been tried. Arndt [8] and Davidson [9] are developing neutron television camera type systems, while at Brookhaven position sensitive 2-dimensional charge division type counters [10,11] were developed.

Such counter systems do provide an efficient way to improve data collection, counting many reflections simultaneously. Depending on the protein size and crystal space-group, data collecting time can be reduced by a factor of twenty and more. This improved efficiency allows the use of smaller crystals which is very desirable since it is often difficult to grow large protein crystals. At the HFBR at Brookhaven, a linear position-sensitive counter system [12] is now being replaced by such a large area detector. This two-dimensional position-sensitive counter has an efficiency of 80%, a spatial resolution of 3 mm and a usable counting rate of $\sim 10^5$ n/s. A schematic of the control system for this protein diffractometer is shown in Fig. 4. The crystal is oriented on a 3-circle goniometer and slowly rotated (stepped) parallel to a principal axis. The control computer keeps track of the crystal's position and calculates the position on the 2D counter where the next reflection should occur. Before a particular reflection (Bragg position) is reached, background data is integrated for a specified angular range and is then subtracted from the intensity integrated during passage of a particular reflection through the sphere of reflection thus providing a good peak to background ratio. At any moment, different areas of the counter will be collecting different background or reflection intensities requiring sophisticated bookkeeping by the control computer. The control computer will also provide initial data processing like absorption and Lorentz factor correction and checks crystal alignment. The whole process can be monitored on the graphic terminal which is particularly useful for initial crystal alignment procedures.

Apart from position-sensitive devices, a large 100 counter diffractometer [14] has been developed for the reactor installation at the Institute Laue-Langevin. Further improvements in data collecting strategies have been obtained through improvements in beam geometries utilizing wide wavelength band width and focusing techniques like curved monochromators [14], multilayer monochromators [15] and Fourier chopper techniques [16]. For best results, the incident beam divergence is matched to the mosaic of the protein crystal and the wavelength band width ($\Delta\lambda$) is chosen for maximum flux at the necessary resolution. While further improvements are still needed, satisfactory progress has now been made to permit routine neutron protein analysis on a time scale not very different from standard X-ray protein investigations.

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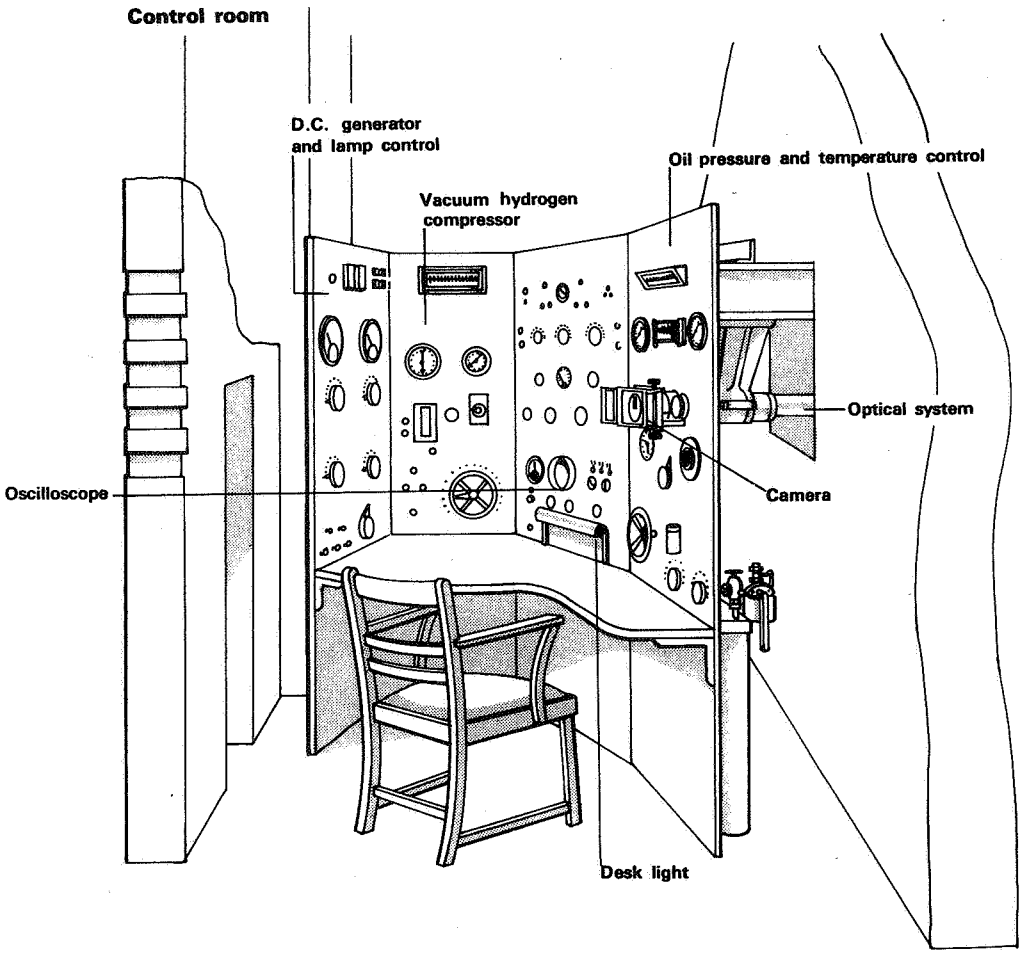
Life with a Svedberg Ultracentrifuge

A. G. Ogston

In the first of two articles on his affair with the Svedberg Ultracentrifuge A.G. Ogston describes the setting up of the machine in the Biochemistry Department at Oxford University and some of the initial problems.



A.G. Ogston FRS, formerly Reader in Biochemistry at the University of Oxford and from 1960–1970 Professor of Physical Biochemistry at the Australian National University, Canberra, is now President of Trinity College, Oxford, U.K.

'The specialist is too apt to do, not what needs doing, but what he knows how'. These words of W.T. Williams give a specific echo to what T. Kuhn says in a more general way, that we are all conditioned by what we have inherited. In 1942 I inherited the use of a Svedberg oil-turbine ultracentrifuge and much of my work in the following 17 years involved its use. In those days the ultracentrifuge was (X-ray diffraction apart) the most expensive of biochemical instruments. Ours was one of only two in the U.K., of less than a dozen in the world, and there was a consequent obligation placed upon us to make its use available to others. A nuisance? Yes, at times. Yet it brought us into touch with, and helped us to contribute to, more problems than we could have initiated ourselves. In our own researches, it was natural that we should make use of the available facility; yet I like to think that we were aware of the problem of the specialist and never undertook work merely with the object of keeping the wheels turning.

The development of the oil-turbine ultracentrifuge from its first beginnings in the 1920s, has been described by Svedberg and Pedersen [1]. By 1937, when J. St. L. Philpot decided to instal one at Oxford, for his research on pepsin, it had effectively reached its final mechanical form. However, developments in its optics were still to come. As ordered from the workshops of the Physical Chemistry Institute at Uppsala, this and the instrument simultaneously obtained by A.S. MacFarlane for the Lister Institute in London came equipped with U.V. absorption optics; but, before its delivery Lamm had devised the refractometric 'scale method'. While waiting for the optical parts needed for the scale optics, Philpot [2] devised and set up the 'diagonal schlieren' refractometric system (in one form or another, to become standard in later ultracentrifuges and in U-tube electrophoresis) finally choosing its 'edge' version from among the possible variants.

It was in this form that, when Philpot left Oxford in 1942, I found myself, with little previous experience, responsible for its use. In 1945, on his return from a distinguished career in the RAF, we were lucky to secure the services of R. Cecil as 'ultracentrifuge assistant'. Between us we ran the machine or instructed others in its use until it was finally dismantled in favour of the more compact and versatile Spinco Model 'E'.

It would be hard for anyone, accustomed only to the boxed-in Spinco, to visualise the Svedberg machine. Majestically, in the centre of a room 13′ × 15′, raised 5′ above floor level on a 30-ton concrete plinth, in a massive cast-steel casing, stood the rotor; 10 kg of machined steel, its graceful tapered shafts carrying journals running in white-metal bearings held in bronze housings and, at their extremities, the two small turbines. From a pit in the floor, which housed the 15 HP driving motor and oil compressor, rose the grey-painted column, cooling the oil destined by way of pipes and channels in the rotor casing for turbines and bearings. In recesses in the wall were the large refrigerator and vacuum pump. The upper half of the rotor casing was pierced by windows, allowing light to pass through the cell at the top of its travel, from the light source, monochromator and collimating lens on the distal side, to the schlieren system on the camera side of the rotor. To the end of its life, the diagonal edge consisted of a steel engineer's protractor, mounted on Meccano, exactly as Philpot originally set it up. The camera end of the optical system projected through an aperture in a brick partition, partly separating the rotor room from the operating area.

This area, some 10′ × 8′ contained, apart from the camera, the switch gear and control wheel (of polished steel) for the compressor motor, regulating the oil pressure and hence rotor speed; a large instrument panel with gauges showing supply voltage and power consumption, rotor speed, gas pressure and temperatures of the cooling-water, oil and rotor; and the large hydrogen cylinder and valves controlling the pressure of gas in the rotor casing. On a wall-table were the Schuster-built travelling microscope and record sheets. From near the camera, strings running over

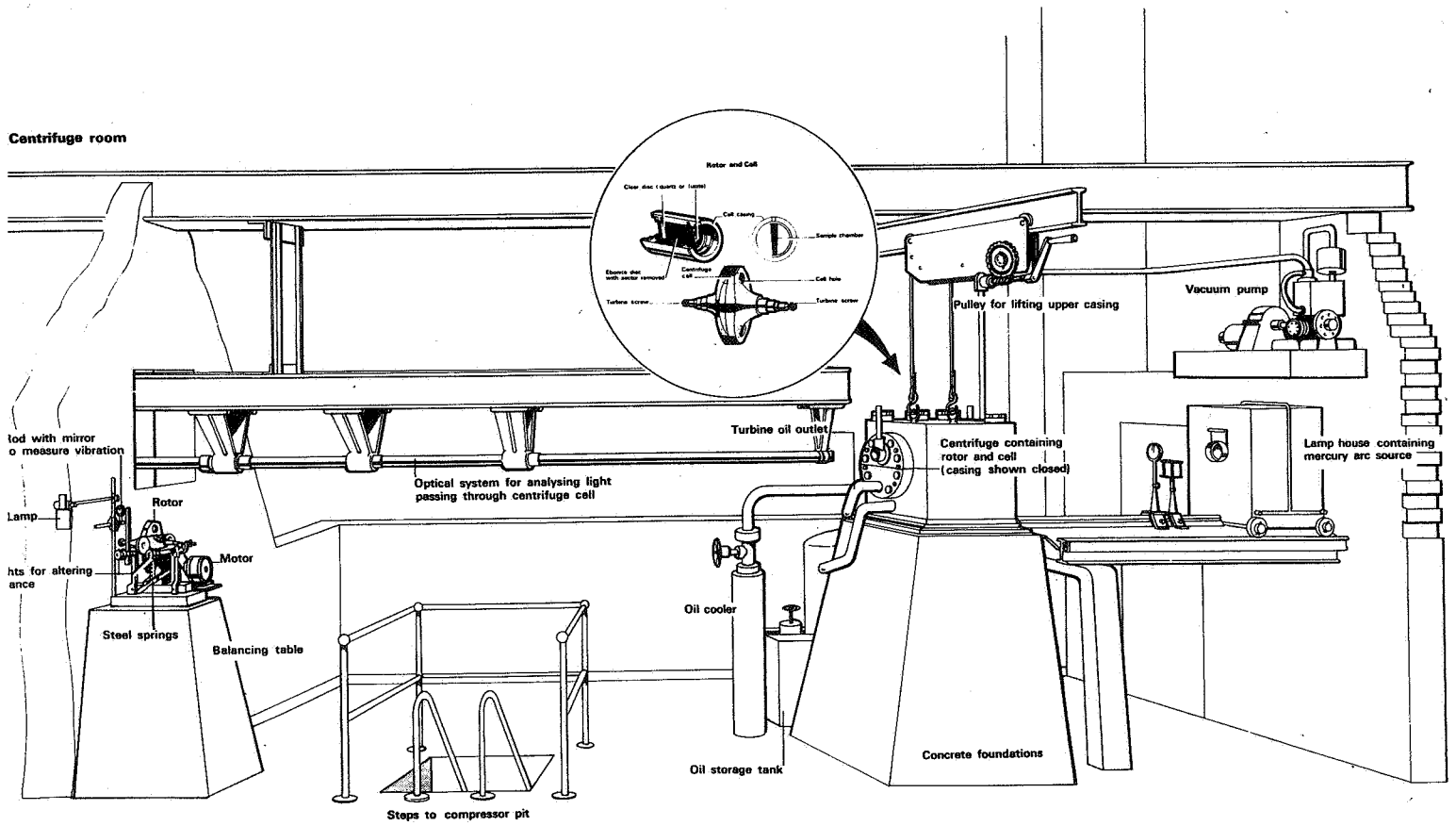


Diagram of the Svedberg ultracentrifuge originally housed in The Lister Institute for Preventive Medicine and now on exhibition at The Science Museum, London, U.K. The ultracentrifuge was housed in two rooms; the centrifuge itself, pumps, motors and the front part of the optical system occupies one, while the control panel and rear of the optical system is housed in a second. The steps in the foreground would lead to the machine pit housing the oil compressor. (Science Museum photo Crown copyright.)

vooden bobbins on Meccano axles allowed the operator to adjust the source lit and the angle of the diagonal edge. All parts except the rotor itself when running were easily accessible.

From the time of starting the refrigerator, filling the cell, adjusting the balance cell, insertion in the rotor, bolting down the rotor case, checking the oil level etc. took half an hour or so. Full speed was attained in about 25 min from starting; stopping took 20 min and cleaning up a further 30 min. We could, with comfort, do two normal runs a day.

A visitor entering during a run would first become aware of the soft, though pervasive note, about b″, curiously changing intensity as he moved his head. The rotor room would be in darkness except for light from the mercury arc, eerily illuminating the shining rotor case and the grey tanks and supply pipes. In the control room, the operator was always in attendance, following the progress of sedimentation by inspection of the schlieren pattern with a hand lens, adjusting the oil pressure and speed, periodically taking photographs and noting speeds, temperatures, hydrogen pressure etc. on the record sheet. With an inexperienced operator there might be some air of tension; otherwise there was an atmosphere of ordered calm. Yet there was always a sense of relief when silence marked the end of a run, free from the whine of the rotor, the sound of the compressor motor and refrigerator, and the tom-tom like throbbing of the vacuum pump.

I must not fall into the error of a parent (or foster parent) by assuming that the least development achievement or deviation exhibited by his child holds the same fascination for others (who have their own babies to delight or to give them trouble) as for him. But, assuming some degree of interest I begin with my child's inborn capacity.

An ultracentrifuge imposes a centrifugal force upon an initially uniform system, causing it to move towards a new state of equilibrium, and one may make use of the velocity of change or of the characteristics of the new state; the former method includes 'velocity sedimentation' and 'approach to equilibrium'; the latter, equilibrium sedimentation' and 'isopycnic sedimentation in a density gradient'. Of these, we made use only of the first. Equilibrium measurements may require periods up to several days, with careful control of temperature and centrifugal field (speed) throughout; neither control was automatic on the Svedberg machine, and would have required constant and meticulous attention of the operator over long periods.

In velocity sedimentation, steady motion of macromolecular solute particles is established under the opposing forces of centrifugal field and of viscous resistance; this is observed as steady motion of a 'boundary' formed by each set of particles present, starting from the meniscus and moving at a rate equal to that of those particles in the region of uniform composition (the 'plateau') ahead of the boundary. From the velocity of a given boundary, with use of accessory measurements (separately made), such as effective density of the particles, diffusion coefficient and viscosity increment, one may obtain information about the mass and hydrodynamic properties of the particles. From the size of the boundary (however observed) one may estimate the concentration of the material that forms it. Types of particle may be recognised from their characteristic rates of sedimentation, and their relative amounts in a mixture (boundary anomalies apart) estimated from the sizes of boundaries formed.

Because of thermal diffusion, a boundary does not remain sharp but ideally has Gaussian form, broadening with time. Measurement of the change of boundary form with time allows interfering factors such as polydispersity of solute or 'self-sharpening' to be estimated. To achieve accuracy in these measurements, we need to have an optical system free from distortion and to know its magnifications accurately; we need accurate measurement of speed and (since it affects the density and viscosity of the solvent) of the temperature of the cell contents. It was, initially, because of doubts about the last of these that Rupert Cecil and I undertook, from 1947 onwards, an investigation of errors [3].

A number of measurements of the sedimentation coefficients of well-defined proteins made on different Svedberg ultracentrifuges had revealed discrepancies far beyond the accepted margin of error. These proved to arise mainly from errors in measurement of the cell temperature. The measuring device was a thermocouple, fixed in one bearing housing with a very small clearance from the rotor surface. This was assumed with support from early measurements in Uppsala to be in radiative equilibrium with the rotor, thence with the cell. In fact this thermocouple measures the temperature of a point in a complex distribution of temperature resulting from generation of heat by friction in the bearings and turbines and with the surrounding hydrogen and its removal by the refrigerator-cooled supply oil. Filling the cell with diphenyl ether, whose melting-point lies near the normal cell temperature, we were able to show that the thermocouple temperature differs by a constant amount up to 4–5°C from that of the cell, provided that a careful routine of speeding-up is followed and provided also that the rotor is left undisturbed on its bearings. Any shift of rotor relative to thermocouple can change the calibration by several degrees. Measurements with this correction agreed excellently with early measurements on a Spinco Mode E, once allowance was made for the effect of adiabatic rotor stretching on the latter.

The shaft of the rotor, magnetised, generated a synchronous A.C. in neighbouring coils, which was amplified and originally fed a vibrating-reed meter responding at intervals of 10 Hz. The operator's aim was to keep variation of speed to within 10 Hz/10 min, the mean value being taken as applying to that interval. Since the centrifugal field is proportional to the square of the angular velocity, the meter allowed more error than we liked. After experimenting with a very sensitive (0.1 Hz) but somewhat temperamental frequency bridge, we finally adopted a 100:1 sonic motor, driven by the amplified A.C., working through a 4:1 reduction gear, one of a pair of relays and 'Post Office' counters, automatically averaging the speed over the chosen intervals.

By replacing the original tungsten light-source with a 500 W mercury arc, we reduced exposure time to 5–10 sec, thus minimising the effect of boundary movement during exposure. Also to reduce reflexions, we replaced the plane quartz windows in the rotor casing, and the single U.V. combination schlieren lens mounted outside it, with a glass pair of collimator and schlieren lenses. We asked several optical firms to make recommendations for these, giving them details of the whole optics. Howard Grubb Parsons suggested, very promptly, that a pair of uncorrected spherical lenses would do, quoting £3 for the pair, complete with mountings. We bought them and they performed perfectly. Those were days!

With these modifications, we could estimate sedimentation coefficients correct to 0.5% and the refractive increments of boundaries to 1%. The only later improvement was the introduction by R.L. Baldwin of a black glass fibre in place of the schlieren edge which further improved the measurement of boundary form. This fibre was attached parallel to the edge of the steel protractor – with Plasticine of coure.

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Life with a Svedberg Ultracentrifuge

A. G. Ogston

In his final article on the Svedberg Ultracentrifuge A.G. Ogston looks back on some of the problems and accidents that beset the machine and its operators and also talks about life in Oxford's Biochemistry Department during the machine's heyday.

My life with the Svedberg was not without its problems and accidents. In this magnificently simple and robust machine, the cells were the one relatively weak point. Unlike Spinco cells, they were screwed up from both ends. This, with the exacting requirement for balancing imposed by the solid rotor bearings, meant that a cell could not be disassembled and reassembled without a day at the Lister Institute in London, re-balancing the cell in the shared balancing machine. So we tightened the cells, with their hand-cut washers, as much as we dared and hoped that breakage of a quartz plate or leakage would not put us back to square one. It is fair to add that an assembled cell might well last several years and many runs.

Of course this meant that cells could be cleaned only by thorough rinsing with water, then acetone and air-drying. And hereby hangs the tale of the Gremlin.

Solute material would appear at the bottom of the cell and diffuse upwards, sometimes interfering with precise estimation of a descending boundary; clearly an evil spirit that had followed Rupert Cecil from the RAF. I am ashamed, now, to think how long it took us to exorcise it; we first blamed the PVC washers and changed to polythene, to no avail. The effect was, of course, due to buffer salts from the previous run, imperfectly washed out, trapped in interstices between quartz plates and the cell body, concentrated by drying and convecting in concentrated solution to the bottom of the cell in the next run. So if we needed a really clean cell, we ran it briefly up to speed filled with carbon tetrachloride at the end of a run before washing and drying. Exit Gremlin.

But this experience enabled us, shortly afterwards, to intervene in a discussion of why a solution of sodium chloride appeared (in the Stern air-driven ultracentrifuge) to produce a rapidly-descending boundary. This was, of course, the reverse effect; convection of trapped water to the top of the cell. It disposed of extravagant ideas of large complexes formed by sodium chloride!

A normal cell could break a plate and lose its contents at full speed without producing serious imbalance. Not so the separation cell, which held 3 ml. It was my fault for not realising that its Perspex body was rated to only 900 rev/min. At near 1000 rev/min the rotor's normal whine suddenly gave place to a harsh agonised scream. I did all I could – cut turbine oil pressure, raised bearing oil pressure, raised hydrogen pressure – to stop the rotor as quickly as possible; but for 15 min the shriek continued, falling in pitch but rising and falling in intensity. I was terrified, even though I knew that the casing would contain even a burst rotor. I allowed no anxious enquirer into the room and crouched behind the brick partition till, at last, blessed silence came.

When we opened the casing, no ruin met our eyes. The white metal bearings had worn and had to be sent to Uppsala for re-lining; the rotor journals and end-bearings needed repolishing; but a journey with the rotor to the Lister Institute showed that it was undistorted. Within a few weeks we were back in business. Of course we had to re-calibrate the thermocouple.

One morning I was summoned early to the laboratory where a horrid sight met my eyes. The machinery pit was full of water to the floor level. I saw only a black, oily surface. During the night water had leaked from a pipe and found its own level. The Museum Fire Brigade was summoned and pumped out the pit, with great enthusiasm. Then the electrician. All winding insulations in the motor were down to zero, but he had hopes. We borrowed electric fires from the Professor's – Rudolf Peters' (now Sir Rudolf Peters) – office and trained them on the motor; gradually, as it dried, the insulation came back. Our (and the Professor's) only fear was that we would freeze to death (it was cold weather) before the motor recovered. In the end, we had to replace only one rusted ball-race.

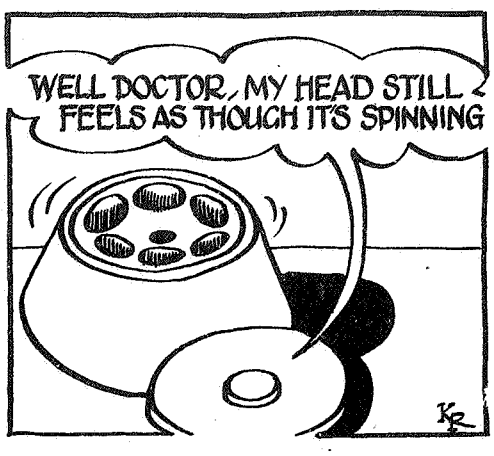
I think it must have been following this cell-burst that, before replacing the rotor, we wanted to check the oil-supply. So Cecil watched over the open rotor casing while I cautiously started the compressor. Instantly, two magnificent jets of thin driving oil converged from the ducts over Cecil's head and shoulders.

For some days we had been puzzled by an unpleasant and increasing smell. We enquired about the drains, and what unpleasant chemicals others might be using. It proved to come from the remains of a large white rat, lost from the animal house, trapped in the pit. When I told the Professor, he said, 'I wonder what Svedberg would say, Ogston!'

Visitors could be something of a menace during runs, distracting the operator and, at critical moments, getting their heads into the light-path. I think we had three during Rupert Cecil's first 'solo' run. When I congratulated him on his unruffled calm, he said: 'Well, it costs much less than a Lancaster!' Our motto at that time came from a cartoon of an ex-RAF type in a signal box saying down the phone: 'There's going to be a wizard prang your end, old boy. I've just pulled the wrong lever'.

Great strength was sometimes needed when dealing with machine and I once very nearly persuaded Cecil to be careful moving an empty hydrogen cylinder because it would be so much heavier than a full one.

We were, of course, a fluctuating group: Rupert Cecil and I were the permanents (and he developed his own lines of research and later had his own research students); others came and went as research students or visitors. Other 'residents' who made use of the ultracentrifuge were: J.P. Johnston, J.E. Stanier (how surprised Karl Meyer was to find her an attractive girl), J.H. Fessler, R.L. Baldwin, O. Smithies, E.F. Woods, J.M.A. Tilley, C.C. Curtain, J.R. Hall, M.P. Tombs, B.S. Blumberg (so far my only Nobel Prize-winning research student), B.I. Aldrich, T.F. Sherman and C.F. Phelps. The Australians amongst them were part of the attraction that took me to Canberra in 1960. We larded our research with lightheartedness. Doggerel verse was written about characters and

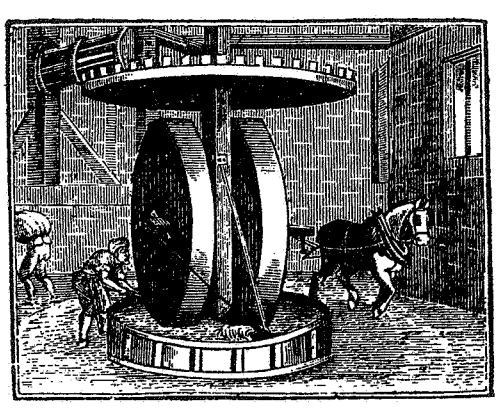


incidents; poetry was read at morning coffee; flute and recorder music was played at the end of the day. R. L. Baldwin, fresh from a demanding M.Sc. course at the University of Wisconsin, thought us culpably non-serious; but he was very soon converted to poetry-reading and weekend brass-rubbing instead of work. We liked to believe that we gave ourselves time to think.

We designed and made, or helped to make (those *were* the days), our own accessory apparatus: a Gouy diffusiometer [1]; a Couette viscometer and birefringence apparatus [2]; an elastoviscometer [3]; a refractometer [4]; and an Indeculator (rotating dialyser) [5]. Our own researches were centred on proteins (particularly the β lactoglobulins) and other macromolecules (after all, we had the Ultracentrifuge). We developed new techniques such as the measurement of sedimentation rate by meniscus-depletion (on insulin fractions, with H.G. Gutfreund and R.L. Baldwin [6]) and (R.L. Baldwin [7]) refined methods of measuring heterogeneity from boundary form. We applied knowledge gained from study of synthetic polymers (with J.F. Fessler [8]) and dextrans [9] (with E.F. Woods and J.R. Hall) to throwing light upon the molecular form and solution properties of hyaluronic acid [10] (with J.E. Stanier, the late J.P. Johnston, J.H. Fessler, C.C. Curtain, B.S. Blumberg, B.I. Aldrich, T.F. Sherman and C.F. Phelps). I must make special mention of the first work that J.P. Johnston and I undertook after the War, which led to explaining the apparent interconversion of proteins in the ultracentrifuge (observed by MacFarlane and by Pedersen) as a boundary anomaly – the 'Johnston-Ogston effect' [11].

And, beyond our own researches, we were able to make contributions, through use of the Ultracentrifuge, to the work of others, including (in alphabetical order) R. Aschaffenberg, D. Bell, B. Bernier, R.K. Callow, M. Dixon, E.O. Field, G.G. Freeman, G.P. Gladstone, H. Gutfreund, D. Herbert, W.E. van Heyningen, D.O. Jordan, D.A. Lowther, R. Markham, V. Massey, A. Neuberger, R.A. Peters, N.W. Pirie, M.R. Pollock, H.J. Rogers, K.M. Smith and T.S. Worth. We were apt to publish our contributions as short addenda to main papers; this allowed us to make what deductions we felt followed strictly from the sedimentation measurements, leaving more imaginative interpretation to others.

An engraving of one of the early horse driven centrifuges.



Of these very various projects, we became particularly involved with three:

(i) E.O. Field and I [12] together made an early attempt (before G.A. Gilbert's penetrating work on reaction boundaries and before the ready availability of computers) to analyse the sedimentation boundaries of haemoglobin under conditions where dissociation was occurring, making allowance for diffusion. This was the beginning of my later interest in complex migration boundaries.

(ii) Because of our experience (through N.W. Pirie) with plant viruses, R. Markham asked us (in 1947) to examine a preparation of Turnip Yellow virus [13]. Although this was apparently homogeneous by electrophoresis and electron microscopy, two distinct components were seen in sedimentation. Partial separation (with the Separation cell) of these, and rerunning, showed that this was not due to an aggregation-disaggregation reaction. Further examination showed the lighter fraction to be pure protein 'coat' lacking a nucleic acid core. This appears to have been the first report of deficient virus particles. The observation was of interest also in showing that electrophoresis may be an incomplete criterion of homogeneity, and that in this case the nucleic acid and its counterions must lie entirely within the shear-boundary of the particles.

(iii) A. Neuburger asked us to examine the newly-isolated 'neutral salt-soluble collagen'. J.H. Fessler undertook this and it proved the beginning of his major research interest. It showed up also a deficiency of the Svedberg machine (later affecting Tilley's work on β lactoglobulin) that, because of the large heat and power (some 7 kW at full speed) dissipation, the cell temperature could not be held lower than 25°C. Our first runs looked quite satisfactory, giving a plausible concentration dependence of sedimentation rate; but, in the viscometer at 25°C, solutions became clouded with aggregated collagen. On more careful examination of the ultracentrifuge plates, we saw that a progressive diminution of boundary area had occurred, due to formation of fibres and their rapid, unnoticed, sedimentation to the bottom of the cell. Consequently, our concentration-dependence was spurious, and Fessler had to obtain use of a Spinco to make measurements at lower temperature.

On my first return from Australia, in 1962, it had gone. 'The sun still warms its native ground, The Dodo is not there' [14]. There I enjoyed, though never quite so wholeheartedly, living with a Spinco: perhaps I should say 'sleeping with' for, in spite of its automation, we still insisted that an operator should be on hand during all runs, even if these extended over several days; these meant hard nights, wakened hourly, in a sleeping bag. The Spinco's flexibility allowed us to do things that could not be done with the Svedberg. Yet I regretted the latter's massive accessibility and the indestructibility of its steel rotor – no worry there about scratches or about metal fatigue.

Would 'love' be too strong a word? Like a child amongst adults the Svedberg ultracentrifuge really helped to bind together our small community. Perhaps not quite like a child. I remember finishing a run at 4 p.m. on a summer vacation afternoon and thinking 'Two hours working-time yet: what shall I do?' There were several non-urgent things I could have done. And then I thought, 'Why don't I go home?' The answer was: 'Because it's less trouble to stay here than to go home and play with the kids'. So I went home.

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