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Development of Synchrotron Radiation as a High-Intensity Source for X-ray Diffraction†

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Interest in the molecular mechanism of muscle contraction led to the search for an intense source of X-rays of 1–2 Å wavelength so as to be able to examine the rich X-ray diffraction patterns given by muscles during contraction. This led to the first X-ray diffraction experiments using synchrotron radiation, carried out by Holmes, Rosenbaum and Witz at DESY, Hamburg, in September 1970. In the following years, the EMBL Outstation, to utilize synchrotron radiation for biological structure determination, was established at DESY and preliminary experiments on muscle were also carried out at NINA (Daresbury). The development of time-resolved techniques for muscle diffraction was first started in the MRC Molecular Biology Laboratory in Cambridge, using rotating-anode X-ray tubes, and was then greatly extended at the EMBL Outstation, Hamburg, using the storage ring DORIS. This was a very successful venture, and helped to drive the whole technology development and to interest other potential users in the technique.

Keywords: muscle; mirror monochromators; structural change; time-resolved studies.

1. Early work in the search for higher intensity

The limitations of laboratory X-ray tubes as sources of X-rays for diffraction experiments were particularly apparent to us as workers in the muscle field in the 1950's and 1960's. Muscles are relatively weak diffractors (compared even with protein crystals) since they contain up to 80% water, and are much less highly ordered. Consequently, X-ray patterns from a surviving muscle would have taken many weeks to record if one had used radiation from a conventional fixed-anode X-ray tube, and, even with a special fine-focus device and a miniaturized low-angle slit camera, it took several hours or several days, respectively, to record the equatorial and meridional diagrams in the first experiments on live relaxed frog muscles (Huxley, 1951, 1952, 1953). Since the most important questions to be answered concerned contracting muscle (once the main features of the resting structure had been determined), and since an isolated muscle could be kept contracting for only a few seconds at a time (at intervals of a few minutes for recovery), it was impractical to answer even the most basic question – is the overall average pattern different during contraction? – without a substantial improvement in the available X-ray intensity. Moreover, since the changes in muscle structure which produce contraction take place on a time scale of milliseconds or less, experiments to determine their time courses, and to detect transient changes, were far out of range.

A considerable improvement in intensity available from normal laboratory systems was effected by technical developments that we introduced in the early 1960's. One of us (KCH, in collaboration with W. Longley) took the electron gun and focusing assembly from a Beaudoin fixed-focus X-ray tube and grafted it onto a rotating-anode X-ray tube of the type developed by Tony Broad at the MRC Molecular Biology Laboratory in Cambridge from an earlier design of Taylor at the Royal Institution. This made possible a ten times greater anode loading (intensity per unit area of the focal line on the anode) than with the conventional electron guns at that time. Then, together, we made the important discovery that if we combined a totally reflecting bent glass mirror (of the type developed by Franks) with a focusing monochromator (quartz crystal) (the focusing mirror being the first element in the system, close to the X-ray tube), it was possible with careful collimation to use the full aperture of the crystal to collect the incident X-ray beam over a relatively wide angle from the X-ray tube focus. We could focus it to a narrow line (or to a small spot with the mirror focusing as well) and yet have a relatively clean background. Low-angle X-ray patterns extending into reciprocal spacings of several hundred ångströms could be recorded with very high resolution, with a large gain in available intensity over previous systems, so that informative two-dimensional diagrams of the myosin and actin layer lines could be recorded in ~15 min (Huxley, Brown & Holmes, 1965). Experience with the mirror monochromator

† This article describes our own personal recollections and activities during the early phases of the development of synchrotron radiation as an X-ray source for diffraction experiments. It is not intended to be a review of all the developments during that period, and does not cover work at other sources. §1 of this article is written jointly by HEH and KCH; §2 is by KCH; §3 is by HEH.

system proved to be very important in the subsequent development of synchrotron radiation for X-ray diffraction.

However, even though we could now obtain average patterns during a 1 s tetanus of frog muscle (by repeating the contraction 900 times), the process took a total time of 30 h (2 min intervals between contractions) and required great care with muscle preparation and handling. The experiments yielded many valuable results (Huxley & Brown, 1967; Huxley, 1968), but still left us a long way from being able to collect true time-resolved X-ray data during contraction.

Our next move was to obtain further increases in intensity from the rotating-anode X-ray tubes. The limitation in intensity is imposed by the temperature rise of the surface of the copper anode during the time interval that it is passing under the focus of the electron beam. After passing through the beam, the heated region of the copper anode rapidly cools again by conduction (laterally and to deeper layers of the copper anode, and ultimately to the cooling water) before returning again under the electron beam one revolution later. The controlling variable is the time spent in the electron beam; hence, the surface velocity of the rotation anode needs to be as high as possible. Existing bearings and vacuum seals operated successfully with anodes spinning at ~1500 r.p.m., and so, in order not to exceed that rotational rate, but still obtain a higher surface velocity, we developed a much larger diameter anode, going from the 4.5 in diameter of the Broad tubes (by that time being manufactured commercially by Elliott Bros.) to a bicycle-wheel-like structure, ~24 in in diameter, which we operated inside an old high-speed centrifuge containment vessel, in case of anode explosion. It was a terrifying device to operate, and although it led ultimately to the very useful 'big wheel' rotating-anode tubes produced by Elliott Bros., it served to convince us that the path to much larger gains in X-ray intensity must be elsewhere. The basic problem is that the maximal permissible current loading in the focus increases only as the square root of the linear velocity of the surface, while the mechanical stress on the anode due to centrifugal forces increases as the square of the velocity. Thus, there is an unfavourable fourth-power relationship between the necessary mechanical strength of the anode material and any gains in X-ray output, and the possibilities for further improvement are rapidly exhausted.

Consequently, our thoughts turned to more exotic physical processes by which X-ray beams might be produced, and one of us (KCH) considered the possibility at that time (1965–1966) that a useful X-ray intensity might be obtained from synchrotron radiation, which was known to produce radiation over a broad range of wavelengths, and was already serving as a source of UV light of variable wavelength. However, calculation showed that the X-ray intensity available would be little better than the output of our present X-ray tubes and cameras and so we continued to pursue incremental improvements in instrumentation, and (HEH) began using electronic X-ray detectors (proportional counters) to improve signal detection over film, and to facilitate time-resolved measurements.

In 1968 Kenneth C. Holmes moved to Heidelberg, where he became Director of the Biophysics Section of the Max Planck Institute for Medical Research (which had become a well known centre for muscle research under the previous Director, Professor H. H. Weber) and continued his X-ray diffraction studies on tobacco mosaic virus and on insect flight muscle. In 1969 Gerd Rosenbaum joined his group as a graduate student, having previously worked (in physics) at the DESY synchrotron radiation laboratory in Hamburg (F41), where an active program of spectroscopy in the vacuum UV was being pursued. In discussions with KCH it emerged that the updated characteristics of the DESY synchrotron were such that it should in theory now provide a considerably better power output than any available laboratory X-ray tube, and Rosenbaum and KCH realised that the mirror–monochromator system, which they were using very successfully on laboratory X-ray tubes, was especially well suited for monochromatizing and focusing the synchrotron beam. Moreover, it was clear that future generations of particle accelerators would provide vastly greater increases in X-ray intensity.

At that time there was a committee, of which one of us (HEH) was chairman, which was charged with producing a report detailing the scientific justification for setting up, somewhere in Europe, an international laboratory for molecular biology. The basic argument was that such a laboratory would facilitate the many technological developments which could now speed up the advance of biological science, particularly in the field of structural biology, but which were difficult or impossible to pursue with the limited technical resources of individual biology laboratories. It seemed clear to us (KCH was a consultant to the committee) that the future use of synchrotron radiation, which would require the administration of a large centralized facility and a major commitment in instrumentation, was an extremely good example of the necessity for this form of European collaboration, and we made a very strong case for it being a major feature of the new laboratory. We were very sure that it offered great possibilities. These and other arguments were in the end successful in persuading all the governments concerned to go ahead with the European Molecular Biology Laboratory (EMBL), and to set up an outstation at DESY, Hamburg, to construct and operate an X-ray diffraction facility there. However, long before this official sanction arrived, Holmes and his colleagues had embarked on experiments, on the new source.

First, it had to be established whether the expected X-ray intensity could in fact be obtained. Together with Dr J. Witz, an expert on monochromator design, they constructed an experimental camera consisting basically of just a curved quartz-crystal monochromator, together with a means of measuring the output flux on film and of recording a simple one-dimensional equatorial X-ray diffraction pattern from insect flight muscle. They arranged with the DESY management to carry out a pilot experiment on an available beam port in the F41 bunker for a few double shifts of beam time (16 h), and this first attempt to record X-ray diffraction

patterns with synchrotron radiation (in September 1970) was remarkably successful. They found that their calculations of the expected flux were indeed approximately correct, and that even the initial very crude experimental system gave an X-ray pattern that was ten times stronger than that obtained with the most powerful laboratory X-ray source. Moreover, they showed that another one or two orders of magnitude of improvement would be available with doubly focused camera arrangements.

These results were published in *Nature* in April 1971 and this new application of synchrotron radiation was born (Rosenbaum, Holmes & Witz, 1971). However, there was a long way to go before the full possibilities of the new technique could be realised.

2. DESY beamline and establishment of the EMBL Outstation

Insect flight muscle changes its structure on adding adenosine triphosphate (ATP) (Reedy, Holmes & Tregear, 1965) (the hydrolysis of ATP drives muscle contraction). Moreover, it is highly crystalline. Therefore, the Heidelberg group hoped that insect flight muscle might provide an alternative system to frog muscle for studying the cross-bridge cycle by X-ray diffraction. However, we met all the intensity problems encountered by HEH for frog muscle but in a more acute form, since the muscle specimens are much smaller than the frog muscle. This drove us to reconsider synchrotron radiation. Since the initial calculations the energy of the machine had moved to 7.2 GeV and the beam current was often in excess of 10 mA. Therefore, significant gains were to be expected from using synchrotron radiation.

On the basis of the initial experiments in the bunker of the F41 group outlined in §1, Dr Haensel and the directors of DESY (in particular Martin Teucher) encouraged us to set up a bunker for X-ray diffraction experiments on biological samples. This was built by DESY during the summer shutdown of 1971 and was known as 'bunker 2'; a little later this became the initial headquarters of the EMBL Outstation at DESY (Fig. 1). Above the experimental hall two offices and a room for biochemistry were added later. Inside the experimental hall a massive neutron-proof concrete wall separated the operators from the beam. Therefore, all adjustments had to be made by remote control without direct visual interaction.

In collaboration with John Barrington-Leigh, Gerd Rosenbaum set about building a beamline which took the form of a fully remotely controlled optical bench (Barrington-Leigh & Rosenbaum, 1974, 1976) (Fig. 2). The optics were based on a Guinier monochromator to focus the fan of radiation from the synchrotron in the horizontal plane and 2 $\times$ 20 cm adjustable bent mirrors to focus the much smaller divergence in the vertical plane. The mirrors (fused quartz) were nearest to the synchrotron and were housed in a helium-filled box separated from the machine vacuum by a beryllium window. Otherwise, beams were accommodated in vacuum tubes fitted with Mylar windows. The length and thickness of the mirrors were a compromise between stability for polishing and stiffness for bending. Long mirrors need to be thick to achieve the necessary stability and can become too thick to bend. It turned out that our mirrors were too thin since they deformed permanently after

Figure 1
The building (bunker 2) which housed the world's first synchrotron X-ray laboratory. The curve of the synchrotron (DESY) can be seen behind. A remote-controlled optical bench with mirror–monochromator optics was installed in this bunker during 1971–1972. An upper storey containing offices and a biochemical laboratory was added later.

a few months bending. Furthermore, it proved difficult to obtain mirrors polished to the necessary flatness: optical mirror manufacturers had no way of monitoring the micro-flatness necessary for X-ray mirrors. Here we were considerably helped by the pioneering work of Franks (Franks & Breakwell, 1974). Movements were controlled by about 100 small DC motors with potentiometers as position sensors, which led to an impressive run of cables. DC motors were chosen rather than stepping motors because they are light: the whole apparatus was built on a mini budget and the apparatus dare not become massive. An SIT-vidicon camera was used to observe in line the image of the direct beam formed on a caesium iodide crystal. Two other steerable TV cameras fitted with zinc sulfide screens were mounted on a parallel optical bench. These could be used for monitoring slits. The Guinier monochromator (quartz) was cut at 7° to the surface. In order to compress the beam this was used in the anti-Guinier geometry. This results in a wavelength inhomogeneity across the converging beam. However, this effect is unimportant for the apertures being used. The angle of the latter part of the optical bank to the direct beam was fixed for $\lambda = 1.5$ Å. Since the optical elements were ~40 m from the tangent point of the synchrotron and focused within 2–3 m, a demagnification of ~15 was achieved. The electron beam of DESY was relatively compact so that a focused beam of dimensions 200 × 250 μm could be obtained. With a flux of $\sim 10^9$ photons s^{-1} and excellent optical properties this was a very good beam for low-angle scattering. The flux density was two orders of magnitude better than could be achieved with the best rotating-anode tubes. Images were registered on film or on one-dimensional single-wire (Gabriel & Dupont, 1972) position-sensitive detectors. The beamline was in operation in 1972 and for a couple of years remained a unique facility.

Using this beamline the Heidelberg group (in collaboration with Richard Tregear from Oxford) studied the diffraction from insect flight muscle. The excellent collimation led to detailed fibre diffraction pictures which yielded new structural information (Holmes, Tregear & Barrington-Leigh, 1980). Time-resolved experiments were set up with oscillating insect flight muscle. The muscles were attached to a vibrator and oscillated at 5 Hz, at which frequency they generate considerable work if provided with ATP. At low amplitudes of oscillation it was expected that the cross-bridges might be partially synchronized so that one should be able to record diffraction patterns from various parts of the crossbridge cycle. The diffraction was recorded, a layer line at a time, on a position-sensitive detector and the output switched into one of 32 bins in synchrony with the oscillation. Data with usable statistics could be obtained from the equator in ~15 min. However, on account of the available intensity, the measurements remained confined to the strong equatorial reflections (Barrington-Leigh &

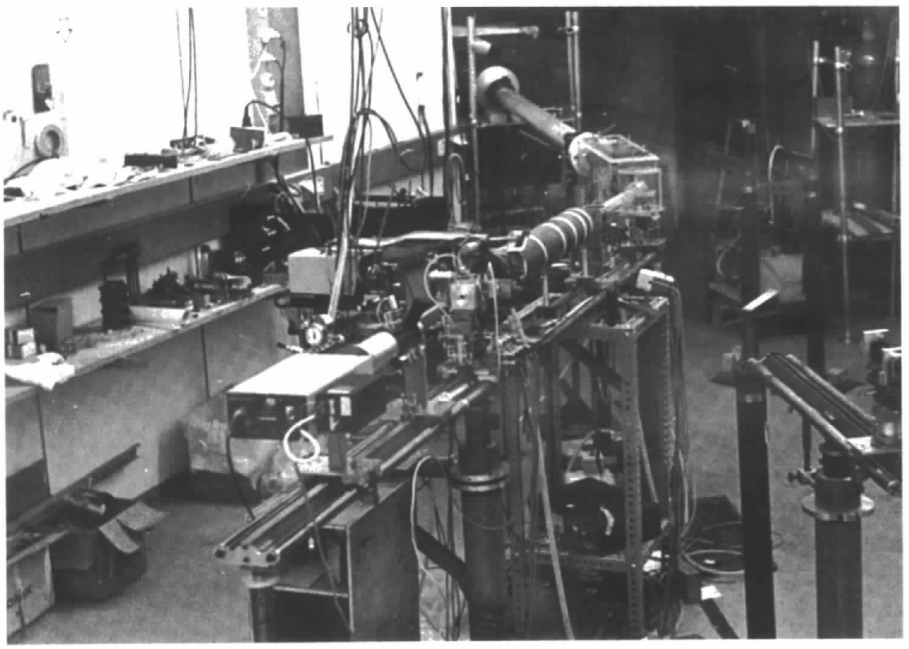

Figure 2
The mirror–monochromator optics installed in bunker 2 on the DESY synchrotron. Two 20 cm bent mirrors (housed in a helium-filled plexiglass box which is visible towards the rear) focused in the vertical plane. They were followed by a Guinier curved crystal quartz monochromator used in the 'anti-Guinier' position so as to compress and focus the beam in the horizontal plane and deflect it through 26°. The beam left the synchrotron vacuum through a beryllium window which can be seen at the rear. The optics were ~40 m from the tangent point of the synchrotron. An in-line TV camera (front) could be used to observe the direct beam impinging on a caesium iodide crystal. The cooled specimen holder can be seen in the middle of the picture. A second TV camera mounted on a track parallel to the optical bench could be used for inspecting the positions of apertures using a zinc sulfide screen. All adjustments could be remotely controlled. Movements were effected by small DC motors using potentiometers for positional feedback. The apparatus was built in the workshops of the Max Planck Institute for Medical Research, Heidelberg.

Rosenbaum, 1976). It turns out that these reflections alter little between resting and contracting insect flight muscle and, therefore, in this case are not very useful for monitoring the crossbridges. The insect flight muscle experiments needed a storage ring.

Since the intensity was not adequate to allow a time-resolved study of the meridional reflections (which do alter with crossbridge orientation), attempts were made to 'freeze' the crossbridges in alternative conformations by the use of non-hydrolysable analogues of ATP (Goody, Holmes, Mannherz, Barrington-Leigh & Rosenbaum, 1975) and then take photographs. Quite large changes in the diffraction pattern were induced by certain analogues. However, subsequent work showed that a large part of the changes resulted from alterations in the pattern of binding of the crossbridges to actin, rather than in an underlying change in the crossbridge orientation. Nevertheless, the changes in the fibre diffraction pattern could be used for monitoring the binding of ATP analogues to crossbridges *in situ*, which yielded data which are difficult to obtain in any other way (Goody, Barrington-Leigh, Mannherz, Tregear & Rosenbaum, 1976).

From the beginning it was hoped and planned to set up the DESY X-ray diffraction laboratory as an outstation of the projected EMBL. Even before the pilot experiments in the F41 bunker, two of us (Gerd Rosenbaum and KCH) had made proposals to the EMBL planning committee for the founding of an X-ray synchrotron radiation facility. The most likely form for this initiative appeared to be an EMBL Outstation at DESY. This suggestion was warmly received by Hugh Huxley and John Kendrew, the Director General designate of EMBL. However, the nascent DESY facility could not wait on the formal grounding of EMBL. Therefore, the initial facility was financed by a consortium of DESY (buildings), the Max Planck Institute for Medical Research (equipment) and the Deutsche Forschungsgemeinschaft (salaries). In 1974 the facility was put on a proper basis by being taken over as an outstation of EMBL a few weeks after EMBL's foundation. A year later a contract was signed between DESY and EMBL which entitled EMBL to set up an X-ray facility for the molecular biology users.

From its inception the DESY/EMBL group tried to make the facility available to other interested users. The beamline was also shared with the Bonse group who were developing channel-cut X-ray monochromators. The beam was able to record the Debye–Scherrer rings from tubulin in the mitotic spindle, foreshadowing an important later application of synchrotron radiation to study the mechanism of polymerization of tubulin. Studies of the low-angle fibre diagram from rat-tail tendon (collagen) were also undertaken and the synchrotron beam yielded unsurpassed data. Moreover, the group shared experiences with Hugh Huxley and Uli Arndt in Cambridge who were setting up a similar beamline at NINA.

The initial success at DESY sparked worldwide interest. In June 1972 there was a historical meeting in Brookhaven at which most of the subsequent applications of X-ray synchrotron radiation were discussed (see Barrington-Leigh, Holmes & Rosenbaum, 1973). The most important application for biology later proved to be protein crystallography. Early tests of protein diffraction on the DESY source (Harmsen, Leberman & Schulz, 1976) showed improvements compared with conventional sources but the gains were limited. The flux was about ten times better than with a conventional source. At this stage one had failed to appreciate that the parallel collimation of the beam was giving an unusually good signal-to-noise ratio. This was the

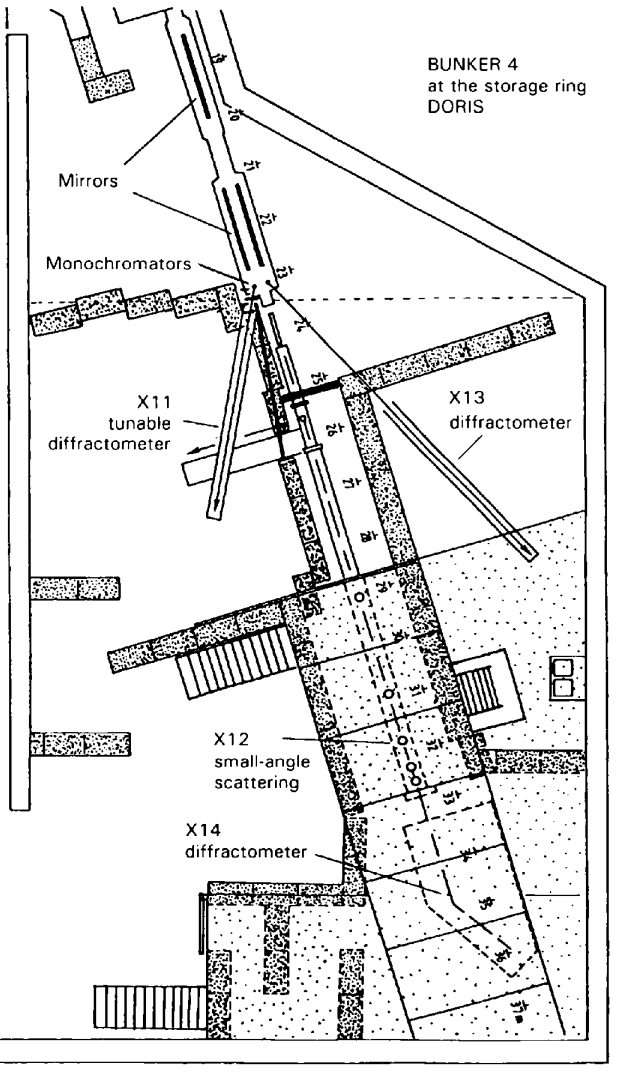

Figure 3
A diagram of the initial layout of the beamline and diffractometers in bunker 4 at the storage ring DORIS. Three sets of mirrors were housed in a common vacuum box which also housed two monochromators for the *X*11 and *X*13 diffractometers. Each mirror assembly consisted of 6 × 20 cm mirrors which could be individually adjusted and (later) computer controlled. The *X*11 bench could be turned to allow wavelength selection. At the rear (*X*14) a diffractometer was initially installed for muscle work, but was eventually abandoned in favour of *X*11 and *X*13; a third mirror system in the vacuum box was then used to provide a 'white' beamline in this sector.

property of synchrotron radiation which ultimately made it the source of choice for all kinds of protein crystal data collection. At about the same time studies on the Stanford storage ring SPEAR (Phillips, Wlodawer, Yevitz & Hodgson, 1976) showed gains for crystal diffraction even with a non-focusing monochromator which indicated that storage ring sources were going to be of considerable importance in protein crystallography. These authors made use of the ability to 'tune' the wavelength across an adsorption edge to demonstrate the potentialities of synchrotron radiation in exploiting the effects of anomalous dispersion.

During this initial period plans were being laid at DESY to make use of the much stronger synchrotron radiation from the storage ring DORIS. Experiments on SPEAR showed how important it was to use a storage ring rather than a synchrotron which dumps the beam 50 times a second. Styles had altered in the elapsed couple of years so that the experimental facility at DORIS was housed in a small experimental hall rather than a bunker. Nevertheless, it was known as 'bunker 4'. Duly equipped with offices, a seminar room, a workshop and a biochemistry laboratory, this building became the home of the EMBL Outstation in 1975. DORIS is a colliding beam facility with electrons and positrons circulating in opposite directions. The beams into the EMBL bunker (Fig. 3) were from the positron ring. The first beamline set-up ($X11$, designed by Rosenbaum, later taken over by Bartunik) was a mirror–monochromator combination with 6 × 20 cm mirrors and a bent germanium monochromator. The bench carrying the specimen and detector could be rotated around the monochromator as a pivot so as to vary the wavelength. Each of the mirrors could be individually bent, although this proved mechanically difficult because of the large stresses involved. The electron beam in DORIS was considerably larger in cross section than that of DESY, so that fine-focused beams such as we were used to on DESY were not attainable. In fact, it turned out that it was not really worth bending the mirror segments at all; aligning them appropriately without bending produced as fine a beam as one could obtain. A second optical system, $X13$ (Bordas *et al.*, 1980), similar in design to $X11$, was soon added. The two shared a common mirror box and mirror design. Improvements included the use of a triangular germanium monochromator. This was later incorporated into $X11$. These beamlines were the work-horses of the DORIS facility for a number of years and were also used by HEH and his group (see below). The DORIS facility in bunker 4 expanded steadily and became one of the most widely used biological facilities in the world.

3. The long road to success

3.1. *Early work at Daresbury*

Work had begun in the MRC Molecular Biology Laboratory in Cambridge in 1971 to take advantage of the synchrotron radiation available from NINA at Daresbury. Like the machine in Hamburg, this was an electron synchrotron used by physicists for atomic particle collision work, and did not have a stored beam but one that was wound up to full energy every 20 ms and then deflected into a target. Consequently, it only became a significant emitter of X-rays during the last few milliseconds of each cycle. Nevertheless, calculations indicated that it should provide more photons than we were currently able to obtain from the mirror–monochromator camera on our 'big-wheel' rotating-anode X-ray tube.

A remotely controlled low-angle X-ray camera was therefore designed and constructed by Haselgrove, Faruqi, Huxley and Arndt (finally written up and published in 1977; Haselgrove, Faruqi, Huxley & Arndt, 1977). It consisted of two mirrors in tandem (total length 40 cm) as the first element, focusing in a vertical direction, and a bent quartz or germanium crystal monochromator giving focusing in a horizontal direction. The length of the monochromator crystal was only 7.5 cm (quartz) or 6 cm (germanium), for that was what was available at that time. The faces of the crystal were cut at an angle of ~8 to the reflecting planes (Bragg angle for 1.5 Å X-rays ~13) so as to give lateral compression of the beam by about a factor of three. The crystal accepted an incident beam ~20 mm in width and gave an output beam ~6 mm wide, which was a suitable size for the quite large muscle specimens. These were placed halfway between monochromator and detector, which was at the focus of the optics, 3 m from the crystal. Stepping motors controlled the positions of three sets of collimating slits (before the mirrors, between the mirrors and mono-chromator, and immediately before the specimen), and other motors provided the necessary adjustments to align and focus the optical elements by remote control (using a television camera and fluorescent screen), after preliminary adjustments had been made with a laser alignment system while the X-ray shutter was closed.

The first experiments were made on the NINA beamline at Daresbury in late 1973 and continued at irregular intervals (when beam time was available) over the next three years. It was a learning experience, and a rather painful one. Electron synchrotrons were extremely temperamental machines in those days, and in any case were being operated primarily for the benefit of the particle physics experiments. Therefore, the proportion of useful beam time was quite small, often with seemingly endless and unexplained waiting periods. But worse still, even when the beam was on, the intensity of our X-ray patterns was usually inferior to what we could obtain with rotating-anode tubes. There were several reasons for this, which took us quite a long time to unravel.

First of all, we were located as the end station of a beamline, which was advantageous in some ways since we did not have to worry about our apparatus blocking off the beam from users further down the line. However, it placed us 47 m from the tangent point, with an acceptance aperture ~1.5 mm vertically and 20 mm horizontally. This meant we were accepting only about one sixth of the beam vertically (we found that gold-plated mirrors deteriorated too rapidly

to be very useful) and only $\sim 4.2 \times 10^{-4}$ rad horizontally. But worse still, we found that when the beam was being extracted for collision experiments (as it was most of the time), the beam at our tangent point became deflected quite early on in the acceleration cycle, so that the average X-ray intensity at $\lambda = 1.5$ Å was only a few percent of its peak value without extraction. Also, the peak intensity itself varied very sharply with the peak energy at which NINA was being run, decreasing by a factor of almost 15 between 5 and 3 GeV.

A further factor, which we appreciated only gradually, was just how remarkably efficient were the very compact low-angle X-ray cameras we were using on the rotating-anode tubes. We were recording the images on film at a specimen-to-film distance of ~10 cm, yet were able to resolve the myosin 429 Å layer-line repeat perfectly well, and could record very useful pictures in as little as 10 min of total exposure (or about 600 1 s tetani). If we used longer cameras in the laboratory, *e.g.* 50 cm or 1 m specimen-to-film distance, we obtained very much more elegant pictures but at the expense of increasing the exposure time to up to 30 h (Fig. 4). This meant that such photographs could never be taken of contracting muscle, even during a long series of contractions. With the optical system at the synchrotron, we were constrained by geometric and mechanical factors to the use of the longer specimen-to-film distances, so while under optimal conditions we could now record patterns with shorter exposure times than our laboratory cameras of similar length would allow, we still could not approach the speed of our shorter laboratory cameras, which it was essential to do in order to observe two-dimensional patterns from contracting muscle.

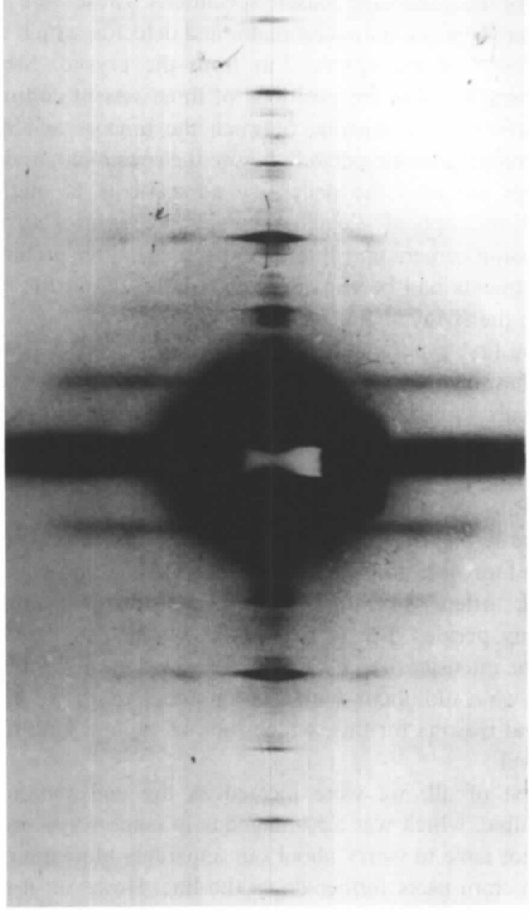

Figure 4
Low-angle X-ray diffraction pattern from frog sartorius muscle in live resting state. Laboratory X-ray source (*GX6*) mirror–monochromator camera. 50 h exposure.

Therefore, while we remained convinced of the potential value of synchrotron radiation, we were forced to conclude that we would need to use the radiation from a storage ring, which would be a much stronger and continuous source, if we wanted to carry out time-resolved measurements on the myosin and actin layer-line patterns. Such a source was soon to be available in DESY, Hamburg, where the EMBL Outstation had now been set up, and where beamlines were being constructed on the electron/positron storage ring DORIS.

3.2. *Early developments in electronic data collection*

During this whole period (1971–1977), one of us (HEH), assisted by Dr John Haselgrove and Dr A. R. Faruqi and by Chris Bond, all at the MRC Cambridge, had been making a parallel effort to develop time-resolved techniques using rotating-anode tubes as X-ray sources, and electronic X-ray detectors. Such measurements could still only be carried out on the stronger reflections in the muscle X-ray pattern, namely the two inner equatorial reflections ([10] and [11]) at spacings of ~345 and 200 Å, and the strong myosin meridional reflection at 143 Å. The experiments required very long series of contractions to accumulate enough counts, but they did begin to yield interesting results and they were particularly valuable in that they gave us a great deal of early experience in the technology of using electronic detectors to collect time-resolved data (in 10 or 20 ms time slots), in synchronizing the repetitive cycles of data collection with various physiological manipulations of the muscles, and in handling, storing and displaying the resultant streams of data (Bond & Faruqi, 1976; Faruqi, 1975*a*,*b*; Faruqi & Huxley, 1978*a*,*b*; Faruqi & Leigh, 1975; Huxley & Haselgrove, 1976). This experience was extremely valuable to us when we started to perform experiments at the Hamburg storage ring.

Briefly, our first experiments were to measure the time course of the large changes in intensity of the [10] and [11] equatorial reflections that we had found to occur between resting and isometrically contracting muscles (Haselgrove & Huxley, 1973), and to see how it was correlated with the onset and decay of tension in the muscle. This required us to make measurements with a time resolution of 10 ms or better, which we were able to do by optimizing the camera design (two mirrors in parallel), tweaking the X-ray tube (transient increase in current during the 1 s or so each 2 min when data was being recorded), and, most notably, by using a position-sensitive detector of the early Gabriel design

(Gabriel & Dupont, 1972) (a later version of which we used extensively in experiments at Hamburg). The version developed by Faruqi operated at 3 atm of xenon–methane and gave a spatial resolution of 100 μm, suitable for our muscle cameras, and was interfaced to our electronics so as to collect time-resolved patterns. With up to 10^9 photons s^{-1} in the beam incident on the specimen, we could obtain a few thousand counts per second in the equatorial reflections that we were measuring, so that a few hundred contractions would give us a few seconds of total exposure in each of our 10 ms time slots, enough counts to plot out informative time courses of intensity change (Huxley, 1975; Huxley & Haselgrove, 1976). We also began to make measurements during active shortening of the muscles at various speeds, storing the data from four time channels whose position could be adjusted to coincide with periods of rest, isometric contraction, shortening, and post-shortening isometric (Huxley, 1979). All these techniques we were able to transfer rapidly to the later experiments with synchrotron radiation, when the incident flux was 100 times or more greater, and we were able to switch our attention to the much weaker, but even more interesting, parts of the pattern.

3.3. *Early work using the Hamburg storage ring (DORIS)*

Experiments by the Cambridge MRC Group (now just Huxley and Faruqi, later joined by Kress) at the Hamburg storage ring DORIS began in the summer of 1977, shortly after the first beamline into the EMBL Outstation facility from that ring (bunker 4) had come into operation. However, despite our high hopes that now, at last, we would quickly be rewarded with the much desired very high intensity beams, we had still to endure many more fruitless days and nights on the beamlines over the next year or two. Initially, we set up our own camera (the one we had been using at Daresbury) at the end of beamline 12, the last station on a beam port which was divided into sectors, there being both right- and left-deflecting monochromators for the upstream beamlines *X*11 and *X*13, as well as an upward deflecting system for cameras on a mezzanine floor. We were still some 32 m away from the tangent point, with our two 20 cm-long mirrors and 7.5 cm-long quartz monochromator, so did not expect to obtain a very bright beam. But for several sessions, the beam was extraordinarily faint, the result, as was eventually found, of an obstruction somewhere upstream in the beamline!

The major problem during the first two years, however, was the unreliability and unpredictability of the circulating beams (electrons and positrons – we were on the positron beam) in the storage ring. The ring's primary use was for colliding beam experiments, and as an intermediate storage device for positrons for collisions in the big storage ring PETRA, for the high-energy physicists were carrying out very advanced and exacting studies on exotic particles, which required constant adjustments and interruptions to the operation of DORIS. Moreover, it was, in the early days anyway, almost impossible to obtain more than the most fragmentary information as to when we might hope to receive beam again, though that did improve considerably as the years went by. So the initial setting-up period of the beamlines, when we were still feeling our way into unknown territory, was rather lengthy and at times almost unbelievably frustrating, as we struggled to gather some morsels of data. It became clear after some months that even when everything was operating properly, the flux through our rather diminutive and distant camera was very much smaller than through the full-scale camera on *X*11, and the later one on *X*13, so we switched our operations to those lines, having at least gained some first-hand experience with our own instrument.

The camera on *X*11, designed by Rosenbaum and Harmsen (Rosenbaum & Harmsen, 1978), comprised an eight-element totally reflecting glass mirror of total length 160 cm which could accept the full vertical spread of the synchrotron beam, followed by a germanium curved crystal monochromator. With this camera we were delighted to find that good-quality layer-line patterns from muscle could be recorded on film in a few minutes, compared with about 24 h for a pattern at a similar camera length using a high-power rotating-anode tube. However, all our real experiments were now being performed with electronic detectors of various kinds, and our first real success at the storage ring was with an X-ray image intensifier TV detector which had been brought to Hamburg by Jim Milch visiting from the Reynolds' laboratory in Princeton (Reynolds, Milch & Gruner, 1978). This gave us a two-dimensional display of the myosin layer-line pattern in muscle, with an integration time of ~1 s, so it was possible to see the layer lines fade out, more or less in real time, as the muscle was stimulated tetanically, and then re-appear again as it was allowed to relax (Huxley, Faruqi, Bordas, Koch & Milch, 1980). This was a memorable experience for us all, after yet another all-night session in bunker 4!

We were able to obtain somewhat better time resolution, using the same detector, by recording the pattern during a 150 ms time slot set at a particular time during contraction, but because of readout time limitations only one such time slot could be recorded during each twitch. This was repeated about six times at 1 min intervals to give approximately one second's worth of data, and then the timing switched to another point in the contraction cycle. This was very inefficient and laborious, but it did give us some time-resolved two-dimensional layer-line data, albeit rather noisy. We had only limited opportunity to use this detector, and did not realise then how long it would take before a high-counting-rate two-dimensional detector with a rapid readout system would be available. We were not alone in this, and when I outlined the essential requirements of such a system for muscle work – maximum total count rates of up to 10^6 counts s^{-1} or more, time resolution 10 ms or better, and spatial resolution of a few hundred micrometres – at a detector meeting in 1978, I received several very optimistic forecasts of when one might be available, ranging from a few months to one or two years! In reality, it took ten years or more to produce a satisfactory one, and, in fact, detector

development always seems to have lagged behind the experimental capabilities of the X-ray source.

3.4. *Success at last in Hamburg*

We were able to do much better with one-dimensional electronic detectors. We had already gained some experience with these on a rotating-anode X-ray tube, as I mentioned earlier, and now Gabriel had designed and built a much improved instrument using the delay-line readout technique (Gabriel, 1977) in which the position of the burst of ionization from a photon arriving at some point along the length of the counter was decoded by measuring the arrival times of the centre of gravity of the pulses appearing at either end of the delay line connected to the cathode elements. Using electronics developed at CERN and at the EMBL in Heidelberg, and data-handing systems designed and built in the EMBL Outstation in Hamburg (Bordas *et al.*, 1980), the one-dimensional patterns could be collected in a continuous series of time slots of any desired width during each contraction of the muscle, the slots being synchronized to the stimulus, and repeated and accumulated until a sufficient number of counts had been collected. Such a detector could operate at total count rates up to $\sim 2 \times 10^5$ counts s^{-1}, but the patterns became distorted if the count-rate limitation was exceeded. Thus we were always pressing the design groups to produce detectors with higher and higher count-rate capabilities and with the maximum serviceability.

We used this detector on the new beamline *X*13, which had been designed very much with work on muscle in mind (Hendrix, Koch & Bordas, 1979). Like *X*11 it had an eight-segment 160 cm total length glass mirror array giving focusing in the vertical direction, but now had a bent triangular single-crystal germanium monochromator some 18 cm in length, which gave excellent focusing in the horizontal direction, and high intensity (Figs. 5 and 6). The dimensions of the X-ray beam at the sample were approximately 2 mm (vertical) × 5 mm (horizontal) and at the detector (~2 m from the sample) approximately 0.5 × 1.5 mm. The total flux at the specimen was of the order of 10^{11} photons s^{-1}, and good data would be collected in an experiment lasting a few hours.

This was an excellent system, and the data-collection electronics allowed us to inspect the accumulated one-dimensional pattern in any chosen time slot as the experiment progressed. Our earlier experiences in collecting time-resolved muscle data on rotating-anode tubes using electronic detectors and data-handling systems (see references above) were very valuable to us, both in knowing how best to perform the experiments and in being able to interact efficiently with the instrumentation experts at the EMBL and in Heidelberg and Grenoble. There was very good contact between the providers and the users, enhanced by many long nights of triumph and of trauma on the beamline together!

3.5. *Evidence needed about muscle*

The data that we wanted to obtain from muscle were basically of four kinds. First, we wanted to know the exact time course of the characteristic changes that took place in the various parts of the X-ray pattern during the transition

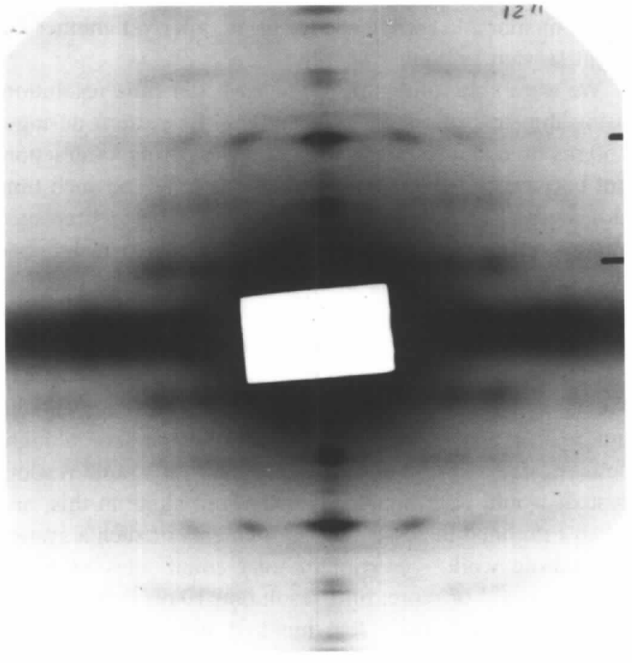

Figure 5
X-ray diagram from resting muscle; synchrotron radiation source (beamline *X*33 on the storage ring DORIS at DESY Hamburg). 12 min exposure (film).

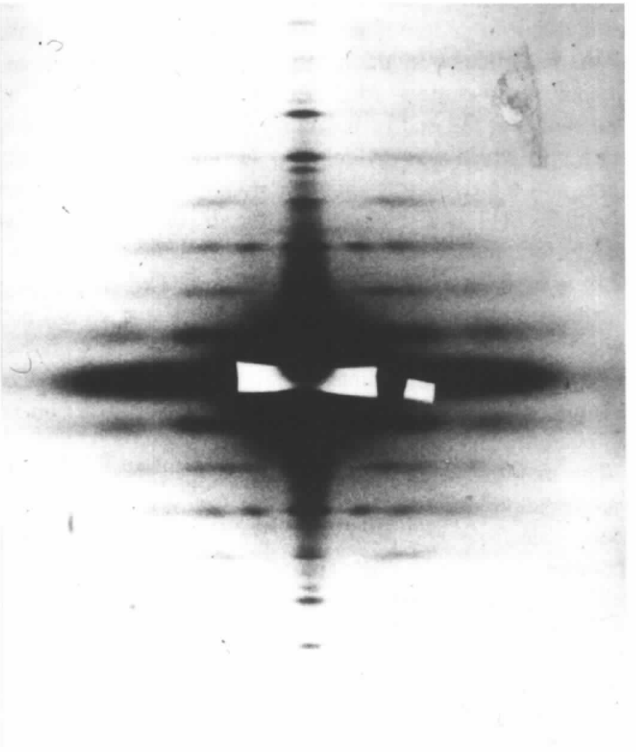

Figure 6
X-ray diagram from frog muscle, laboratory X-ray source (*GX*6), mirror–monochromator camera. 24 h exposure.

from rest to full activity (and their reversal during relaxation), in relation to the time course of tension development and its subsequent decay, during a twitch or a short tetanic contraction of a muscle. We had postulated that muscle force was produced by crossbridges from the myosin filaments first attaching to actin filaments and then changing shape or orientation, in a repeated cycle so as to produce a sliding force between the filaments (Hanson & Huxley, 1955; Huxley, 1969), but for many years there was no actual direct evidence that crossbridges moved in the required manner. We and others (Huxley, 1975; Matsubara & Yagi, 1978; Podolsky, St Onge, Yu & Lymn, 1976) had been able to make time-resolved measurements on the equatorial reflections during tension development using rotating-anode tubes, since these reflections are very strong, and the changes in them are very substantial (a factor of two or more). These experiments showed a close synchronization between what we interpreted as lateral crossbridge movement to attach to actin, and the onset of tension. In fact, the structural change seemed to lead tension slightly, by ~10–15 ms, as though an initial attached state of crossbridges had to be established before they could undergo subsequent structural changes to develop tension. However, the force-producing changes would also have to involve movements in an axial direction, and these would only show up in the myosin and actin layer-line reflections, most of which were one or two orders of magnitude weaker than the equatorial reflections. Therefore, studies of their time course could only be performed effectively with synchrotron radiation.

Secondly, we wanted to see what evidence we could obtain for a specific attached configuration of tension-generating crossbridges. Our model of crossbridge action (Huxley, 1969) postulated that myosin heads attached to specific sites on the actin filaments and then either tilted, or changed their shape in an equivalent way, so as to produce a forceful axial displacement (by an order of 5–10 nm) of the distal end of the head, where it attached to the S2 part of the myosin molecule and thence to the fixed backbone structure of the thick filament. This meant that, during contraction, part of the myosin head structure would conform to the same helical and subunit repeat parameters as the actin filaments, resulting in an enhancement of parts of the actin layer-line pattern. Indeed, such a 'labelled-actin' pattern is prominently displayed in the X-ray diagram from rigor muscles, when all the crossbridges are attached to actin, so it seemed reasonable to expect to see some sign of an equivalent pattern in contracting muscle, especially as we had already shown (Haselgrove & Huxley, 1973) that the equatorial pattern indicated close lateral proximity of many myosin heads to actin in contracting muscle.

Thirdly, although the crossbridge model predicted that in a contracting muscle the actin myosin crossbridges would be distributed through a range of configurations, since their cycling was asynchronous (to produce a steady average force in the muscle as a whole), there was good reason to believe that they could be partially and momentarily synchronized by applying a small but very rapid quick release, *i.e.* a length decrease of 1% or less of muscle length, to an otherwise isometrically contracting muscle (Huxley & Simmons, 1971). It might therefore be very informative to see what changes could be seen in the X-ray diagrams during these very brief mechanical transients.

Fourthly, we and others had also put forward a model for the on–off switching mechanism in striated muscle – the so-called steric blocking hypothesis (Huxley, 1972; Parry & Squire, 1973; Vibert, Haselgrove, Lowy & Poulsen, 1972) – in which azimuthal movements of tropomyosin strands in the long-pitched grooves in the actin filaments controlled the access of myosin heads to the specific binding sites on actin monomers to which they had to bind for force development to proceed. We had seen evidence in static X-ray patterns that some such change in tropomyosin position occurred, but the patterns were very faint, and we had some doubts as to whether they would show up above the noise level in time-resolved measurements. There had been strong criticism of this theory from several quarters, and we were anxious to find out if we could confirm our original ideas.

These questions defined the four main types of information that we knew it was crucial to obtain. The X-ray technique offered the most direct way – in fact, almost the only direct way, in which structural information could be obtained about how the force for contraction was produced by an intact functioning muscle. The only way that we could obtain this information was by using synchrotron radiation as an intense X-ray source, but the whole technology had first to be developed. Thus, this had been the motivation that had driven us to apply all the pressure we could to make sure that the technology did get developed; by helping to get the EMBL and the EMBL Outstation at DESY Hamburg started; by having an EMBL Instrumentation Committee set up and ensuring that they were fully aware of the great possibilities of synchrotron radiation in X-ray diffraction; by making sure that the detector designers and the electronics and computer experts were fully aware of the challenging problems that the experiments presented in high-speed counting and on-line data analysis and storage; and by showing by our own investment of effort that we needed the data very badly, and believed the technique could be made to work. And, of course, by obtaining some results.

3.6. *Muscle experiments*

Since it quickly became apparent that two-dimensional detectors with the requisite high counting rates and high time resolution would not be available for some considerable time, we collected data from the various relevant parts of the muscle X-ray diagram in separate experiments, using a one-dimensional detector placed so as to record a slice of the appropriate position and orientation. Collection of equatorial and meridional data was straightforward. Layer-line data were collected in a series of strips parallel to, and at different distances from, the meridian, or with the detector placed along a particular layer line, to record changes in radial distribution directly. Data could be recorded in up to

256 successive time slots, whose width could be set to any required value from 0.5 ms upwards.

Initially, we studied the time course of the intensity changes in the myosin layer lines (Huxley *et al.*, 1980; Huxley, Faruqi, Kress, Bordas & Koch, 1982) when we found we could obtain adequate counting statistics for the time resolution we needed – 5 or 10 ms – by summing the patterns from a series of less than 100 twitches or short tetani, at intervals of 30–60 s between contractions to allow time for recovery. This gave us several thousand counts in each reflection (after background subtraction) in each time frame. We found that the large decrease in intensity of the off-meridional layer lines was a genuine affect, even in a completely unfatigued muscle, and that its time course remained closely synchronized to the onset and decay of tension over a range of temperatures between 2 and 10 °C where the half-times for the changes varied by a factor of four. This was strong evidence that the myosin crossbridges did undergo pronounced axial and/or azimuthal movements during tension development, as they would need to do if they interacted with specific sites on actin, and that they quickly returned to their regular helical positions around the thick filament backbone as soon as contraction was over. Indeed, the intensity decreases during the onset of contraction preceded tension development, there being a 10–20 ms difference in their half-times. This provided further evidence that it was crossbridge movement which led to tension production, and not *vice versa*. Interestingly, the intensity of the 14.3 nm meridional reflection, arising from the axial repeat of groups of myosin heads on the thick filaments, was found to increase during contraction, when it was corrected for changes in width across the meridian. This indicated that the heads must be more perpendicularly oriented relative to the filament axis during contraction, or more regularly ordered axially on the 14.3 nm repeat, or both. These were all very encouraging findings.

However, we were very disappointed to find that, even in the layer-line patterns collected from our best experiments from excellently oriented muscles in prime condition, we were unable to see any clear evidence for a measurable intensification of the inner regions of the inner actin layer lines, so prominent in rigor muscles. We know that there was a faint reflection present in the inner region of the first actin layer line (at ~36 nm axial spacing) in resting muscle, but no evidence that it became measurably stronger during contraction. Of course, our data, collected with a one-dimensional detector, were not very satisfactory for looking for reflections in unknown positions on other actin layer lines, but it was clear that either the actin-attached myosin heads had very considerable azimuthal freedom of movement or there were relatively few of them (*e.g.* if there were 20% of the rigor number the intensity would be only 4% of that present in rigor, a signal which would be undetectable in our experiments). This provided even stronger motivation for us to press for the development of good two-dimensional detectors.

Our next experiments, looking at the effect of mechanical transients on the X-ray reflections from an isometrically

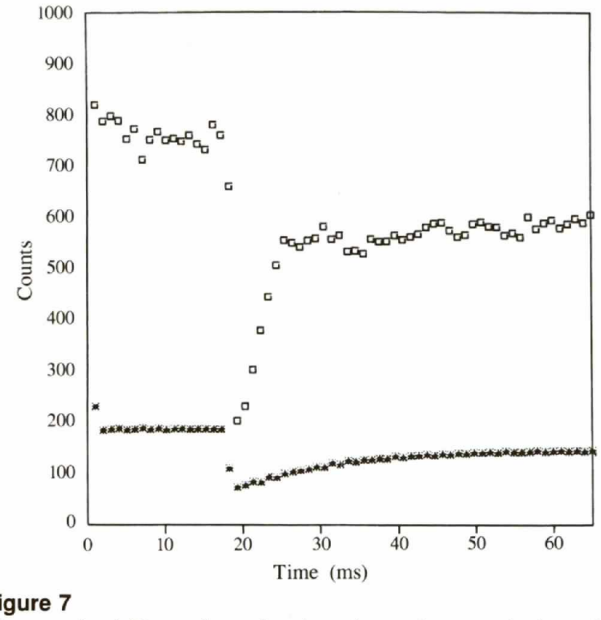

Figure 7
Time-resolved X-ray data, showing abrupt decrease in intensity, and partial rapid recovery, of 14.3 nm meridional reflection (open squares) in 1 ms time channels, produced by small rapid length decrease applied to contracting frog sartorius muscle. The sudden drop in tension (asterisks) precedes the intensity decrease by ~0.5 ms.

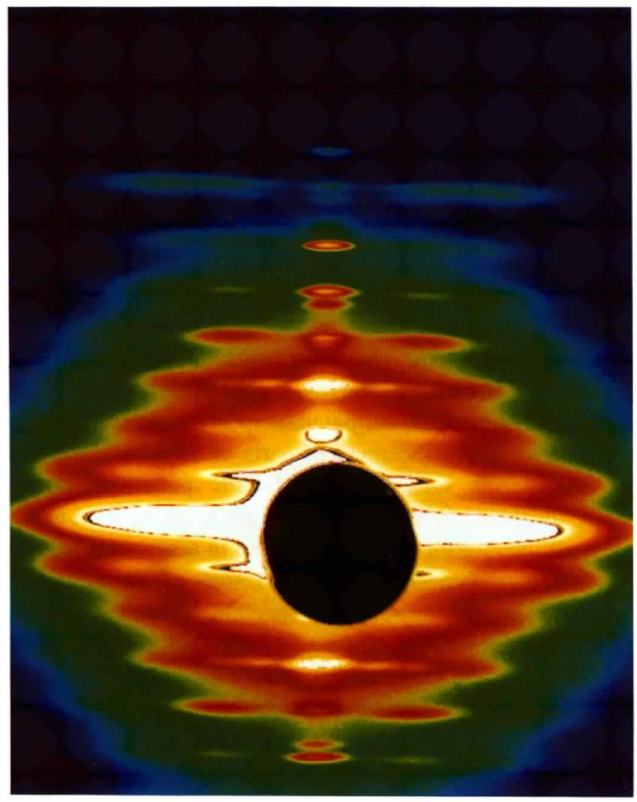

Figure 8
X-ray diagram from frog muscle. Synchrotron radiation, using beamline F1, 24-pole wiggler, at CHESS (Cornell Storage Ring). 1 s exposure (imaging plate).

contracting muscle, gave much more positive results (Huxley *et al.*, 1981, 1983). These experiments provided new and interesting technical challenges, for they required 1 ms time resolution to begin to see any changes properly, so that only 1 ms worth of data could normally be collected per time frame every contractile cycle, *i.e.* once every minute or so. Thus the only parts of the layer-line pattern we could look at closely were the stronger meridional reflections. However, the improved X-ray cameras and improved running conditions were now giving us 10^4 counts s^{-1} or more in the 14.5 nm meridional reflection, so that we could accumulate 10^3 counts or more per millisecond time frame in an experiment involving 100 cycles of contraction and quick release.

Another problem was that these experiments were being carried out on quite large whole muscles (in order to obtain sufficient counts), whereas the quick-release experiments which had originally shown the interesting tension transients when studied at high time resolution (Huxley & Simmons, 1971) were performed on single muscle fibres. However, Dr R. M. Simmons was able to design an electromechanical device powerful enough to impose these very rapid length changes on a whole muscle in an accurately controlled manner.

The experiments showed that a rapid shortening of a muscle, by ~5–10 nm per half sarcomere (*i.e.* 0.5–1% of sarcomere length), produced an almost immediate large decrease in intensity of the 14.5 nm meridional reflection, down to 20–30% of its isometric value, followed by a rapid partial recovery of intensity over the next 6 ms or so, and then a more gradual recovery back to the original value as tension redeveloped, during the next 50 ms (Fig. 7). A similar drop in intensity (but without the rapid recovery) was produced by a small rapid stretch applied to an isometrically contracting muscle. These were important results, because they showed for the first time that a structural change was taking place in the myosin crossbridges as adjacent actin and myosin filaments moved past each other, during active shortening, by 5–10 nm. It would be difficult to think of a more direct demonstration of the involvement of crossbridges in force generation and filament sliding, a concept which some workers had continued to question.

The most straightforward interpretation of the large decrease in intensity of the 14.5 nm meridional reflection is that the reflection is generated by an elongated part of the crossbridge structure, which, in an isometrically contracting muscle, is oriented predominantly perpendicular to the filament axis, leading to sharp concentrations of density at the 14.5 nm periodicity defined by the thick filament backbone. Upon a quick release, the crossbridge tilts as it goes to the end of its working stroke, generating force again if the release is not too great (and if this transient mechanical event can be resolved – possible in a single fibre but not in a whole muscle). Applying extra force, in a quick stretch, would tilt the crossbridge in the other direction. In either case the projected mass of the crossbridge becomes more extended in an axial direction, so that the sharp concentrations of density at 14.5 nm intervals become smeared out and the intensity of the 14.5 nm meridional reflection decreases. A more complicated explanation could involve axial displacement of part of the crossbridge away from the 14.5 nm repeat as it went through the working stroke. Whatever the case, crossbridge movement closely synchronized with filament sliding must be taking place.

This result, and the fact that a 1 ms time resolution could be obtained in the X-ray patterns by using synchrotron radiation, did provide an important demonstration of value of the technique, many of the details of which we reviewed at that time (Huxley & Faruqi, 1983).

The next topic that we investigated concerned the activation mechanism in striated muscle, which we thought might be based on azimuthal movements of the long tropomyosin molecules which followed the long-pitch helical grooves in the actin filaments. This could give rise to the increase in intensity rather far out on the second actin layer line (at 18 nm axially and 0.023 $Å^{-1}$ radially) which we had been able to observe on film in particularly long exposures of contracting muscle (Huxley, 1972). This was a region of the diagram which was normally too far off axis to show up in data collected at the 2–3 m specimen–detector distances that we habitually used with synchrotron cameras (in order to optimize resolution and signal-to-noise ratio). However, as soon as we displaced our beam tube in the appropriate direction, we found that we could record the reflection very readily from contracting muscles. Our work was made much easier by a new detector, designed and built in the EMBL Outstation in Hamburg by Jules Hendrix (Hendrix, Fuerst, Hartfiel & Dainton, 1982). This was similar to one developed at the MRC in Cambridge (Faruqi & Bond, 1980; Faruqi & Huxley, 1981). It was still only a one-dimensional detector, but it was a multiwire one, *i.e.* it consisted essentially of 128 single-wire detectors 1 mm apart and 15 mm in length, each with its own counter so that the total counting rate could be as high as 10^7 counts s^{-1}, and the full available intensity in most patterns could be recorded without attenuation. Also, the width of the rectangular counting area (15 mm) made it a very efficient collector of integrated layer-line data when it was placed across the layer-line pattern at the appropriate distance from the meridian.

We had by now (1983–1984) moved to the new EMBL beamline *X*33 in the HASYLAB at DESY, Hamburg, which gave us much better working conditions than the very cramped and awkward-to-access quarters of the old *X*13, and also gave us somewhat higher intensity (probably well in excess of 1×10^{11} photons s^{-1} onto the specimen under the best beam conditions), so that we now scanned our muscles (which were mounted vertically) up and down in the rectangular shape synchrotron beam (5 mm × 1 mm at the specimen) to spread out possible radiation damage over a large area of muscle. Up to 200 contractions could be recorded without any sign of such damage.

The first thing we noticed about these new reflections was how rapidly they appeared after stimulus of the muscle

(Kress, Huxley, Faruqi & Hendrix, 1986). It was fascinating to see the peaks appearing, as the data accumulated during an experimental run, in the time frames occurring *before* any tension was developed by the muscle, but at a time when we knew that the activation process must be well advanced. Moreover, we were able to show that in muscles stretched to sarcomere lengths at which no overlap was present between actin and myosin filaments, and no active tension was developed, a large part of the intensity increase on the second actin layer line still occurred. This showed that the structural change was not merely due to myosin head attachment to actin, but that a large part of it was the result of autonomous structural changes in the thin filaments, brought about by calcium release in the activation process. Clearly, the steric blocking mechanism was alive and well!

3.7. *Wider uses of synchrotron radiation*

By this time, X-ray diffraction using synchrotron radiation had become a well established technique, with dedicated storage rings being built and operated in many parts of the world. As the operation of the synchrotron sources became more reliable, and the beamlines and cameras became more user-friendly, the user community began to extend to people for whom a more powerful radiation source had at first seemed more of a convenience rather than an absolute necessity. Protein crystallographers, whose numbers of course vastly exceeded the muscle community, began to appreciate the high speed and convenience of data collection at the storage ring sources, and soon began to realise that their data were better too, since radiation-induced damage had less time to fully manifest itself. Also, it became possible to work with much smaller crystals, especially as the advantages of rapidly freezing them (before data collection) became apparent. It was ironic that the muscle experiments, which to a considerable extent had driven the technology needed for the use of synchrotron radiation, became almost a victim of their own success, for it became increasingly difficult (though not impossible) to obtain beam time because of the pressure from other users!

Further progress in the muscle work now needed two-dimensional detectors capable of high total counting rates, and even higher incident beam fluxes than we had been using at Hamburg, preferably on a beamline devoted to and optimized for fibre diffraction work. In search of such an ideal, and for other reasons, we drew down our operations at Hamburg, and began to explore possibilities elsewhere (Fig. 8). But that is another story. The EMBL Outstation at DESY, Hamburg, was what had made it all possible for us. It was a very exciting and rewarding experience.

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