PREFACE

Frog and rabbit are the principal animal species studied in this monograph. The passage from one species to another may make it difficult to follow the reasoning, but this passage is necessary because most of the experimental results concerning physiological properties (e.g. isometric tetanic tension and velocity of shortening) were obtained with intact frog fibres, whereas most of the enzymatic results concerning MgATPase activities were obtained with demembranated rabbit fibres.

This book comprises a long line of reasoning, with many interdependent and complex sections. I therefore recommend reading the titles of the most complicated chapters and sections carefully to facilitate understanding. Consulting the Index could also be useful.

In addition to reviewing and reanalysing the results of studies of my own and many other independent groups, I also report unpublished results for experiments with half-fibres (intact fibres split lengthwise) from white skeletal muscles of young adult frogs and with permeabilised fibre bundles from red skeletal muscles of young adult rats. From this many-faceted 'treatise', a hybrid model emerges, combining the swinging cross-bridge/lever-arm processes and lateral swelling mechanisms.

In these new experimental findings, the relative resting force, recorded at pH 7 and 10°C, in half-fibres from white skeletal muscles (*iliofibularis*) of young adult frogs (*Rana pipiens*), held around the slack length, increased very slightly when the bulk ionic strength was lowered from 180 mM to ~40 mM. Between ~40 mM and ~30 mM, the relative resting force increased very rapidly with further decreases in ionic strength, peaking at high levels, between ~30 mM and ~20 mM. Below ~20 mM, the relative resting force decreased sharply. The dependence of the relative resting force on ionic strength, the existence of a maximum and the rapid decrease at very low ionic strengths demonstrate that strong radial repulsive electrostatic forces are exerted between the myofilaments under resting conditions (see below concerning the conversion of radial forces into axial forces). These radial repulsive electrostatic forces are also effective in half-fibres (and all types of fibre, whether intact or demembranated), under isometric tetanic contraction conditions, and present qualitative characteristics similar to those at rest (only some quantitative features differ).

In another set of experiments, myosin heads were cleaved enzymatically (i.e. digested with α-chymotrypsin) from the rest of the thick myosin filaments, in permeabilised fibre bundles from red skeletal muscles (*tibialis anterior*) of young adult rats (Wistar), held around the slack length, in a buffer mimicking the physiological resting medium, at room temperature. Very small, sometimes tiny, but detectable, transitory contractures resulted from these enzymatic cleavages, demonstrating that thin actin and thick myosin filaments are

tethered by a small number of 'resting' (weakly bound) cross-bridges, exerting strong radial tethering forces (together with weak radial attractive/compressive forces) that counterbalance the radial repulsive electrostatic forces, under resting conditions.

Based on these two series of experimental observations, a hybrid model of muscle contraction is proposed, in which the radial tethering forces decrease drastically once contraction is triggered, leading to net radial expansive forces between the myofilaments. Under both resting and contracting conditions, the net radial repulsive (expansive) forces are turned into axial forces. Indeed, in the 1970s, based on theoretical and logical reasoning, mechanisms for this conversion were proposed that are valid at rest and during contraction, regardless of volume variations. In this hybrid model, under standard conditions (e.g. 10°C, slack fibre length, pH ~7, ionic strength ~180 mM), part of the isometric tetanic tension (~40%) results from lateral swelling mechanisms (the usual name for mechanisms involving radial expansive forces inducing axial contractile forces) and another part (~60%) results from swinging cross-bridge/lever-arm processes plus, possibly, the impulsive mechanism developed by Elliott and Worthington plus, possibly, the 'step-wise' mechanism developed by Pollack's group, and other models (e.g. ~30% of swinging cross-bridge/lever-arm models, ~10% of impulsive model, ~10% of Pollack's model, and ~10% of other models). In this work, I neglect the 'unconventional' models and use only swinging cross-bridge/lever-arm mechanisms, with a proportion of ~60%.

The experimental findings, summarised here, and the hybrid model developed and discussed in this book provide the pretext for a long critical and constructive review and an analysis concerning many well-known properties of contracting muscle fibres, as well as complex phenomena (including unexplained, forgotten, ignored, even 'mysterious' experimental and semi-empirical results). Most of the 'forgotten' observations were not accounted for by swinging cross-bridge/lever-arm models and were, therefore, rarely taken into account in the many discussions presented in publications concerning muscle contraction and its molecular basis. By contrast, I think that the hybrid model answers many of these awkward questions.

In 2000, Cyranoski published a commentary paper concerning the symposium held in Osaka (Japan) on in vitro motility and its possible link to muscle contraction. The author provided a severe, but lucid, analysis of the approach of Yanagida and his coworkers (see also Chapter 9 in this book for supplementary arguments). Cyranoski also cited Molloy, who claimed, during the symposium, that 'The tightly coupled lever-arm idea is simple, predictive and inherently testable because of its more restrictive nature' (as opposed to 'the loose-coupled thermal ratchet model' defended by Yanagida and his group). Thus, in 2000, the general view, expressed by Molloy, was apparently clearcut. In this monograph, I demonstrate that the situation is much more complex than previously thought by many specialists in muscle contraction and in vitro motility.

The starting point for writing this book was essentially the conclusion of Cyranoski (2000), who cited A.F. Huxley: 'I came here confused about actin and myosin. Now, I am still confused, but at a higher level'.

The need to reopen the question of the universality of swinging cross-bridge/lever-arm theories and to search for innovative ideas, based on new experiments, has been expressed by Bryant et al. (2007), who wrote: 'the basic actomyosin motor has been embellished, altered, and reused many times through the evolution of the myosin superfamily'. More recently, Grazi (2011) wrote: 'With time clever hypotheses may be accepted as "facts" without being supported by solid experimental evidence. In our opinion this happened with muscle contraction where pure suggestions still occupy the scene and delay the progress of the research'. In the last few years, many authors, including Bryant et al. (2007), have focused on myosin VI and discovered unexpected properties of this motor protein, studied in vitro by brilliant techniques. The titles of the articles by Spudich and Sivaramakrishnan (2010) and Sweeney and Houdusse (2010) were particularly 'explosive': 'Myosin VI: an innovative motor that challenged the swinging lever arm hypothesis' and 'Myosin VI rewrites the rules for myosin motors', respectively.

I have made use of the time available to me since my retirement to read as many papers as possible in the domains of muscle contraction and in vitro motility, with the aim of resolving the confusion. I provide in this work my own analysis

of the various questions posed in the muscle and motility areas and suggest a synthesis, including the hybrid model. Moreover, to the best of my knowledge, the most recent monographs describing the traditional mechanisms of muscle contraction only are those by Bagshaw (1993), Simmons (1992) and Squire (2011).* They have the same titles and resemble descriptive textbooks, and the many experiments published during the last 30 years or so have never been critically analysed. In any event, this long and complex book will be a useful working tool for specialists in muscle contraction, professors, doctors in medicine, and graduate students.

Some important keywords emerge from this monograph that may help the reader to understand this work: head–head dimers, thick myosin filaments, radial repulsive electrostatic forces, radial tethering forces and translation of radial forces into axial forces.

This monograph was completed between 2008 and 2011, and the bibliography concerns the period before 2008–2011 (the first reference dates back to 1911). There are 1000–1100 references. In the addendum, I propose a supplementary list of about 200–250 references, corresponding to the period between 2008 and 2012–2013, with a few references from 2014 and 2015.

Finally, this book may be seen as an analysis and a synthesis of many experimental, theoretical and semi-empirical studies published over the last century or so. Even the most firmly 'unconvinced' reader will enjoy reading the long reference list.

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* After the acceptance of this monograph, Rall published a book on muscle contraction (2014; see Addendum). These two works are clearly complementary, with little or no overlap.