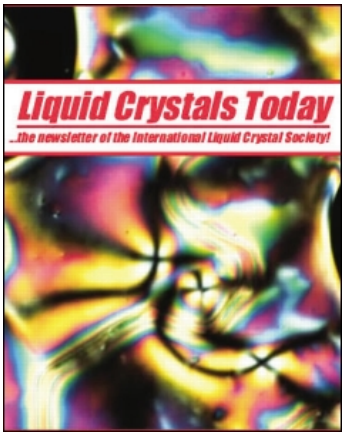

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A personal history of the early days of chromonics

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A personal history of the early days of chromonics

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''I have gathered a posie of other men's flowers and nothing but the thread that binds them is my own'' (Montaigne).

Keywords: Chromonic mesophases, lyotropic liquid crystals

1. Preamble

I stress that this is a personal account (i.e., probably selective, biased and unreliable) of my involvement in the early history of chromonic phases – how they came to be defined and identified. I shouldn't use the word 'discovered' because almost all of the separate pieces of the jigsaw had already been recognised and described (some almost a century earlier). They were scattered over half a dozen separate fields, ranging from drugs to dyes and nucleic acids, and it was simply a matter of putting the pieces together to see the overall picture. It was rather like Victorian explorers mapping Africa, entering ancient cities and surprising the people who lived there, by telling them that, up to that moment, they had been officially unknown.

2. The beginning

My story starts at 8:30 a.m. one morning in 1979. At that time, I was a young lecturer in the Astbury Department of Biophysics in the University of Leeds. I had just arrived at work and looked in my pigeon hole in the foyer. It was usually empty. In those days when everything had to be handwritten first and then typed up by a secretary, no one received much mail and I got very little. This particular morning there was one envelope. It was postmarked ''London''. Inside was a glossy leaflet advertising a four-day, hands-on course on the optical microscopy of liquid crystals to be held at the McCrone Research Institute in about a month's time. The name McCrone was vaguely familiar – then I remembered where I had seen it before. The 'quality' Sunday newspapers had featured his work on the ''Vinland Map and the Tartar Relation''. The Vinland map (figure 1) purported to show that the Vikings had reached mainland America in the 16th century, but he concluded that at least parts of the document were relatively recent forgeries (1). The map was discovered bound together with the mis-named *Tartar Relation* – this is an account of the epic journey in 1245 of Giovanni da Pian del Carpine to visit the Great Kahn (a Mongol emperor who was not at all pleased to find that he had been confused with a Tartar). Walter McCrone was later to become even more widely known for his work on the Turin shroud. If you look on bookshop shelves next to the Da Vinci Code and other Dan Brown books, you will probably find a paperback about the Shroud; in the index of this, there will be a number of references to Walter's forensic examination.

The principal lecturer on this course was listed as Dr N. H. Hartshorne. I knew Norman Hartshorne from my undergraduate days when I was a chemistry student at Leeds and he was a Reader in the department. I calculated that he must have been at least 69 by that time. Four years earlier, in his last few months before retirement, he had supervised my final year research project on chemical microscopy. He was the epitome of a gentleman scientist: tall, distinguished, courteous, gentle but tough, in the way that made his First World War military medal appear unsurprising. I felt privileged to have had 1:1 tuition from him. After his retirement from the university, he was still very active and, for many years, he continued to run Royal Microscope Society summer schools on optical microscopy together with Alan Stuart, the co-author of his definitive book, *Crystals and the Polarising Microscope* (2).

At that time, I was trying to dig myself into liquid crystal work. Like many others of that generation, my inspiration had been Jim Fergason's article in the August 1964 Scientific American. It caught my imagination in a way that no other paper had. I felt it hinted at far more than it actually said. To me it implied widespread roles for liquid crystals in the structuring of biological systems. To the growing irritation of my colleagues, I began to see the involvement of liquid crystal phases everywhere in biology. The two major topics of research in the

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Figure 1. The Vinland Map. This is a mediaeval-style map which surfaced in the 1950s. It purports to be a 15th century *mappa mundi* redrawn from a 13th century original. It found bound with a previously unknown but apparently genuine tract dating from 1440, called the 'Tartar relation'. The feature of interest in the map is the large Island on the left, labelled *Vunlanda Insula*, with a brief comment that it had been discovered by Bjarni and Leif (Eiriksson) and their companions – apparently proving that western Europeans were aware of at least part of the North American continent 60 years before Columbus. Walter McCrone became well-known for his forensic examination of this map using optical microscopy. He found crystals of the anatase form of titanium dioxide in the ink, which he argued could only have come from a modern source – and considered the map to be a forgery. However, subsequent investigators have found TiO_2 in this form in contemporary documents judged to be genuine – and have argued that it could have been produced by mediaeval ink manufacturing processes. After intensive investigation, the authenticity of the map is still an open question (1).

department at that time were the structures of plant cell walls (Prof. R. D. Preston) and insect cuticle (Dr K. Ruddall). It seemed to me that, in both cases, the architecture that my colleagues were seeing in the electron microscope could be explained if the components had been aligned in a cholesteric liquid crystalline phase. After all these years, it now seems to be generally accepted that the helicoidal structure of insect cuticle (which gives rise to the beautiful iridescent colours and metallic sheens) originates in the helicoidal structure of a cholesteric phase of the chitin/protein composite. However, the mechanism for the alignment of cellulose microfibrils in plant cell walls is still an open question. The literature is full of references to the possible roles of microtubules, but I have not seen the words 'liquid crystal' used in this context.

3. Walter McCrone and Norman Hartshorne

Somehow I had to get a place on the McCrone course. But it was extremely expensive (more than a month's salary at that time, as I recall) and my head of department, who (with good reason) considered me to be an ineffectual Maverick, would not countenance paying for me. I wrote back saying that I was desperate to come, but I could not raise the funds. Was there any way I could attend? I pointed out that I had done a course with Dr Hartshorne and he might remember me from his time at Leeds. Would it be possibly for me to come if I promised to be inconspicuous? I would bring my own sandwiches, not drink the coffee or eat the biscuits, and sit unobtrusively at the back – or, alternatively, I might possibly be able to help demonstrating in the laboratory sessions, since I knew how to set up a microscope with Köhler illumination and I could explain how polarisation colours arose, and I knew a little about liquid crystals and could explain the geometry of nematic disclination lines and smectic focal conic structures. (Actually I wasn't confident about any of these things, but I knew that they were covered in Hartshorne's book and I was keeping my fingers crossed that I would be able to master them in time, if the need arose.)

The next day as I walked into the department, the secretary called out to me, ''John, there's a telegram for you in the office''. This was the only telegram I have ever received. Before then, the only telegrams I had seen were those folded slips of paper kept in the sideboard drawers of aged female relatives next to faded photographs of young men in First War army uniform. They all started with the words, ''The War Office regrets to inform you that…''. This one, however, said ''Hartshorne ill – come and give course''.

I went and gave the course as best I could. Hartshorne was not well, but he invited me to stay with him for a day beforehand to talk things over. With typical old-world courtesy, he met me at the railway station with sandwiches and a flask of tea, and then we drove to 'Ivy Cottage' his beautiful half-timbered house in Sussex for a day beforehand. He came with me to London the next day and, ironically, it was me at the front and him sitting at the back, keeping quiet. He was far too much of a gentleman to correct my mistakes publicly, but the occasional intent look, raised eyebrow and puzzled frown said it all. I was relieved when the course was over and I hoped that I had successfully bluffed my way through it as far as the rest of the audience were concerned.

The book that was given out at the course was a hardback volume, bound in orange cloth, written by Hartshorne and published by the McCrone Institute (3). In retrospect, I consider this virtually unknown book to be one of the most seminal books on liquid crystals ever written. The early chapters are nothing unusual – an introduction to polarisation microscopy, followed by the standard descriptions of nematic, smectic and cholesteric textures. All of the familiar drawings of Dupin cyclides and polygonal textures are reproduced there; but it is the last chapter that contained the hints of things to come. Under the heading '*Some unusual mesophases*', Hartshorne described the optical study of two novel and puzzling mesogenic systems. The first of these was the anti-asthmatic drug, disodium cromoglycate and the other was di*iso*butylsilane diol. Although neither term had been coined at that time, the first was a '*chromonic*' system and the second was a '*discotic*' system. Note that this was six years before Chandrasekhar's classic work on hexa-substituted benzene discotics. The phase diagram and characteristic optical textures of discotic phases were all there (but that is a story for another day – here we are concerned with the drug mesophases).

4. Roger Altounyan and Intal

The distinctive Armenian name, Altounyan, may be familiar to those interested in children's literature (4). The young Roger Altounyan was one of the children who were the inspiration for Arthur Ransom's classic *Swallows and Amazons* (figures 2 and 3). This story, set in the inter-war years, describes a family of children and their friends on holiday in the Lake District (not far from my home town of Barrow-in-Furness). The children are allowed complete freedom to explore and sail on the lake (a fictional combination of Lake Windermere and Coniston Water), the premise being that, if they are sensible enough, they will survive (and if they aren't…). In later years, Roger worked at Bengers research labs (part of the Fisons group), and he suffered from asthma. His goal in life was to find a cure, and he was single-minded. One of the problems with studying asthma was that no experimental animals could be found that suffer from this condition and that can be used to test the effectiveness of possible treatments. There are no convenient asthmatic mice or rats, guinea pigs or even lower primates. Altounyan therefore experimented on himself and a team of volunteers. Every week, a grimly committed group would arrive at his laboratory and induce asthmatic attacks by sniffing a witches' brew of rodent hairs and other irritants. When the gasping started, they would work their way

Figure 2. The young Roger Altounyan, as painted by his mother. He was the model for Roger in Arthur Ransome's classic children's story, *Swallows and Amazons*.

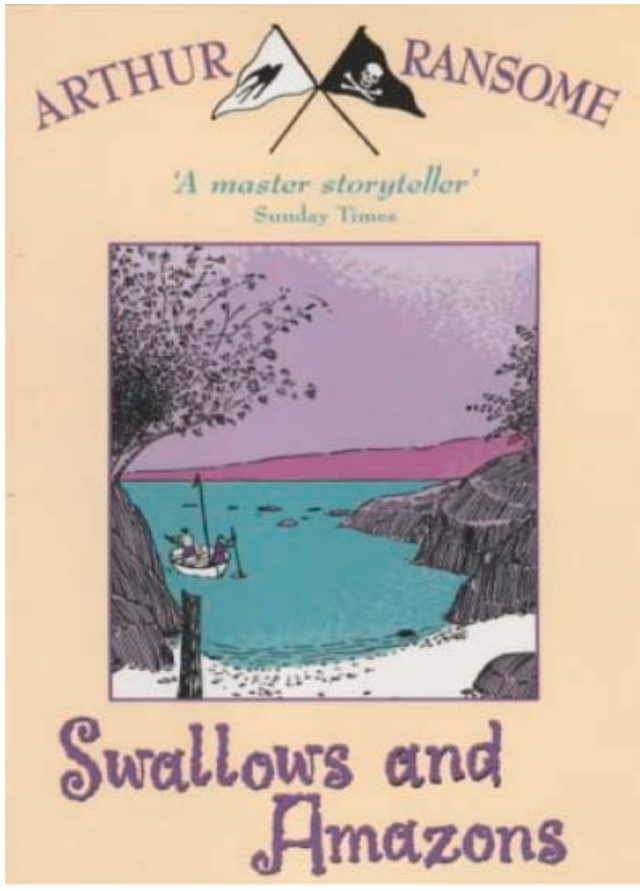
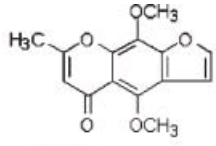


Figure 3. Swallows and Amazons.

through a row of compounds in the hope of finding something effective.

As a starting point, Altounyan studied an old Middle Eastern folk remedy for renal colic called khellin (figure 4). He hoped to find an analogue with lower toxicity and better efficacy. Month after month went by and none of the dozens of khellin derivatives examined looked promising – and, not surprisingly, the management of Fisons began to grow increasingly alarmed when they found out the full scope of the work. There was a real possibility that one of the volunteers might die during the lab tests. It would be prudent to put a stop to Altounyan's reckless activities. The protests that they were genuine volunteers[*] who were well aware of the risks they were taking, were overridden by the warnings of legal advisors that the disclaimers they had signed might not be sufficient to prevent distraught relatives from suing should any deaths occur. In any case, it would be appallingly bad publicity. Altounyan protested (and was sacked).

Altounyan's colleagues and his loyal guinea pigs were not deterred.[†] They simply waited until the management had gone home in the evening and then sneaked into the lab by the back door and carried on. Luckily, no one died. Such persistence deserved success and, in 1967, after nearly 100 disappointing failures, a successful derivative was found (5). The world was immediately turned upside down. Altounyan was reinstated. Within a year, the new drug (FPL 670) a bis-chromone derivative, was in production (figure 5). It was given the trade name '*Intal*' (from 'interfere with allergy'). It was also known as *Chromolyn* in the US. It made a fortune for Fisons and is still marketed in ton quantities, world-wide.

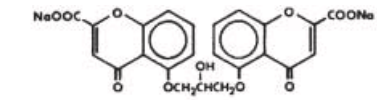


Khellin

Figure 4. Khellin and Ammia *visnaga*. The first effective commercial anti-asthmatic drug, disodium cromoglycate (marketed in the UK as *Intal* and in the US as *Chromolyn*), was developed from an ancient middle-eastern folk remedy for renal colic called 'khellin'. This is the Moorish name for the Egyptian plant, Ammia *visnaga* (popularly known as the toothpick weed because of its long sharp, stipules).

[*] Unlike the United States and many other countries, Britain has never recruited volunteers from prisons for medical testing in exchange for the tacit promise of shorter sentences – but it has used military conscripts as 'volunteers' (on occasions, without explaining the full level of danger involved – and sometimes with disastrous results).

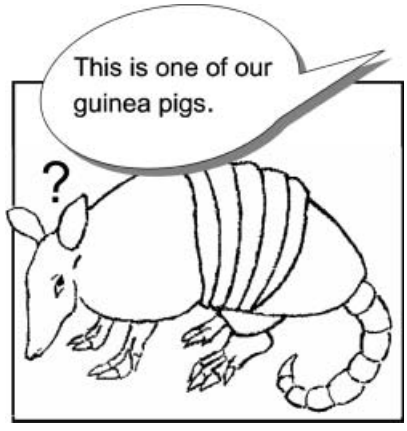
[†] The selection of experimental animals for medical studies is sometimes bizarre. The reason why the word 'guinea pig' has become generic for any experimental animal, including humans, is interesting. They were the most convenient animals to study scurvy. And they were widely used in the early years in studies of vitamin C deficiency. Some decades later, when attention was focussed on leprosy a search was made for a suitable experimental animal. Mice and guinea pigs are not vulnerable to leprosy and the most convenient research animals for this condition were found to be armadillos. If the timing had been slightly different, we would now presumably call human volunteers 'armadillos' instead of 'guinea pigs'.



Disodium cromoglycate

trade names: *Intal* & *Chromolyn*

Figure 5. Intal and spinhaler. Altounyan had a distinguished wartime career in the RAF and his knowledge of aerodynamics proved useful when designing the spinhaler for delivering a dispersion of Intal to asthma sufferers.



5. Harthorne's models for the N and M phases

It was discovered that the Intal/water system formed two strange lyotropic mesophases, and it was quite reasonable to suspect that the unique mesogenic property might be in some way responsible for the unique anti-asthmatic activity.[‡] Fisons therefore commissioned the McCrone institute to examine it, and they brought in Norman Hartshorne as a consultant.

The phase diagram of the Intal/water system as deduced by Hartshorne and Woodard has the multi-peritectic form shown in figure 6. There are two mesophases (6). That formed at lower concentration shows optical textures of the type that were then regarded as characteristic of thermotropic nematic phases (figure 7a). Hartshorne naturally argued that there must be some form of nematic ordering and, accordingly, he labelled this the N phase. The second mesophase, formed at higher concentrations, showed a variety of paramorphotic textures depending on the past history of the sample (figures 7b and 7c). Hartshorne attached particular significance to the herringbone texture that is normally characteristic of the amphiphile middle (hexagonal) phase when it is produced by concentrating a more dilute sample. Accordingly, he called this the M phase.

Walter McCrone and his co-workers turned to X-ray diffraction to obtain some information about the molecular ordering in the two mesophases. Jim Nelson was their X-ray person. He obtained powder type patterns (i.e., from samples with randomly aligned domains) from both mesophases. The N phase pattern contained only two features, a diffuse inner reflection in the 30 Å range and a moderately sharp outer reflection in the 3.4 Å region. The M

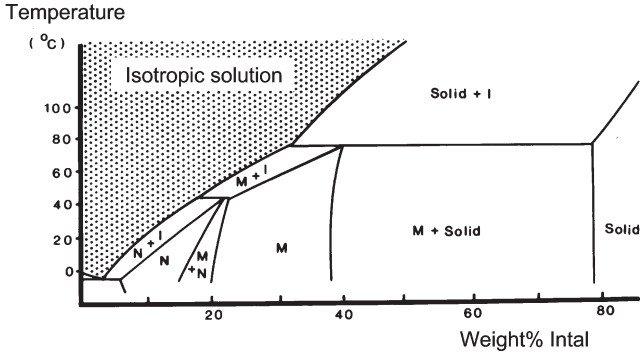


Figure 6. The phase diagram of the Intal/water system as determined by Hartshorne and Woodard. Note the multi-peritectic form of the phase diagram – in contrast to the multi-eutectic form characteristic of conventional amphiphile/water systems.

[‡] We now know, of course, that its mesogenic properties were far from unique, and that many similar compounds have anti-asthmatic and anti-allergic properties.

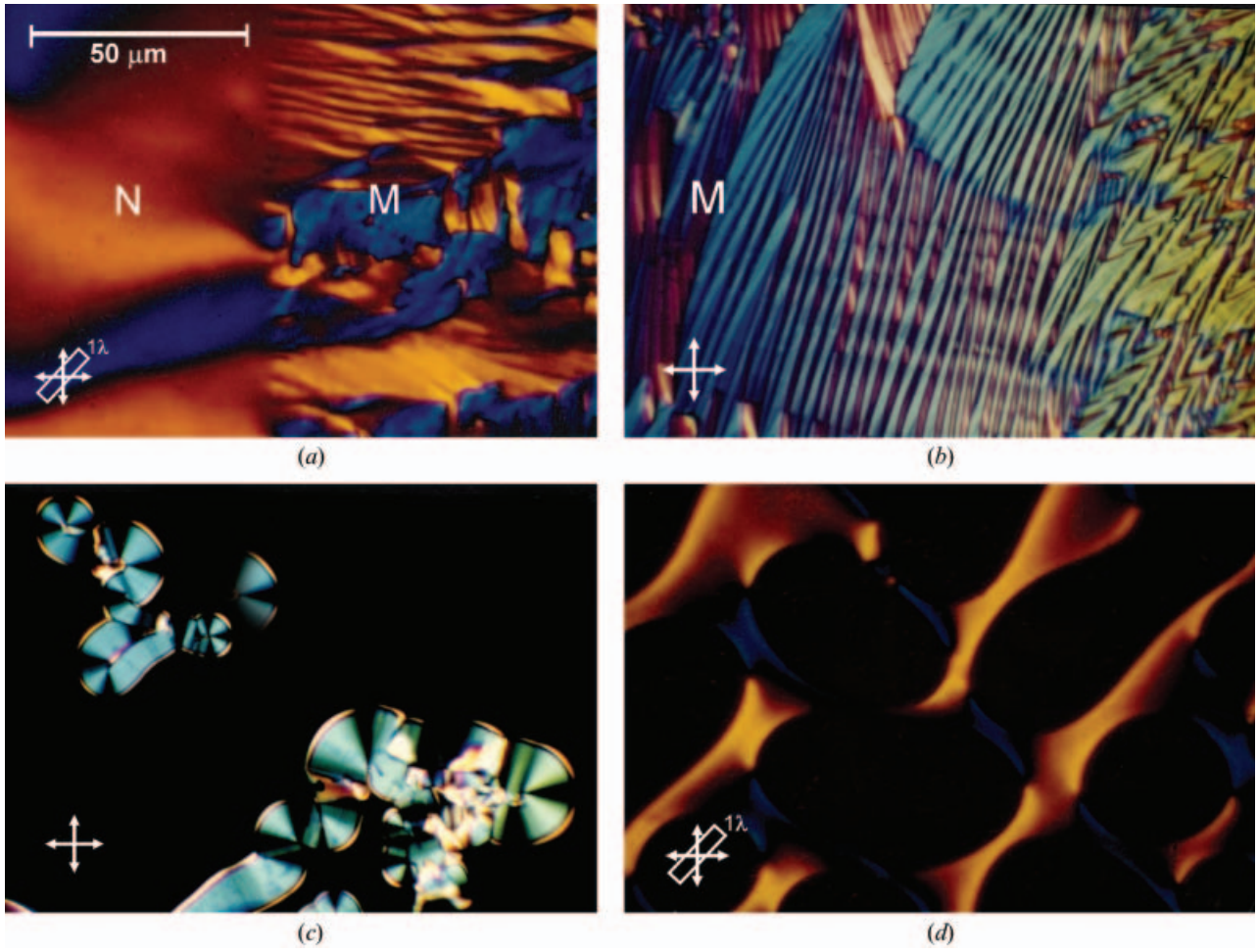


Figure 7. Optical textures of the Intal/water system. Both mesophases are optically negative – indicating a parallel, or approximately parallel arrangement of the molecular planes perpendicular to the director. (a) The N/M boundary* (with the concentration of Intal increasing from left to right). With crossed polars and a 1 λ plate. The schlieren texture of the N–phase is on the left and a herringbone texture of the M phase is beginning to develop, on the right. Note the way in which some features of the texture are extended across the well-defined boundary (- presumably indicating the continuity of some features of the structure from the N- to the M-phase). (b) A well-developed herringbone texture of the M phase when grown by allowing the N phase to become more concentrated by evaporation. Crossed polars. (c) Birefringent M ribbons, which are formed by cooling the concentrated isotropic phase below the clearing temperature. Crossed polars. (d) The reticulated texture formed at the N – I transition on heating (but not cooling). Crossed polars with a1λ plate. As far as I am aware, a reticulated texture of this kind is a unique characteristic of chromonic systems. When originally described by Hartshorne, this pattern of optical textures was thought to be unique to the Intal/water system. It is now regarded as characteristic of chromonic systems in general.

phase pattern was rather more detailed. It showed the same outer reflection, together with two sharp inner reflections at about 40 Å and 30 Å. He noted that these were more or less in the 1:1/$\sqrt{3}$ ratio, characteristic of a hexagonal lattice. This appeared to confirm that there was some structural similarity with the conventional amphiphile hexagonal phase, and implied that the mesophase consisted of cylindrical units in a water continuum. However, I could not see any way in which Intal molecules could form circular cylinders of the size required by the M-phase diffraction pattern. In 1970, I went to London to see Jim to ask if he had had any further thoughts about the M phase structures but, by this time, he had moved on to other topics and could not add anything to what had been published (figure 8).

I was puzzled as to how molecules that looked so unlike those of conventional amphiphiles could possibly form cylindrical micelles of the required dimensions. Surely micelles could only be formed from polar molecules with distinct hydrophilic properties at one end and hydrophobic properties at the other? The whole rationale for micelles is that they are structures that keep hydrophobic parts of molecules out of contact with water. Furthermore, the molecules (or at least parts of the molecules) must

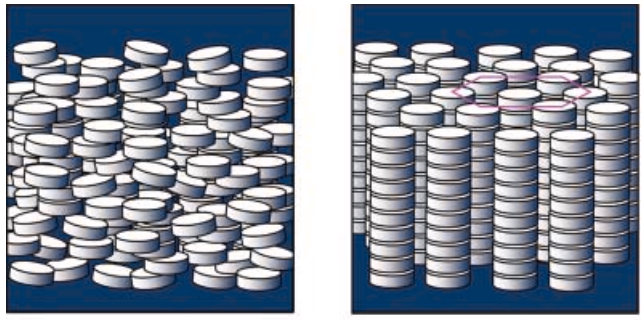


Figure 8. Models for the N and M phases of Intal proposed by Hartshorne and Woodard. Each molecule, together with bound water molecules, is pictured as being an effectively-circular disc. In the M phase these discs aggregate in cylindrical stacks which pack in a hexagonal lattice. The N phase is described as *''— a structure in which planar molecules are arranged with their planes parallel or approximately parallel to one another and which is nematic in the sense that the distribution of centres of the molecules is random and that they have freedom to move past one each other in directions parallel to these planes.''* (6) [Note that this was written in 1972 – six years before Chandrasekhar's work on discotic phases.]

be flexible in order to fill the awkward space at the centre of each cylindrical aggregate. The accepted view is that micellar mesophases cannot form at temperatures below the Krafft point, where the alkyl chains freeze and lose their flexibility. However, there is nothing flexible about the chromone rings of Intal. And why is the 3.4 Å reflection common to both phases? Hartshorne had no explanation for this. He simply said that this reflection must represent "some predominant inter- or intra-molecular repeat distance" (a fairly safe bet). Since this spacing was comparable to the thickness of an aromatic ring, it struck me that the most obvious explanation was that both systems consisted of stacks of molecules.

I was sure that the form of the phase diagram was trying to tell us something. All the phase diagrams of lyotropic liquid crystal systems I had recalled seeing at that time had been of the poly-eutectic type, with the peak of each one-phase region corresponding to the optimum concentration required to produce a particular type of micelle. I had seen multi-peritectic phase diagrams for other systems in physical chemistry text books, but not for mesophase systems. The cases that seemed to be closest to home were for inorganic salts, which formed a succession of hydrates with increasing water content. It seemed that the Intal phase diagram was implying that there was some *progressive* build up of structure across the phase diagram. With some trepidation, I therefore proposed rather different models from those of Harthshorne and his colleagues (7). I suggested that, in both cases, the molecules are aggregated into columns, and that the N phase consists of a nematic array of these columns and the more concentrated M phase has them arranged in a hexagonal array. Like Jim Nelson, I was still thinking in terms of effectively cylindrical columns and, bearing in mind the V-shaped conformation of the molecules in the crystalline solid that my colleagues, Stavros Hamodrakas, Sandy Geddes and Bernard Sheldrick had found (8), the only way I could see Intal molecules forming stacks with a more or less circular cross-section was for the hollow cylindrical structure shown in figure 9.

There were obvious problems with this model. Firstly, the proposed structure would require unit cell dimensions significantly larger than those implied by the two inner reflections given by the M phase. I had a vague idea that one might be able to construct an explanation in terms of systematically-absent reflections resulting from the high symmetry of the pattern (possibly coupled with some 'accidental' absences).

And then there was the problem of explaining why the molecules would want to stick together in this pattern. I needed a rationale for both the lateral and longitudinal ordering. As far as the longitudinal stacking was concerned, I had no idea whether it was reasonable to suggest that face-to-face interactions between the chromone rings were sufficient to hold the columns together at the temperatures involved. As far as the ring structure was concerned, I had vaguely heard of salt bridges (in the context of protein structure) and I thought that electrostatic interactions involving sodium ions sandwiched between the ionised acid groups might stabilise the proposed model.

It was embarrassing to find, shortly after the paper had been published, that this was not a very convincing proposal. If I had checked with my colleagues, I would have found out that, for salt bridges to be effective in globular protein structures,

Figure 9. The hollow square model for a chromonic column. I proposed this model in 1980 when I was under the impression that hexagonal symmetry must necessarily imply a packing of cylinders with more or less circular cross-section. (The planar L-shaped slabs represent the bischromone structure as indicated by an X-ray diffraction study of the crystalline solid. The spheres represent the Na^+ counter-ions.) It does not appear to be compatible with the X-ray diffraction pattern of the Intal M phase and I later retracted it in favour of the model with statistical six fold symmetry shown in figure 10. Interestingly a similar hollow chimney model (– which, in this case, is compatible with the X-ray spacings) has been proposed by Gordon Tiddy for a dye/water mesophase.

they must be buried in a hydrophobic environment. They do not occur in an aqueous environment. There would be no driving force, since all the ionic groups involved could interact with water molecules with more or less the same energetics. I should have known better. (There seems to be a moral here – use the right jargon and you can get away with anything – for a short time at least.)

6. Intal: Teresa Attwood

At this stage I was joined by a research student, Teresa Attwood (now Professor of Bioinformatics at Manchester University) a young lady of remarkable enthusiasm and drive. We repeated the optical studies of the Intal/water system, and examined the textures of all the one–phase and two-phase regions. We also took X-ray diffraction patterns of the two mesophases. For this we used equipment that had been developed by Bill Seeds in the laboratory (and previously, in Kings College London) for obtaining the diffraction patterns of fibrous material. This camera had a much finer beam than that used by Jim Nelson. In addition, we put the samples in narrower tubes (0.1~0.2 mm diameter), and found that there was an appreciable degree of spontaneous alignment because of epitaxial interactions with the walls. Because of the alignment of the samples, our pictures were much more informative. They showed that the high angle 3.4 Å reflections are axial, and the sharp low angle arcs are meridional (figure 10). These features are compatible with the proposal that both mesophases consist of more or less the same molecular stacks.

I had previously done some X-ray work for George Gray on the thermotropic systems that gave smectic B and E phases, and I had come to the conclusion that the smectic E structure had the rectangular herringbone array of molecules within the layers and the smectic B was locally similar, but owed its hexagonal symmetry to the dynamic disorder: localised areas of the S_B showed orthorhombic symmetry, but the time- and space-average array was hexagonal. (Every year at the BLCS winter workshop at Hull University, Rob Richardson, of Bristol University, gives a very elegant demonstration of precisely this phenomenon using an optical diffraction analogy.) The hollow

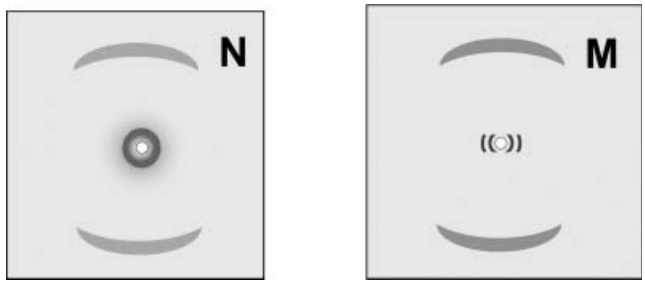


Figure 10. Stylised sketches of the X-ray diffraction patterns of the N and M phases of Intal. The diffraction patterns of both mesophases have invariant axial arcs which indicate a prevalent repeat distance of 3.4 Å parallel to the director. Since this dimension corresponds to the thickness of an aromatic ring, it is taken to indicate that the molecules aggregate in untilted stacks. The position of the diffuse inner equatorial reflection of the N phase varies with composition. The positions of the two sharp equatorial reflections of the M phase also vary with composition – but they correspond to spacings with a constant ratio of $1:\sqrt{3}$ – and are taken as indicating a hexagonal array with centre-to-centre spacing of 30–40 Å.

cylinder model was beginning to look untenable (and unnecessary). Furthermore, Yu and Saupe had published a short paper describing an NMR study of the Intal mesophase (9). They could find no evidence that implied there were hollow water-filled columns (but were characteristically too courteous to state that it was a silly idea anyway). Accordingly, we retracted it and proposed a dynamic disordered herringbone array of columns, structurally analogous to the arrangement of molecules in an S_B layer (figures 11 and 12). As far as I am aware, this has become the accepted view of conventional M-phase structure. (Interestingly, some dyes do appear to form hollow chimney aggregates, but their diffraction patterns indicate a much larger lattice (25–27).)

Some of the liquid crystal papers of this time read rather strangely in retrospect. There was no understanding of chromonic systems, and those were the days of the almost universal acceptance of a two–state theory of liquid crystal textures – i.e., if the system was non-chiral and it wasn't a nematic schlieren and you didn't know what it was, you declared it to be a smectic focal conic.

At this stage, we knew little more than the trade names of other anti-asthmatic and anti-allergic drugs. Terri found that these had structures of the same general form as khellin and Intal – i.e., they were relatively small molecules (with Mr values in the 200–500 range), with one or more planar aromatic cores, little or no aliphatic features, and sufficient hydrophilic groups around their peripheries to make them soluble in water. Perhaps we were looking at a general property of molecules of this type? We wrote to other drug companies begging samples of drugs similar to Intal, and it did not take long for us to find that the Intal/water system was by no means unique.

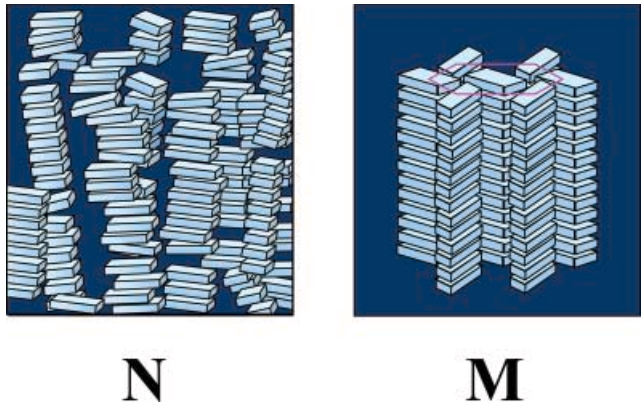


Figure 11. Current models for the chromonic N and M phases. Both mesophases have molecules stacked in columns. The N phase is a nematic array of these columns, with orientational but not positional order. In the M phase they pack in a lattice with statistical six-fold symmetry.

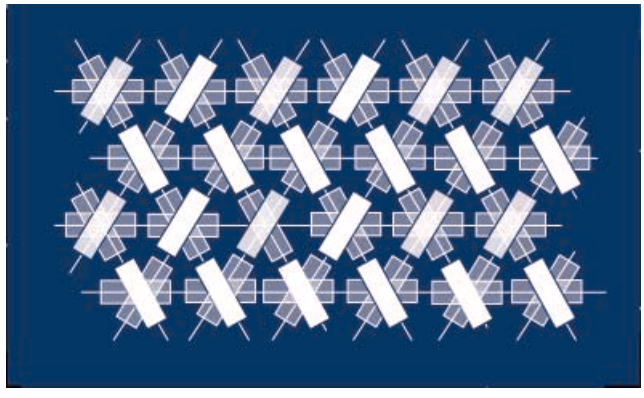


Figure 12. The hexagonal symmetry of the M phase. This sketch shows the M phase as viewed parallel to the column axes. The bold rectangles give a snapshot view of the structure. The paler rectangles show the alternative orientations of the columns. The structure is a dynamic pattern with time- and space-averaged six-fold symmetry (- analogous to that postulated for the arrangement of molecules in the layers of an S_B phase).

Half a dozen other anti-asthmatic or anti-allergic drugs had similar phase diagrams, optical textures and X-ray diffraction patterns. We were looking at a widespread phenomenon (10).

7. Mesogenic dyes: Frank Jones

The next part of the story concerns my friend, the late Dr F. Jones. Frank was a synthetic organic chemist in the Colour Chemistry Department at Leeds. When Leeds University was founded (as the Yorkshire College of the Victoria University at the end of the 19th century), Leeds was a major centre of the British textile industry, and Colour Chemistry was one of the key departments. (It was one of the first departments in the university to be given the right to award honours degrees.) We had friends in common and I often ate lunch with him in the Senior Common Room.

I recall one afternoon sitting in his office on the ground floor of the Colour Chemistry department. After about half an hour's conversation, I became aware of some increased activity in the Food Science building next door, about 8 ft from his window. Frank pointedly ignored this – even when smoke and then flames began to pour out of the building, and the temperature was perceptibly rising. We continued to talk. It was his room after all, and etiquette demanded that I waited for him to decide when we should leave. In the end, a fireman came and threw us out, shaking his head in a mildly despairing way. Clearly a man who had a framed congressional award from the US government hanging on his wall[§] and who had lived through the blitz, was not going to be fazed by a small building burning down.

During the Easter vacation of 1983 Miss Attwood and I drove the 200 miles from Leeds through the midlands and then along the A44 through the beautiful countryside of central Wales to the BLCS conference at Aberystwyth. At breakfast on the first morning, I was surprised to see Frank there, because I had no idea he was interested in liquid crystals. Then we saw his poster of a dye/water system. Terri and I glanced at each other meaningfully. We both saw the implications: apart from the fact that they were bright red, the whole range of textures looked remarkably similar to those of Intal. There were hints of nematic schlieren patterns, M-phase ribbons and herringbone textures, and even reticulated N+I two phase regions. However, the textures were nowhere near as clear and well-formed as those of the drug/water system.[**] They looked as if a short-range domain pattern had been superimposed. We suspected that this might be the result of impurities in the material, and we were told that commercial dye preparations are usually very impure and, in particular, contain large quantities of salt – remaining from the salting out stage of the preparation. We could have attempted to test our suspicions by purifying a dye – but it was much easier to do the converse and add salt to a drug/water system to see if the textures deteriorated as expected. They did (11).

Dyes, in general, have molecular structures similar to those of anti-asthmatic and anti-allergic drugs. We were looking at an even more widespread phenomenon. The drugs of the Intal type are essentially dyes that absorb in the UV – just out of the visible range – and we both knew the story of Ehrlich searching through hundreds of dyes in the quest for the 'magic bullet'. And there was the almost converse story of how the 18 year old Perkin had attempted to create the British synthetic drug industry with the synthesis of quinine (by oxidising allyltoluidine), but had produced a purple compound, and opted for creating the British synthetic dye industry instead (figures 13 and 14). Drugs and dyes were close cousins and, if I had been more alert, I would have had the wit to study the dye literature well before it was thrust into my face in this fashion.

We found that there was an extensive literature (notably from Scheibe in Germany (12) and Balaban and King, and Jelley in Britain (13, 14)), on liquid crystalline phases of dyes. Naturally, it mainly centred on changes of colour with concentration, explained in terms of the aggregation of dye molecules. There were vivid descriptions in the literature of opalescent textures (some dating virtually all the way back to the days of Reinizter), together with models for molecular aggregation (analogies with stacks of coins or packs of cards) in normal and tilted columns (H and J aggregates, respectively). But, in general, workers in the dye industry rarely saw the pure material in a concentrated solution. They saw either dilute solutions in the dye vats or the drums of dry powder. And the polarising microscope was not a common feature in the production sheds. ICI told us how some of their products would thicken and solidify in the vats, and would have to be dug out – this was reminiscent of early descriptions of the problems with delivery of Intal via the spinhaler, when the powder would 'clump' on exposure to moisture, blocking the

[§] I never did ask him what he had done to earn this.

[**] If we had come across a purified dye sample at this stage, for example a food colorant like Edicol Sunset Yellow (E110), we would have had no reason whatsoever to question the correspondence of the two sets of mesophase systems.

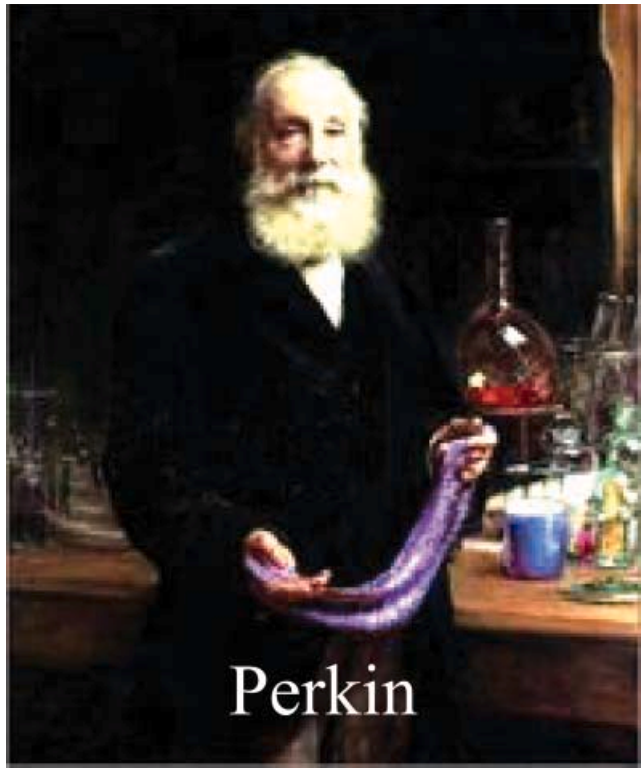


Figure 13. Perkin with a hank of mauvein-dyed silk. This portrait shows Sir Wiliam Perkin looking the very model of a successful Victorian industrial chemist. His initial intention was to found the synthetic drug industry by synthesising the anti-malarial drug, quinine. His naïve attempts produced a stable purple compound – so he founded the synthetic dye industry instead. His son (A. G. Perkin) continued in the family business and later became professor of Colour Chemistry and Dyeing at Leeds. A sealed sample of his father's original mauveine is on display in the Foyer of the current department in Leeds.

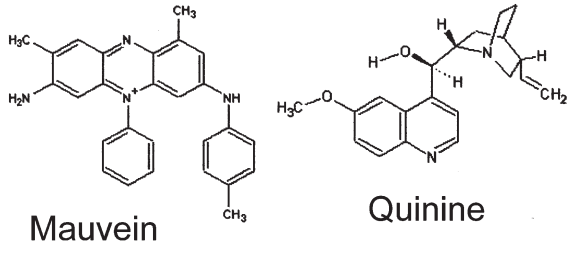


Figure 14. The structures of mauveine and quinine.

spinhaler (obviously because the moisture was inducing mesophase formation).

8. Where the word 'chromonic' came from

In the introductions to our first few publications, Terri Attwood and I referred repeatedly to "that family of lyotropic mesophases formed by many drugs and dyes, which form well-defined liquid crystalline systems that were not of the conventional amphiphile type – in which the molecules have rigid planar aromatic cores rather than flexible aliphatic chains – and where the solubilising groups were arranged around the periphery of the molecule rather than being concentrated at one end." We could not go on repeating all this every time. We needed a name.

I gave this some thought – and, one Sunday afternoon, when Miss Attwood was visiting us for dinner, and I was pottering in my garden for a few minutes before the meal was ready, it came to me that the name 'chromonic' was very suitable. It had respectable historical origins in the bis chromone structure of Intal (there was a clear precedent in the word cholesteric) – and fortuitously, all of the connotations were appropriate, since it implied dyes and (via chromosomes[††]) nucleic acids. Rarely has a neologism been so apt and would be so readily accepted – or so I thought. Actually, it was to be twenty years before the word achieved any level of acceptance and respectability.

[††] Chromosomes were given this name not because they are coloured – but because they can be readily stained (with haematoxolin) to become coloured.

9. Gordon Tiddy

At this time there was little interest in chromonic phases in Britain. The McCrone Institute had moved on, and neither the drug nor the dye industry seemed to consider that these systems had any obvious technological potential. Elsewhere, there was however some activity, principally at the Liquid Crystal Institute at Kent State (see for example refs (15–17)). In addition to the NMR studies of Yu and Saupe, Labes and others studied optical textures showing unusual high-strength disclinations in samples of the chromonic N phase and had patented the use of Intal as a substrate for assaying small quantities of chiral dopants in an elegant optical method. Luz and others had estimated the degree of aggregation of chromonic stacks.

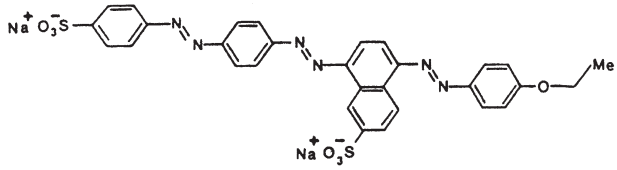
The most prominent figure in the mainstream of lyotropic liquid crystal studies in Britain was Gordon Tiddy. (If you were organising a conference with a section on lyotropics, the question was always who should be asked to speak second.) He was originally sceptical about the concept of chromonic systems being distinct from conventional amphiphiles – but intrigued. We agreed on a test where a chromonic mesophase and a conventional amphiphile mesophase were mixed. We chose a chromonic M phase and a lyotropic H1 phase that existed over more or less the same temperature and concentration ranges, and attempted to gauge the degree of miscibility. The form of the phase diagram and the X-ray diffraction patterns of the mixed systems appeared to indicate that the two types of mesophase kept strictly apart. Gordon was convinced – nevertheless, it took us a further 5 years to get the results published (18)!

Of course, one isolated example is scarcely proof of a general phenomenon and, as far as we were aware, there had never been any discussion as to whether the miscibility criterion was applicable to lyotropic systems. Furthermore, we accepted that, even for thermotropic systems, the miscibility criterion does not apply in converse: i.e., if two mesophases are not completely co-miscible across the entire concentration range, it can not be taken as proof that they are of different kinds. However, it was the fact that there seemed to be no detectable mixing whatsoever of the two systems that we found convincing.

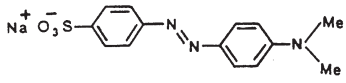
10. Chromonic dyes: Jane Turner

The next part of the story concerns Jane Turner (then Jane Moore) a student financed by a CASE studentship with what was then ICI dyes at Blackley, on the outskirts of Manchester. It was owing to Frank's good offices that I obtained this grant.

I spent the first couple of months telling Jane everything I knew about liquid crystals and optical microscopy, phase diagrams and optical textures of drug/water systems. She was then to work with Bill Fern at Manchester for a few weeks. There seemed to be an all male workforce in the research labs and I wanted to forestall any problems she might have. I made her take everything she would need (microsope, Mettler hot stage, slides, cover slips – even droppers and tissues) so that she would be able to start work immediately without having to ask anyone for help. I told her that she should be polite but firm. She should stress that it had been agreed that she could have the run of the shelves to test everything for liquid crystal-forming properties and not let them use her to make the tea or do the typing. (In the event, I needn't have bothered with any of this nonsense. Everyone was friendly, helpful and very professional.) As she drove off, I asked her to phone me that evening and tell me what she had found. Fearing no immediate success, I stressed that all she needed was just one single new mesogenic dye. We could build a thesis on that if necessary. In the middle of the evening there was phone call. "I have found thirty!"



Sirius Supra Brown RLL



methyl orange

Figure 15. Examples of chromonic dyes.

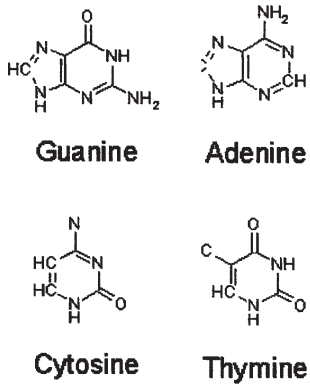


Figure 16. Purine and pyrimide bases of DNA.

As we had found earlier, the large amounts of salt left over from the salting-out process tends to convert a respectable, low viscosity, nematic dye-water system into a soggy mass that looks like damp sawdust. Jane's work at ICI was highly successful. She seemed to have a remarkable ability to see the mesogenic potential in these materials. She was able to visualise what these materials would become when they were cleaned up by dialysis. We were not able to publish the full extent of her work and to name compounds, but a brief statement that she had discovered numerous mesogenic dyes was published in Mol. Cryst. Letters (19). As her investigations proceeded, more interesting textures and patterns of mesophase formation emerged – implying that the full repertoire of chromonic structures extended beyond the classic N and M phases.

11. Nucleic acids: Isodesmic aggregation

A further piece of the jigsaw was thrust into my face in 1990, when my department, the Astbury Department of Biophysics, at Leeds was eaten up by a larger Biochemistry Department and, instead of teaching final year biophysics students about Fourier transforms of helices, I found myself timetabled to teach enzyme kinetics to first year biochemists. I had to learn some basic biochemistry, fast. Whilst reading up about the structures and properties of nucleic acids, I came across a description of a study of the aggregation of the purine and pyrimidine bases in solution (20). Until then, I had not appreciated that molecules of these aromatic bases will spontaneously stack in solution on their own. They do not need sugar/phosphate chains to hold them together (although, of course, these are necessary to preserve the base sequence and hence the genetic message).

Osmotic pressure studies of nucleosides in aqueous solution had led to the conclusion that the molecules stack in columns. Furthermore, sedimentation equilibrium experiments indicated that the process is reversible and that the addition of a base to an existing stack always involves the same free energy increment, irrespective of the length of the column. This implies that column elongation is an additive rather than a cooperative process (in contrast to micelle formation by conventional amphiphiles). The term *isodesmic* (which I had not come across before) was used to describe this behaviour. (The first time I saw anyone else use this word in a mesophase context was in the book, *The Colloidal Domain* by Evans and Wennerstrom (21).) And again, in contrast to conventional amphiphile behaviour, both ΔH and ΔS are negative – i.e., the aggregation process is enthalpy-driven and entropically opposed.

Whatever the forces are between aromatic molecules in aqueous solution (whether they are regarded as π–π interactions, dipole/induced-dipole effects, 'hydrophobic forces' or enhanced van der Waals interactions) they are clearly of the same order as kT, and hence are of the correct strength to explain the temperature ranges of chromonic phases. It would seem that, at the conditions within living organisms, nucleic acid structures like DNA and RNA can be regarded as side-chain chromonic liquid crystal polymers.

One of the factors that complicated early structural studies of DNA was the polymorphism of the material. The interpretation of the first diffraction patterns, taken by Astbury and Bell, was made

difficult because they had not realised that they were looking at a mixture of forms (22, 23). The later work of Rosalind Franklin, using X-ray cameras with controlled humidity levels, showed that there were (at least) two polymorphs. Both forms are double helices, with the two chains lying antiparallel. The more hydrated B-form, occurs in living systems, and the A-form is stable at lower levels of hydration. It is the B form that is usually pictured in biology text books. This has about 10 base pairs per turn, and the bases lie more or less perpendicular to the helix axis. The half-way point along each base pair lies more or less on the helix axis. The A-form is more tightly wound: there are about 11 base pairs per turn; the bases spiral around the axis and are tilted an appreciable angle to the normal.

Clearly, the stacking forces have features in common with other "hydrophobic interactions". They are non-directional enough to allow the bases to slide over one another easily at the A/B transition. In contrast to the impression conveyed by molecular models, DNA is far from being a rigid molecular assembly. From a structural, as distinct from a functional, point of view, it behaves like a stack of greasy plates held loosely with two helically-wound sugar phosphate ropes.

11.1 *Miscibility*

Miscibility, in one form or another, appears to be a general property of mesophase systems. The ease of intercalation[‡‡] of aromatic molecules, such as antibiotics and dyes, into DNA led us to suspect that the intercalation of similar molecules into chromonic stacks would prove to be a general property of these systems. In a preliminary study, Terri Attwood found that the chromonic phases of two drug/water systems are completely co-miscible.

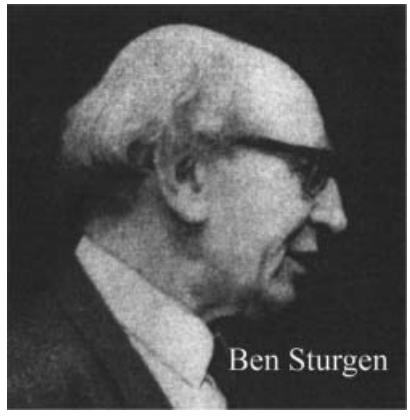
In a later study, Kevin Mundy, Joanne Sleep and I examined the intercalation of ethidium bromide in some chromonic drug mesophases (24). This reagent is widely used in biochemistry as a marker for DNA. It readily intercalates between the bases of the double helix structure and, although not fluorescent on its own, when intercalated, fluoresces pink under UV. We were gratified to find the same pattern of behaviour when ethidium bromide is added to a chromonic drug system.

12. Chromonics as a well-defined family

By 1990, a picture of chromonic systems as a well-defined family of liquid crystalline phases had developed. They can be regarded as the lyotropic counterpart of thermotropic columnar phases and are, in virtually every respect, distinct from those of conventional amphiphiles. They are formed by rigid planar aromatic molecules (such as drugs, dyes and nucleic acids), with solubilising groups at their peripheries (rather than flexible aliphatic molecules with hydrophilic heads and flexible hydrophobic tails). They stack in an isodesmic way, forming columns rather than micelles, and do not have an optimum aggregate size, corresponding to a micelle (and hence have no CMC). They have peritectic rather than eutectic phase diagrams. The distinction between H and J aggregates of dyes, and the polymorphism of nucleic acids, suggests that there are a number of chromonic phase structures with both normal and tilted stacking. Recent studies (by Gordon Tiddy and others) have suggested a variety of more complex chromonic phases in dye/water systems, including brick-wall layers and hollow chimney structures (25–27).

13. Final words

I am deeply touched to have been asked the give the Ben Sturgeon lecture – and I am grateful for the opportunity it has given me to reminisce and to acknowledge the talented colleagues I was fortunate enough to work with.



[‡‡] The word 'intercalation' has an interesting history. It has the same root as the word 'calendar' (and ultimately the Roman, K*alends*: the first day of the month). Before the Gregorian calendar (with its present system of leap years) was adopted, the precise length of a year was not known and the calendar tended to drift away from the seasons. In order to place the vernal equinox back to the same date, extra days were 'intercalated'.

To conclude, a hitherto undiscovered first draft of the Bard's 18th sonnet[§§,***]:

To a Chromonic Phase

Shall I compare thee to a mesophase
Of phospholipid, or a sodium soap?
Nay thou art distinct in many diff'rent ways,
Of similarity, there is no hope.
Sans[†††] *smectic layers, and focal conic fan*
No rounded micelles nor a CMC
No Grandjean planes or cyclides of Dupin
No point of Krafft to mark a boundary.
Thy planar rings in columns spinning free
In drugs and dyes, in R- and DNAs
Make phases N or M (or O and P)[‡‡‡]
In isodesmic, peritectic ways.
So long as men can breathe, so shall we see
Thine aromatic core and well-stacked symmetry.

Acknowledgements

I have endeavoured to check that this account tallies with the memories of the other people involved in this story. I am indebted to Prof T. K. Attwood, Dr J. E. Turner, Prof G. T. J. Tiddy, Prof R. J. Bushby, Prof D. Wray, Prof J. Griffiths and Dr Ian Moxon for reading the manuscript and for their critical and helpful comments.

Further Reading

There are three general reviews of chromonic phases which together cover the principal points in this article and which give a fairly comprehensive list of references:-

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[§§] "Shall I compare thee to a Summer's day"
[***] The Shakespearean sonnet has three, ten-syllable line ABAB quatrains followed by a rhyming couplet (– and an obligatory hint of *double entendre*). Apparently the Bard could knock one of these out without pausing to take breath. Lesser mortals may however, find it more challenging.
[†††] 'Sans', meaning 'without' was used in Shakespeare's day. It has since fallen out of use in English but remains, of course, in French.
[‡‡‡] I have not mentioned these chromonic modifications in the text. The O phase has an orthorhombic arrangement of stacks (loosely analogous to the smectic E ordering) and the P phase is the name used for the combined N and M phases when there is no perceptible first order transition.