while working on quantitative amino acid excretion in West Australian marsupials experienced a similar high incidence of the disease in quokkas housed in small cages and he mentions slight differences in the disease in animals kept in large yards. The disorder was abrupt in onset and was often precipitated in apparently normal animals by the stress of capture. Although administration of vitamin E to quokkas with the disease at the South Perth Zoological Gardens had failed to change the condition Robinson thought that biochemical tests might show some change. Accordingly 2 animals with the disease were fed relatively small quantities of *α-tocopherol* for a period of 2 weeks. The supplement was administered 3 times weekly and doses equivalent to 20 mg per kg body weight were given. One animal became very weak and died within 30 days while the other showed some clinical improvement. He found that with the administration of *α-tocopherol*, urinary excretion of creatine was lowered, β alanine excretion ceased and the ratio of L-methyl histidine to glycine excretion fell abruptly. Robinson suggested, from these results, that the cause of the disease was a complicated deficiency of vitamin E but was unable to prove this.

It was also at this time when paralysis in the quokka was first observed overseas. Carmichael and Reed, (1958), report that in November, 1957, 8 quokkas were received by the Smithsonian Institutional Zoological Park, Washington D.C. A week after arrival one quokka was found dead from fighting and the autopsy revealed infestation with large numbers of the nematode worm *Austrostrongylus minitus* and smaller numbers of *Dipetalonema annulipapillata*. The remaining animals were given antibiotics and anthelminhics (piperazine). A short time later a second quokka died from an intestinal intussusception and it was noticed that most of the remaining animals were exhibiting an exfoliative type of skin lesion. Scrapings from these did not reveal the cause. Necropsy findings on another animal dying at this time were negative, except for parasitism. Between February and late May 1958 the remaining five quokkas developed progressive posterior paralysis. The skin trouble had virtually disappeared and the appetites remained generally good. In addition to the vitamin and mineral supplements which these animals were receiviing for several months, injectable forms of vitamin E were administered. During this time all recommended diets had been offered and eaten. Despite the treatments and nutritional changes the paralysis progressed; two of the animals were sacrificed and the remaining ones died. Necropsies were done at the National Institutes of Health, Bethesda, U.S.A., and Armed Forces Institute of Pathology (A.F.I.P.), Washington D.C. The report of one of these necropsies conducted by Dr P. Craig was kindly made available by the A.F.I.P. The main findings were, myopathy, cause unknown, multiple abscesses in skin of thigh; focal myocarditis; adenocarcinoma of lung, heart, brain and kidney.

Barker (1960) quotes a similar paralysis in quokkas in the Zoological Gardens of Alipore, Calcutta in 1959. A number of animals developed hind limb paralysis and skin lesions soon after arrival and again there was failure of all forms of treatment. In 1962 an outbreak of a similar disease in quokkas was reported from the Zoological Gardens at Healesville, Victoria, Australia.

As the problem had increased in magnitude, apparently did not respond to vitamin therapy, and had certain features suggestive of a communicable disease, paralyzed animals were submitted by Barker, to Professor N. F. Stanley at the university of Western Australia, who attempted to isolate a virus. Muscles from affected animals were examined histologically by Dr J. B. Blackwell, Pathologist at the Royal Perth Hospital, who found widespread segmental necrosis and minimal inflammatory change. Two positive results were obtained on animal inoculation. The first was with a suspension of the brain of an affected quokka which produced emaciation in a litter of suckling mice and the second was with a suspension of hind limb muscle which again produced emaciation in a litter of suckling mice. Both these litters showed histological changes in skeletal muscle similar to those found in the quokka.

These results however, were open to some doubt as the agent responsible could not be serially propagated. This impression was substantiated over the course of time as further suspensions from affected quokkas have failed to disclose a transmissable pathogenic agent. Similar material of muscle was examined by Professor R. E. J. ten Seldam, from the Pathology Dept., University of Western Australia, at this time and he concluded that the changes were consistent with vitamin E deficiency although the possibility of a muscle virus infection could not be excluded. Dr M. Sadka, Neurologist, Royal Perth Hospital, who shared this opinion, suggested that sections of muscle be sent to Dr R. D. Adams, Bullard Professor of Neuropathology, Harvard Medical School, Boston. He was also convinced that dietary deficiency of vitamin E was the most likely cause of the disease.

The significance of the quokka myopathy to muscle research

The nutritional myopathy of the quokka highlighted the regenerative potential of muscle, a discovery which is of great significance to human muscle disease. As indicated above, the current relevance of the work is due to the theoretical support it gives to a future cure being found for progressive muscular dystrophy which is at present a serious world-wide disease.

Before the discovery, in 1960, of complete and total regeneration of skeletal muscle voluntary muscle was believed to possess only limited regenerative potential. Notwithstanding this general view there were a number of excellent prior studies in the late 19th and early 20th centuries which demonstrated regeneration of muscle following local injury.

These papers were fully reviewed by Adams, Denny-Brown and Pearson (1962) in their monograph of muscle pathology. These authors also added their own studies on muscle regeneration including the concept that, for regeneration to be complete, the endomysial sheath should be preserved. In the early investigations attention was usually confined to a small selectively injured area of muscle using a variety of physical methods. However, the technique of crushing, cutting or tearing the muscle partly or completely destroyed the supporting fibrous tissue framework. In this situation when regeneration ensued the multiplying myoblasts, being undirected, became admixed with other elements so that such regeneration which did occur became "aberrant" and "disorganised".

Therefore, what was the contribution of the quokka? Principally it was the demonstration of total and overall muscle regeneration observed in paralyzed animals after treatment with vitamin E. A similar phenomenon may have been encountered previously, possibly within nutritional experiments, but if this was so it was given scant attention or not recorded. Indirectly the quokka work also demonstrated that regeneration continued to complete restoration of muscle architecture provided that the cause of the original necrosis was removed or corrected. This fact is now one of the principles of myopathology which was derived from the quokka studies. Most naturally-occurring myopathies are characterized by focal muscle fibre necrosis with preservation of the endomysium. The *polyfocal*, and *polyphasic* myonecrosis which is typical of the lesions in the nutritional myopathy of the Rottnest Island quokka is also the common histopathological denominator of most animal and human muscle diseases. Examples are the muscular dystrophies, polymyositis and the toxic, drug metabolic and endocrine myopathies. In all of these the endomysial and supporting fibrovascular framework is intact so that the situation is more or less akin to that found in the nutritional myopathy of the quokka.

Agricultural veterinarians knew for very many years that vitamin E, and other anti-oxidants, were effective in the prevention of so-called "nutritional muscular dystrophy" in farm animals (Blaxter 1957). However, these substances and selenium, were usually used for prophylaxis in herds and flocks where treatment of an individual animal would not be contemplated for practical reasons.

The principles of pathogenesis of the lesions in the quokka, which rest upon the observations of experimentally-controlled, chronic and continuing, myonecrosis, were first described at a meting of the New York Academy of Sciences in 1965 when they were used to explain the natural history and pathogenesis of muscular dystrophy.

Discussion

Although it has been well known since the last century that the muscular dystrophies are genetically-inherited disorders, the metabolic or biochemical expression of the inherited factor has not been identified. From the precedents offered by the reversible quokka myopathy and other recent work on human muscle regeneration, one may expect similar regeneration in human muscular dystrophy to occur when the biochemical defect is corrected.

The contributions to myopathology of the quokka work are threefold. Firstly, there is the principle that skeletal muscle, throughout the body and in most animal species, inherently possesses great power of regeneration. This potential does not require to be stimulated but always follows necrosis of which it is the natural consequence. Secondly, there is the fact that if the cause of myonecrosis is removed or neutralized, regeneration is full and complete, and thirdly, there is the formulation of a system to explain the pathogenesis and evolution of the histopathology of the necrobiotic myopathies. This incorporates a new nomenclature introduced to describe the microscopic lesions. Using the terms *monofocal*, *polyfocal*, *monophasic*, and *polyphasic* myonecrosis the necrobiotic myopathies can be accommodated within a simple terminology (Kakulas 1975).

An accelerating factor in the pathogenesis of muscular dystrophy is the fact that regeneration is abnormal and abortive in those conditions so that exhaustion occurs sooner. Outfall of muscle fibres follows with fat and fibrous tissue replacement being secondary and passive. To some extent the "end-stage" lesions are also due to hypertrophy, atrophy, incomplete regeneration and muscle fibre splitting, central nucleation and partial denervation: all of which have been reproduced in the quokka. These concepts are now established as the standard teaching for the pathogenesis of the lesions in the human muscular dystrophies.

However, there are still many questions to be answered in the aetiology and pathogenesis of the nutritional myopathy of the quokka and in muscular dystrophy. Useful comparisons of the muscle lesions in the two conditions have been in terms of the common principles which are responsible for the histopathogenesis of the respective myopathies and it is probable that necrosis in both is due to a basic membrane defect. However, apart from this, very little has been gained from the quokka investigation which has shed light upon the aetiology of muscular dystrophy. Vitamin E as an anti-oxidant is known to prevent the peroxidation of membrane lipids and there is evidence that the human dystrophies, especially dystrophia myotonica, are also basically due to membrane disorders (Rowland 1976). This common ground between the animal and human myopathies deserves further exploration.

The quokka myopathy is primarily due to nutritional deficiency of vitamin E, while human muscular dystrophy is genetically determined. However, this does not preclude the possibility that both conditions act within the same biochemical pathway, i.e. the metabolic disorder in the animal is an "acquired" defect of the system, which is responsible for the maintenance of membrane integrity, and that the biochemical disturbance in man is a hereditary "inborn error" of membrane metabolism. The naturally-occurring myopathy of the quokka is clinically, a more acute disorder than the Duchenne and other forms of human muscular dystrophies, otherwise their pathological manifestations are remarkably similar.

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Recent endocrinological research on the Rottnest Island quokka (*Setonix brachyurus*)

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Abstract

Recent endocrinological work on the macropodid marsupial, the quokka, (*Setonix brachyurus*) is reviewed, concentrating on pituitary, adrenal and reproductive hormones. Studies of adeno- and neurohypophysial hormones are in their infancy and the significance of the suite of newly-discovered hormones in the pars nervosa needs to be established. Recent research on circulating levels of progesterone in the quokka have shown that the oestrous cycle and the pregnant cycle are not hormonally equivalent, as was previously thought. The chorio-vitelline placenta of the quokka has also been shown to be capable of progesterone synthesis *in vitro* and there is mounting evidence in this species, and in other marsupials, that the conceptus exerts a strong local effect on the uterus.

Introduction

Despite the fact that the quokka is a member of a monotypic genus restricted virtually to insular habitats in Western Australia, it has proven a particular acccmmodating vehicle for a wide variety of research, due primary to its ready availability and ease of maintenance in the laboratory. Such is the extent of this work since the last major review of the animal's biology in 1959, that this report will be concerned only with pituitary, adrenal and reproductive hormones. Research on the thymus gland and immunology is reviewed by Stanley (this volume) and the ecological relevance of much of these data is discussed by Main (this volume). McDonald (1977) has recently reviewed what little