Computerised Image Analysis of Rabbit Colonic Motility

A thesis submitted to the University of Glasgow for the degree of **Master of Science** in the Faculty of Science

by

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'On no subject in physiology do we meet so many discrepancies in fact and in opinion as in the physiology of intestinal movement.'

Bayliss & Starling, 1899

'Indeed it almost seems as if with the number of observers the multiplicity of the phenomena as well as that of their views has increased even more.'

Fritz Schneller, 1925

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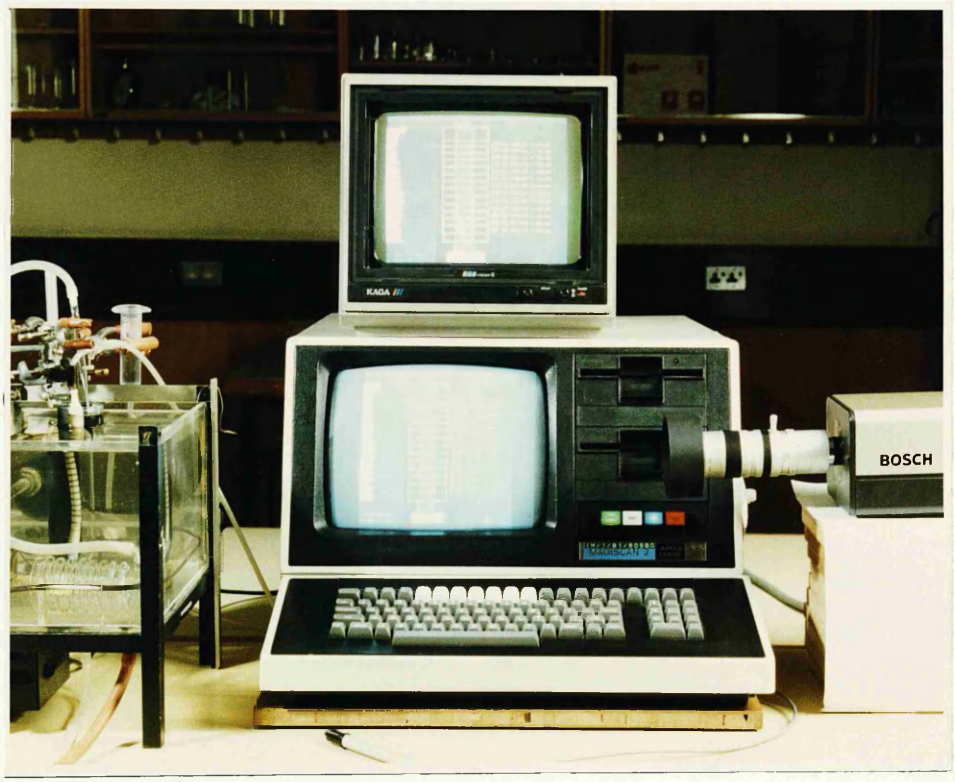


Fig. 1 Photograph of apparatus used in this project, showing Magiscan 2 image analyser, television camera and organ bath. The display on the monitor shows some of the 'windows' used in the analysis and histograms of 'grey values'.

SUMMARY

The aim of this project was to develop a system using the methods and techniques of computerised image processing and analysis in order to quantitatively describe patterns of movement in smooth muscle. The algorithms developed have been applied to the investigation of the co-ordination of motility of rabbit colon *in vitro*.

The MAGISCAN 2 image processor used in this project is designed to analyse single still photographic images from a television camera. In the analysis of movement it is essential to process sequences of images with respect to time. These must be captured at a rate much faster than the period of the movements being investigated. To define precisely complex movements such as gut peristalsis large data sets must be used. Typically this requires the processing of several thousand photographs, the photographs being taken at a rate of three frames/sec over a period of half an hour. The most efficient way to achieve this is to capture, fully analyse and store the derived parameters of each image before the next image is captured.

Software was developed, in PASCAL and machine code, which gave sufficient sensitivity and speed to the MAGISCAN that measurement of the changes in position of the edges of the tissue and of transverse bands marked across the tissue, was possible under experimental conditions. These bands were marked on the tissue using a vital dye. The programs were designed to measure these two movements accurately even if the quality of the image varied.

Although the processing of each image reduces the amount of data that needed to be stored to a few numbers, the amount of data produced from a sequence of images is still immense and some form of synopsis is required. Summaries in the form of graphs of length and width changes were produced. Further analysis was provided by transferring the data from the MAGISCAN to a mainframe computer for univariate and bivariate spectral analysis.

Initial findings indicate that length and width changes in isolated rabbit colon could be measured accurately. These changes sometimes occurred at different frequencies within the same preparation. In preparations where the two changes occured at the same frequency there was no preferred phase difference.

CHAPTER 1. INTRODUCTORY REVIEW OF THE LITERATURE: TECHNIQUES FOR INVESTIGATING THE MOTILITY OF THE GASTROINTESTINAL TRACT

SECTION 1.1 Early Investigations.

From the earliest times people have been curious about the nature of the gastrointestinal tract, probably because this organ was so large, and seemingly alive even after being taken from a recently killed animal. Indeed the positions and shapes made by 'casting the entrails' were regarded as a 'proper method' for prophecying the future. (This could be described as the first systematic investigation of aspects of gastrointestinal motility!)

Alcmaean was probably the first to describe the anatomy of the gastrointestinal tract (circa 500 BC) with Galen (130-200 AD) being the first to try and explain its function (cited by Singer, 1959). Since then physiologists have been developing various techniques and methodologies to record and measure the motility of the gastrointestinal tract. The first part of this thesis reviews briefly the history of the techniques of investigating gastrointestinal motility but it is not intended to be an extensive survey of the literature on function, control and motility of the gut. For this the reader is referred to one of the many reviews on these subjects and in particular to the review of the literature found in H.C. Mckirdy (Ph.D. Thesis, University of Glasgcw, 1968) and to Volume 1 of 'Mediators and Drugs in Gastrointestinal Motility' edited by G. Bertaccini (1982).

SECTION 1.2 Techniques of *In Vivo* Investigation.

Much of the early work on gastric and intestinal motility was purely descriptive, using very general terms. Many of the observations were opportunistic, such as seeing the intestine through an abscess or gastric fistula. Development from this type of investigation led to description of the movement of the intestine of anaesthetised animals whose abdominal cavity had been opened and the contents covered with a balanced salt solution. This work was first carried out in 1871 by Sanders (cited in Hightower, 1968) and has with modifications remained popular.

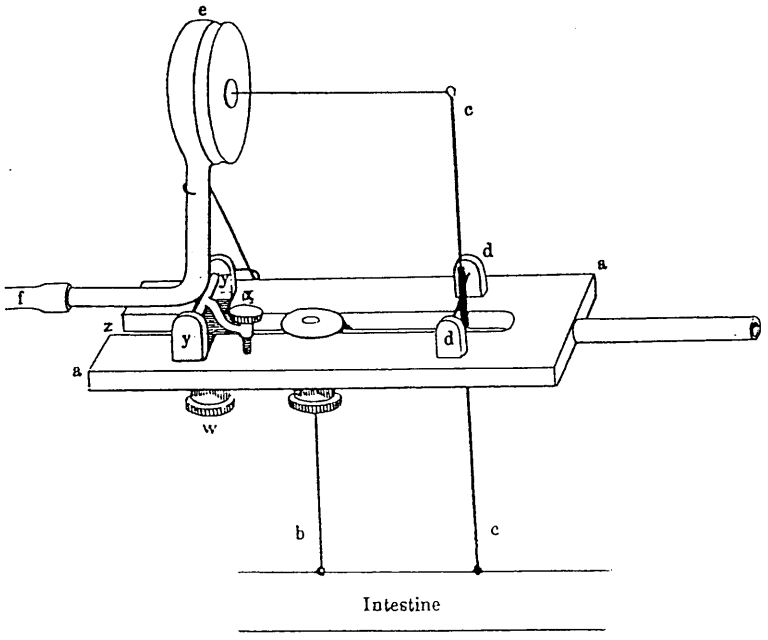


Fig. 2 Drawing of enterograph designed by Bayliss & Starling (1899).

Using enterographs similar to that designed by Bayliss and Starling (1899), basically two levers, at right angles to each other, attached to the tissue (Fig. 2), Elliot and Barcley-Smith (1904) exposed the colon of a number of species to perform what is perhaps the most comprehensive comparison of colonic motility between species. In this study various types of movement were found including anti-peristalsis (backward waves of constriction) repelling food from colon into the ileum. They noted differences in the structure and function of the colon between various types of animals and various positions along the colon.

> 'The colon tends to show a division into three regions of different activities, proximal, intermediate and distal. These are completely distinct in the herbivorous mammal. Anti-peristalsis is constrained to the first region...'
>
> (Elliot & Barcley-Smith, 1904)

Care must be taken in extrapolating between different regions of the intestine, different species and between results obtained from pre-anaesthetised or anaesthetised animals, since some anaesthetics are known to cause changes in gastrointestinal motility (Johnson, 1976). Simply by opening up the abdomen and looking at the intestine Hukuhara and Neya (1968) observed a pacemaker region, which was a zone of pulsating contractions which initiating propagated contractions that could travel in both directions.

In rats and guinea-pigs this region was located at the turn of the ascending to descending colon. Another development of the original 'opportunistic' approach was the surgical implacement of a celluloid window in the abdomen wall of experimental animals. Whilst this allowed more prolonged study than earlier studies, it produced little new and no quantitative data. This technique, developed by Sabotini (1909), was taken furthest by Hukuhara (1931) who took cine pictures of the cat intestine through the 'window' and later analysed the pictures by hand.

Intraluminal balloons, attached to manometers, have been used to measure pressures. The size and shapes of the balloons used can lead to extensive variation in the measured results and balloons have now been largely replaced with perfused catheter systems (Harris & Pope, 1964) and miniature pressure transducers (Millhon *et al.*, 1968, Hollis & Castell, 1972). Singerman (1970) applied time series averaging to the pressure generated by intestinal contractions. The pressure readings from a number of manometers, at different points along the length of the intestine were recorded by the computer. Averaging of this data provided a 'clean' input signal. Similar work has been done on-line by Grundy, Scratchard & Scratchard (1983). Christensen *et al* . (1971) used time series analysis on the data from four tubes implanted *in vivo* in human duodenum. This experiment included the recording of electrical activity and showed that physical and electrical activity were inherently linked. Since a pressure rise in one tube was not necessarily associated with pressure peaks in another tube it was deduced that the functional unit length of human duodenum was smaller than the 2 cm separation between the tubes.

Studying the motility of the intact animal can be done by implanting force transducers onto the extraluminal surface of the intestine (Jacoby, Bass & Bennet, 1963; Pascaud, Benton & Bass, 1978). Some human results have been obtained from patients who have had such transducers implanted during abdominal surgery (Nelsen & Angell, 1979). Such surgical manipulations may have led to tissue damage and abnormal motility patterns. The results from this method were qualitatively similar to the results found from balloon methods, that is to say there were three basic patterns of motility:-

1) low intensity bursts of contraction,
2) general increase in tonus, and
3) an increase in tonus upon which is superimposed bursts of contractions of a greater amplitude.

Raiford and Mulinos (1934a,b) found a novel way around the problem of maintaining an isolated intestinal section by transplanting it into the intact abdominal wall

where it would be nourished by the blood supply and be visible to the investigators. Using a system of levers to record movements it was found that feeding altered the pattern of intestinal motility. In the unfed state the gut was quiescent, but changed to rhythmic contractions when fed. With no neural or physical connections with the rest of the intestine, other than the systemic blood supply, the response to feeding had to be mediated by some blood born agent. This agent would now be considered to be, at least partly, the hormone Gastrin. Raiford and Mulinos added their contribution to the debate on the nature of peristalsis; they found that applying a stimulus to their exteriorised section led to circular contraction both above and below the stimulus.

The use of X-rays to investigate gut motility, though non-invasive, is qualitative and descriptive. This method was introduced by Röntgen (1895, cited in Glasser, 1945) and furthered by Cannon (1902), Luboschez (1931), Steggerda and Gianturco (1936) and others. X-ray investigation is relatively unhelpful in describing motility since X-ray photographs indicate the position and shape of the contrast medium in the intestinal lumen at a given time without relating to times before or after (Fig. 3). Without good reference points on the X-ray image it was difficult to tell much about the movement of the bolus relative to the intestinal wall. (For example, except close to the stomach, it was hard to tell whether the bolus is heading orally or aborally). Cole and Einhorn (1910) used serial Röntgen pictures to give information regarding the changes in contrast media over a given time. More modern radiological technique involving image intensifier systems, offered better resolution of images and lower radiation levels (Wolf & Khilnani, 1966). Despite difficulties results obtained from cinefluorography and digital recording of images began to approach quantitative measurement (Gebauer, Lissner & Schott, 1967; Wolf, Heitman & Cohen 1968).

The rapid and widespread adoption of X-ray techniques for clinical investigation has led to the unusual situation where a large part of our knowledge of gastrointestinal motility is based on studies done on humans rather than experimental animals. Incidentally human intestine, which in most aspects seems similar to intestine taken from experimental animals (Bennett, 1968), is much less prone to degeneration (Bucknell, 1966). An experimental X-ray technique not commonly used clinically is the implantation of small radio-opaque metal markers to the serosal surface of the intestine to record longitudinal movements of the intestine. Tasaki and Farrar (1969) measured intraluminal pressure while taking serial X-rays of intestine with this type of marker attached to it in order to correlate length changes with the pressure increases, which are generally associated with circular contractions. Attaching X-ray opaque substances to the intestine produced trauma, which interfered with normal motility patterns, but if sufficient time was allowed between implant and investigation the effects of trauma were apparently

reduced. The presence of an intraluminal pressure recording balloon could cause abnormal contraction patterns. Although this technique gave some quantitative measure of length and width changes, any changes in orientation in the intestine leads to considerable difficulties in the interpretation of this data.

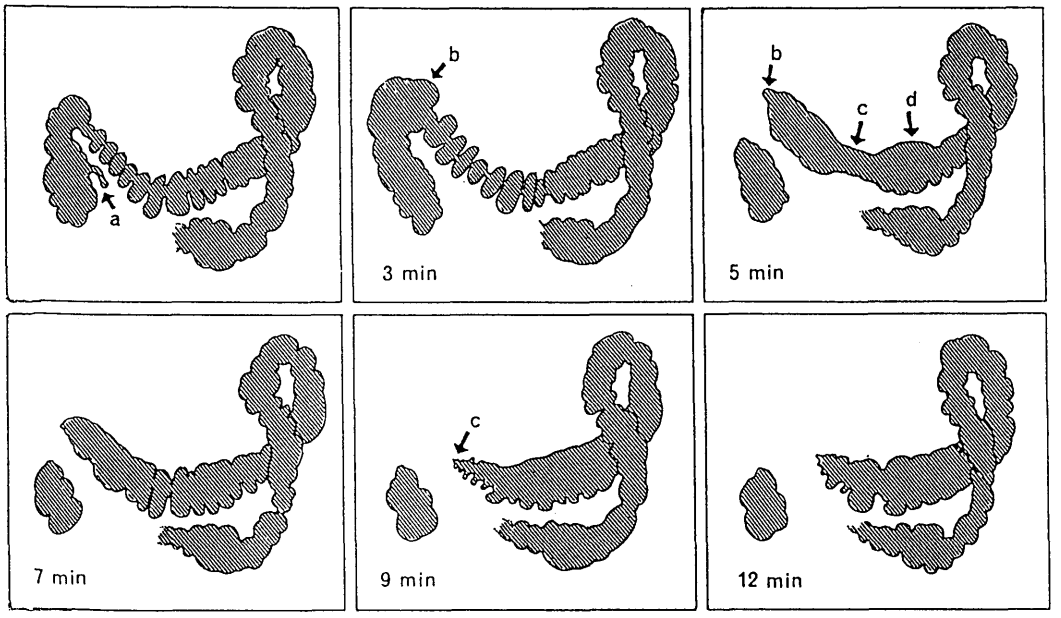


Fig. 3 Drawings made from cineradiographic studies of normal human colon (Davenport, 1977, based on Ritchie, 1968). The sequence shows barium- impregnated ileal contents entering the cecum and moving to the distal half of the transverse colon.

Other means of investigating motility of the intestinal tract or transit of intestinal contents in the intact animals have included the transportation rate of radioactive isotopes (Roswick, Stedford & Brooke, 1969; Walker, 1975; Harvey *et al.*, 1970), radioactive beads (Hinton, Lennard-Jones & Young, 1969), seeds (Burnett, 1923), coloured dyes (Rothman & Katz, 1964), chemical markers (Wilkinson, 1971) which may be isotopically labelled (Hansky & Connell, 1962; Wingate, Sondberg & Phillips, 1972) and magnetic substances such as magnesium ferrite (Benmair *et al.* 1977a, 1977b).

SECTION 1.3 Techniques of *in vitro* investigation

The complete picture of natural motility can be investigated only, if at all, in intact animals. It can be argued however that in whole animal experiments there are too many variables interacting, including the effects of various homoeostatic mechanisms, to easily elucidate the underlying physiological mechanisms. There are therefore advantages in

undertaking an *in vitro* investigation as variables can be controlled to give a more precise and reproducible results. Obviously excised tissue may not have the same properties it had *in situ*. This approach was probably first used (in gastrointestinal investigations) by Haffter (1854), who kept excised bowel sections alive in Locke's solution, but was made famous by the investigation by Bayliss and Starling (1899, 1900) in their well known study of gastrointestinal motility and by Paul Trendelenburg (1917) who did the first *in vitro* study of peristalsis. Since then many others have used isolated preparations in many areas of physiological investigation.

In 1899 and 1900 Bayliss and Starling carried out the first quantitative investigation of gastrointestinal motility, using excised sections of dog and rabbit intestine kept in a balanced salt solution. Using enterographs and kymographs they measured longitudinal and transverse movements. Stimulating the intestine led to contraction above the stimulus and relaxation below it. Various stimuli, including the presence of a bolus in the lumen, elicited this pattern of contraction which they proposed as the mechanism by which intestinal contents were propelled along the gut ('the law of the intestine'). Bayliss and Starling did a number of experiments on these sections, including the effects of pre-anaesthetising the animals with morphia which they found made no difference to the intestinal motility. They also performed a limited investigation of the differences between species, noting that dog colon was generally quiescent while the rabbit colon was much more active. Similarly, in their preparations they looked at the differences between the small and large intestine and found that the small intestine was much more easily stimulated to move. They discovered also that inflating the intestine stimulated contractions to occur as did the more natural stimulus of an artificial bolus. They described pendular movements and occasional regions of circular contractions moving aborally preceded by a zone of relaxation (peristalsis). Bayliss and Starling also found that, within limits, the force of contraction was proportional to distending pressure. Langley and Magnus repeating the work in 1905, could not find the descending inhibition claimed by Bayliss and Starling. A further important factor observed by Bayliss and Starling in this thorough investigation was the effect of the experimental apparatus on the object of experimentation. They noted that the balloon-linked manometer they used to measure pressure in the section actually caused measurable contractions rather than passively recording those contractions already extant.

One of the most important features to arise from the work of Bayliss and Starling (perhaps the most important) was the realisation that their mechanical system for quantitatively measuring the movements of the intestine would lead to the elimination of two sources of variabilty in results: relying on visible inspection only, and allowing preconceived ideas to colour the perception of results. Their admission of and attempt to

prevent experimental or observer error was a major addition to the methodology of biological science.

Trendelenburg (1917) performed his first study of peristalsis on guinea-pig ileum. His technique was later adapted to investigate colonic peristalsis and the technique is still used (Lee, 1960; Mackenna & McKirdy, 1972; Eley, Bennett & Stockley, 1977). Kosterlitz, Pierie and Robinson (1955, 1956) used isometric or isotonic transducers to measure the motility of guinea-pig ileum. The colon is harder to stimulate into peristaltic activity than the ileum but an appropriate stimulus is a semi-solid bolus (Frigo & Lecchini, 1970). In 1924 Alvarez and Mahoney decided, after reviewing the literature to date, that more work needed to be done since many workers had experienced difficulty in showing descending inhibition. Investigating the preceding inhibition using a series of enterographs they concluded that everything from marked contraction to marked relaxation could be found preceding either a bolus or some other form of intestinal stimulus. By cutting and rejoining an intestinal section so that no nerves or muscles were intact between the two parts they showed that apparently normal movement of a bolus could occur. Thus no input was needed from the section above, except for the luminal contents, and a myenteric reflex would seem to be involved. This finding seemed contrary to that proposed by Cannon (1902) who had argued against a pure myenteric reflex explanation of peristalsis and the polarity of the gut on the simple grounds that the food having been placed in the gastrointestinal tract would be rapidly passed through and out.

To investigate this further, Alvarez and Zimmerman (1927) developed a new method of measuring intestinal motility based on analysing images. They placed markers on a section of gut and photographed on cine film the preparation as it moved. Using calipers they measured the changes in position of these markers in a series of 320 photographs which they took of each intestinal section. Thus a quantitative analysis of a whole area of intestine was obtained. From this study it appeared that there was a distension in the intestine in front of the bolus but they interpreted this as being due to liquid being pushed through in advance.

Klinge's (1951) investigation of peristalsis used an interesting approach to measuring the activities of the sections. Shining a strong light on the preparation within a waterbath cast a shadow onto a screen. This shadow, which effectively magnified the preparation and its movements approximately eight times, was photographed and measurements were taken from the photographs.

Brodie (1978), Elder (1980) and Moss (1981, 1982a, 1982b) improved the speed and accuracy of measuring photographs to analyse the movements of the gastrointestinal tract, by using video films and a digitising tablet linked to a mini-computer.

McKirdy (1968, 1972), Mackenna and McKirdy (1970, 1972) had a different approach to using isolated sections. They used a small section of the intestine opened up to make a flat sheet (Fig. 4). This preparation was isometric and therefore any mechanical interaction between width and length changes was removed. They found the two layers contracted in phase; whereas Trendelenberg (1917) had found, using an intestinal segment, that length and width changes were 90° out of phase. In general it is thought that measurements taken using *in vitro* muscle strips are less affected by mechanical interactions between longitudinal and circular muscle layers (Bortoff, 1976).

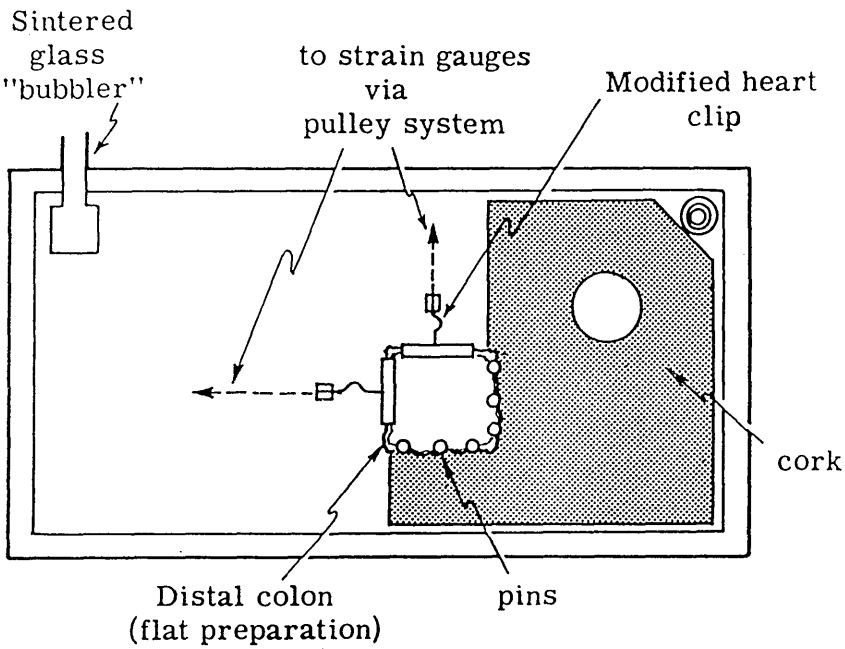


Fig. 4 Drawing of intestinal flat preparation (McKirdy, 1969). The flat preparation, mounted in a Palmer frog bath, is bathed in Krebs solution which is gassed and agitated by the glass bubbler. Two adjacent sides of the preparation are pinned to cork and only these two edges are in contact with cork. The other two sides are clamped along their lengths by small chucks made from Palmer frog-heart clips. The recording is isometric.

SECTION 1.4 Advantages of Image Analysis Investigation.

A review of the literature shows many methods of investigating gastrointestinal motility. In addition the literature contains contradictory findings and interpretations of data. It is

probable that some of the variation is due to taking measurements from different species, regions of the gut, preparations and the feeding state of the animal (Fig. 5).

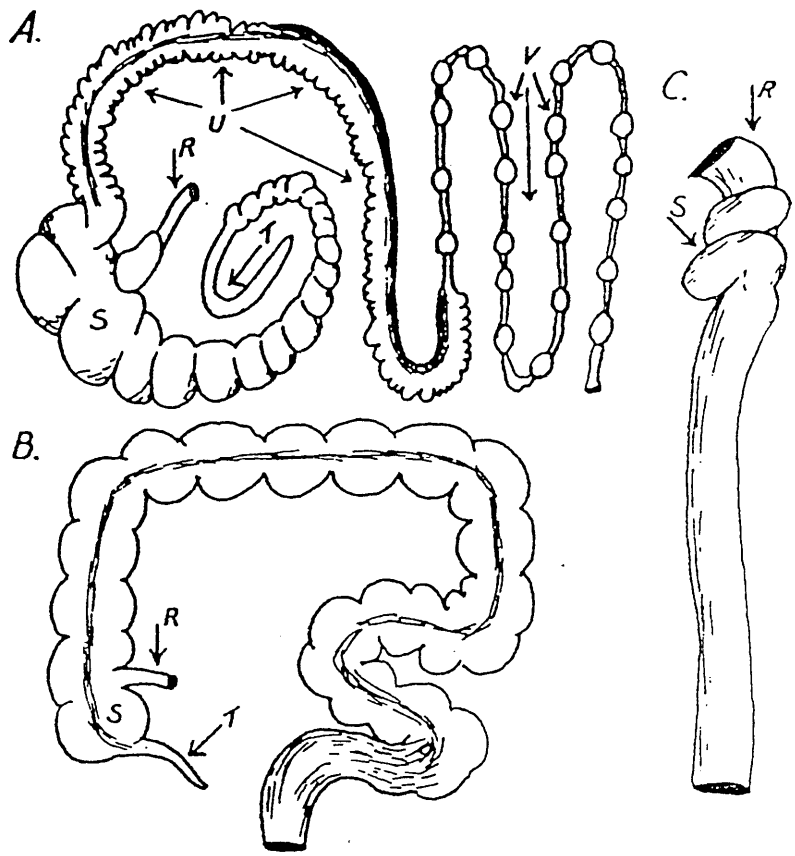


Fig. 5 Drawings of intestinal tissue from different locations in the intestine and different species (Garry, 1934). A, in the rabbit: (R) ileum, (S) cecum, (T) appendix, (U) proximal colon, (V) distal colon. B, in man: (R) ileum, (S) cecum, (T) appendix. C, in the dog: (R) ileum, (S) cecum.

As late as 1982, in a major review of the techniques of studying intestinal motility by Corazziari, it was claimed that there was no adequate way to describe peristalsis other than describing the rate of propulsion of contents, such as fluid or plastic balls (Ishizawa and Miyazaki, 1973a,b; 1975).

Most techniques for investigating motility of the intestine either measure some physical parameter, such as pressure or tension, created by a large segment of intestine or else measure from very small tissue samples or distinct points of the intestine. The claim is then sometimes made that the sum of a number of these small elements approximate the effects of a larger area of tissue. This is probably inaccurate since all tissues interact, especially those tissues like the gut which are linked by a nerve plexus. Recording from a larger section of gut may also limit the sensitivity of the data produced since activity in

one part of the segment could be hidden from detection by the opposite activity elsewhere, thus contraction occurring in one region could mask relaxation in another.

From the time of Bayliss and Starling it has been accepted that the device used to measure motility, be it balloon manometers, enterographs or force transducers, may alter the pre-measured state (A biological 'uncertainty principle'!). To overcome this image analysis of gastrointestinal motility in the quantitative sense was introduced by Alvarez and Zimmerman (1927). Segments of intestine *in vitro* were photographed and subsequently analysed manually. This early work demonstrated some of the main advantages of image analysis over other methods of investigation:

1) no contact is required between tissue being investigated and device measuring it, and
2) the tissue can be treated as the sum of a number of smaller zones.

Image analysis can be applied to the gut by delineating a number of contiguous small areas on a larger segment of intestine. Each of these small elements is studied individually for detail whilst the sum of the actions of these elements indicates the response of the whole segment.

Both marking tissue and sectioning the intestine for *in vitro* experimentation may alter the normal characteristics of the tissue. Image analysis has the advantages that it does not require any mechanical link to the tissue and avoids any variability of the results caused by subjective measurements.

Increased detail from an analysis necessitates increased information handling. Therefore to take full advantage of the power of this technique, the analysis has to be faster. Initially this increase came about in using semi-automated methods with a computer-linked planimeter (Brodie, 1978; Elder & Trueman, 1980; Moss 1981, 1982a, 1982b). Whilst these techniques offer the advantage of combining the ability of a trained observer to discriminate the features of an image with the speed of computerised 'number-crunching', it involves a large degree of subjective decision making.

The next stage forward in the image analysis of the gut came with television linked computerised image processors, such as the Magiscan used in this project. These devices receive input from a television camera, the images from which are digitised and analysed numerically. Various markers on the preparation are found without human intervention, and therefore avoiding possible observer bias. Appropriate programs were developed in this project in order to allow real-time analysis of data. This permitted the study of aspects of motility which previously could not be investigated.

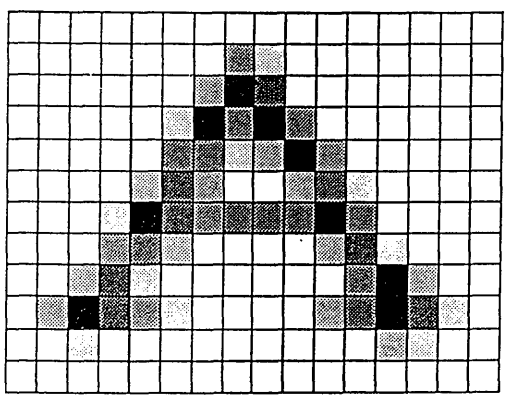
CHAPTER 2. MATERIALS AND METHODS

Since the main thrust of this project has been to develop programs and apply the methodologies of image processing and analysis, this chapter on methods also includes discussion of these aspects.

SECTION 2.1 Fundamentals of Image Processing and Image Analysis.

The basic principle behind any digital imaging device (including the MAGISCAN II used in this project) is that an image can be uniquely described by a two dimensional function F(x,y), where x and y denote spatial coordinates and the value of F at any point x,y is representative of some quality of image at that point; thus the image can be described in matrix form. In digitising visual grey images (black and white pictures) the row and column index of the matrix identify the location of a point in the image and the value stored in this matrix element represents the grey level, that is the light intensity at that point. The elements of this array are called picture elements or pixels and, using a conventional television camera, their values range from 0 (black) to 63 (white) (Fig. 6).

a) b)



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7	7	6	7	7	7	7	7	7	7	7	7	5	6	7	7
7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

Fig. 6 Digitised picture showing pixel values. An example of a digitised image (a) the letter 'A' is constructed from pixels of different grey values, (b) the numerical representation is also shown; since the values range from 0-7 this is 3 bit resolution.

Image Processing is the term used to describe altering an image by some process or system so as to make the data from the image either more readily available or available in more detail. An example is image enhancement in which features of the image are processed to make them more obvious to a human observer or to an analysis program. Another example is feature recognition in which the parts of the image fulfilling a group of

criteria, such as small and round edged, are noted for possible subsequent action. This action may be further image processing or image analysis.

Feature recognition, also termed structural analysis, assumes that the image can be divided, by discontinuities in the image, into a number of regions or structural elements (Dixon, 1977). If these discontinuities are diffuse or 'vague' then processing must be done to identify their location. These processes, known as edge operators, are generally iterative in the case of grey images, that is each relevant element of the image is subjected to repeated processing until some value, possibly the calculated position of the discontinuity, is converged upon.

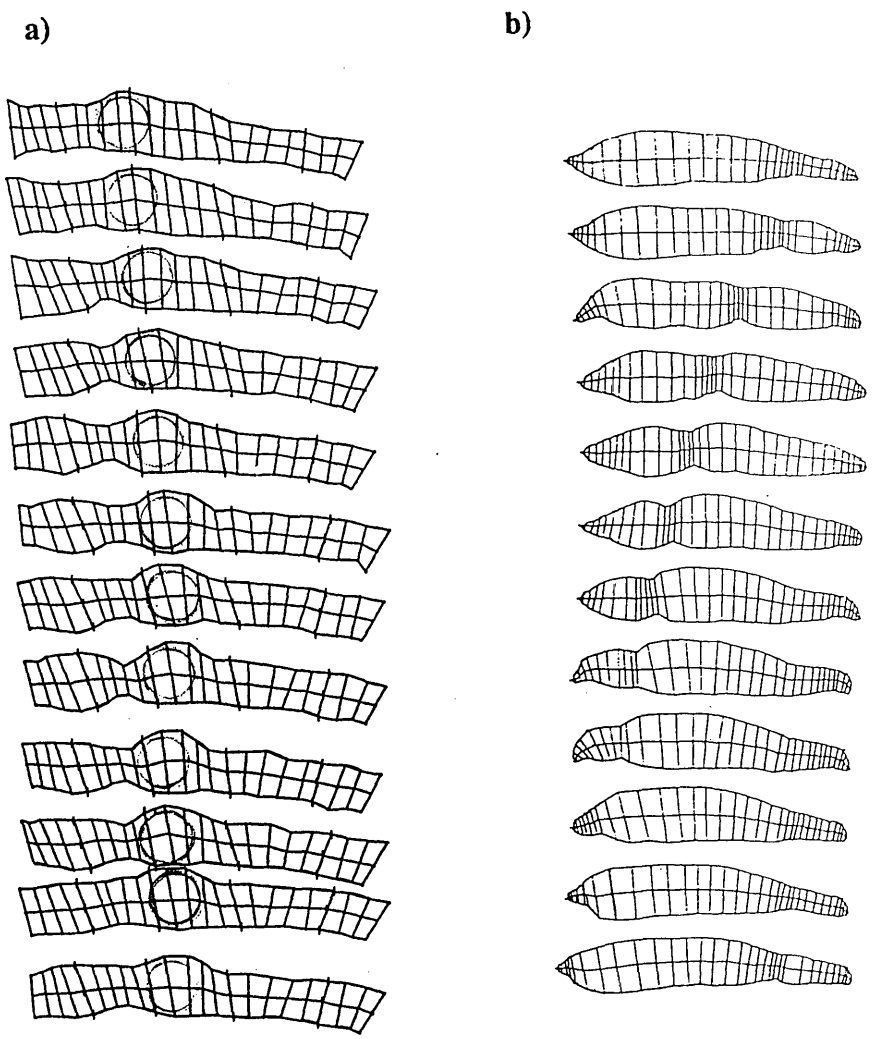


Fig. 7 Drawings derived from measuring pictures using a computer-linked digitising tablet. (a) Intestinal peristalsis (Moss, 1982). (b) Peristaltic locomotion in the marine worm *Polyphysia crassa* (Hunter, Elder & Moss, 1983).

Image Analysis is the term used for the extraction of numerical data from an image or part of an image. This could be, for example, the circumference of the round objects in the image (possibly found by feature recognition) or the percentage of dark stained objects to light stained objects in a microscope field.

The processing steps required in problem solving by man and machine are thought by some to be similar (Poggio, 1984; Ballard & Brown, 1982). Computerised image processing can be compared to the processing carried out by the eye and brain.

Most image analysis and processing used in experimental biology is for the analysis of histological preparations (Bradbury, 1979, 1983; Jenkinson, 1985). Image analysis and processing of the movement of smooth muscle tissue, introduced by Alvarez and Zimmerman in 1927, had to wait for computer assistance before it could be regarded as a efficient means of investigation. Semi-automatic imaging techniques (manual image processing coupled with automated analysis) have been applied to the movements of intestinal tissue (Moss, 1982b), the movements of marine worms (Hunter, Moss & Elder, 1983) (Fig. 7) and the *vas deferens* (Moss, 1984). Only recently has the price of high speed image analysis equipment decreased sufficiently to allow laboratories access to real-time image processing and analysis fast enough to be usable in the analysis of movement (Bell, Boyd & Moss, 1982; Clarke & Moss, 1983; Boyd & Moss, 1984; Moss, 1984; Moss, 1985; Clarke & Moss, 1986; Brodie *et al.*, 1987).

SECTION 2.2 Obtaining and Preparing Tissue.

Tissue was taken from adult New Zealand White rabbits of either sex weighing between 1·5 and 4 Kg. Animals were never sacrificed solely for this work, tissue being obtained from rabbits sacrificed by various workers who did not use intestinal tissue in their work and whose experimental regimes were unlikely to effect the motility of the gut.

All animals were killed by stunning and bleeding from the neck. The abdomen was opened and a portion of colon dissected free. The colon was flushed with mammalian Krebs solution (Appendix A) to remove its contents and thereafter was kept moist with Krebs solution. Care was taken to avoid stretching the preparation. The colon was normally taken from a point near the caeco-colonic junction to a point 30 cm or so below this. Thus the experiments were done on the proximal and early medial colon (Garry, 1933). Before dissecting the segment free care was taken to mark which end of the tissue was orad and which was caudad. This was done by coding the free ends with coloured threads. In determining the presence of anti-peristalsis it is critical to know how the tissue sample was orientated *in vivo*.

A piece of gut about ten centimeters in length was chosen for further cleaning and use. The section had to be clear of any obvious damage and to be free of relatively large anatomical asymmetries, such as those caused by Peyer's patches, which might have led to difficulties in measuring width (any rotation of the preparation might be interpreted as a width change). The lumen of the section was cleaned by slowly inserting a graduated pipette (diameter 8 mm) from the orad end. As the pipette was gently pushed through the section Krebs solution was ejected from the pipette into the section lumen thus lubricating the passage of the pipette. Using this method it required very little force to push the pipette through the section thus minimising the damage to the gut. The pipette was then clamped horizontally so that any non-intestinal tissue still adhering to the section could be easily dissected clear.

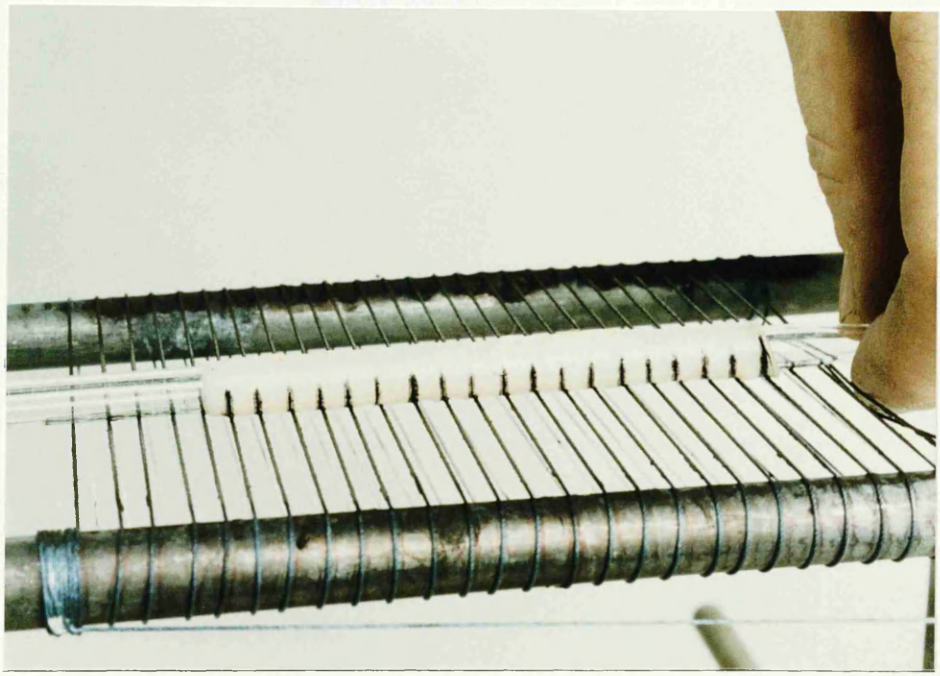


Fig. 8 **Photograph showing method of marking bands on preparation.** Rotating the pipette rolls the segment along dye soaked threads leaving stain rings on surface of segment.

The cleaned gut was inspected for any visible damage that may have occurred during this second dissection. If free from damage the section was marked transversely with bands of the vital dye Janus Green B. This involved adsorbing a concentrated aqueous solution of the stain onto a series of threads 5 mm apart. Excess moisture was removed from the surface of the preparation using absorbent paper and then rolled gently along the threads (Fig. 8). This left a series of bands of stain around the gut. The preparation

was kept for a few minutes in a moist chamber to aid the fixing of the stain before the excess dye was washed away.

A 'T-piece' tube connector was inserted into the orad end of the preparation and tied securely. The lower, caudad, end was attached to a tube connecter weighted with enough plasticine (normally about 3 g) to seal the connector and cause the end of the section to sink. The preparation was suspended in Krebs solution, and gassed by a 95% oxygen 5% carbon dioxide gas mixture, at 37°C. All openings to the lumen of the preparation were closed except one which was attached to a reservoir of Krebs solution. The height of this reservoir could be changed to adjust the intraluminal (and thus transmural) pressure if required (Fig. 1, 9).

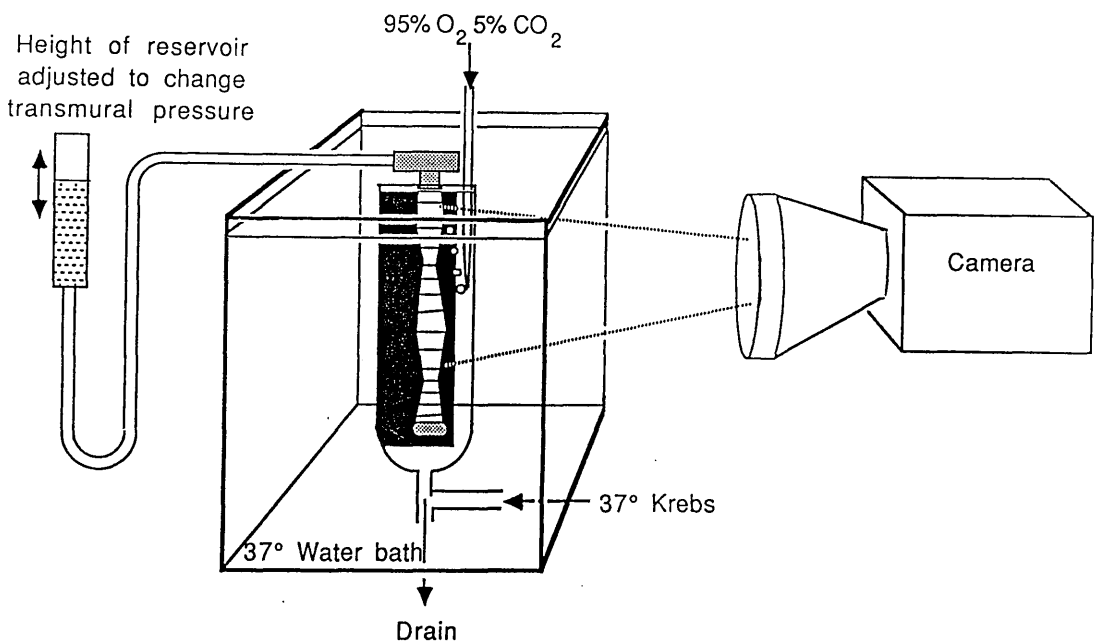


Fig. 9 Diagram showing preparation in tissue bath, c.f. Fig. 1.

The tissue was illuminated as evenly as possible by a number of lamps such that the image of the preparation appeared brightly lit against a dark background. The bands of dye were seen as lines across the width of the preparation and changes in position of these bands reflect the contraction or elongation of the zones between bands. Changes in the distance between the edges of the preparation are changes in diameter. The image analyser uses a television image of this preparation to determine the changes in the width and length of each zone which reflect changes in the lengths of the longitudinal and circular muscle. In most experiments a gut preparation was analysed with about 14 lines marked on it (therefore 13 zones).

SECTION 2.3 Image analysis and processing equipment used

The mage analyser used is the MAGISCAN 2 system (Joyce-Loebl), with input from a television camera with a Chalinicon tube (Bosh, T YK). This is capable of distinguishing 64 different grey levels, i.e. has a 'six bit' resolution. Considered from a logical view the Magiscan consists of two parts. The first logical element is the image analyser running under the U.C.S.D. Pascal operating system, with its own macro-memory (128 KByte) for storage of programs and general data, and microcode-memory (24 KByte) which contains the P-code interpreter and other intrinsic machine code routines. The second element is the image memory (2 MByte) is used for the storing of image data, graphics and in our case also used for storing processed data.

The input from the camera to the image analyser is a standard television signal (25 frame/second). This is converted via an analogue to digital converter to give a 512x512 pixels image of 6 bits which is stored in the memory (Fig. 10).

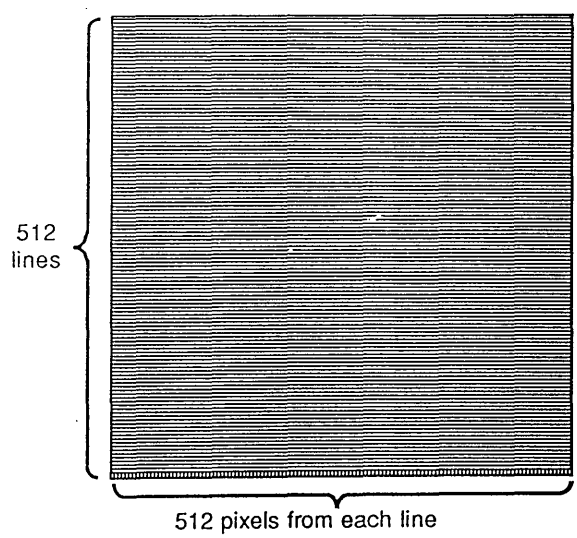


Fig. 10 Diagram showing how a television frame is digitised.
Total data = 512 x 512 = 262,144 pixels. Each pixel represents a degree (0-63) of light intensity.

One captured image takes up almost an eighth of the image memory so that holding more than eight images in memory simultaneously is impossible. The program was therefore designed to process and analyse each image for the data required before the next image is captured. Thus all 'raw' images can use the same part of the image memory, freeing the rest of the image memory to be used to store processed data and graphics. This processed data can be stored on floppy disks from where it can be retrieved and

displayed on the MAGISCAN or transferred to some other device (Fig. 11).

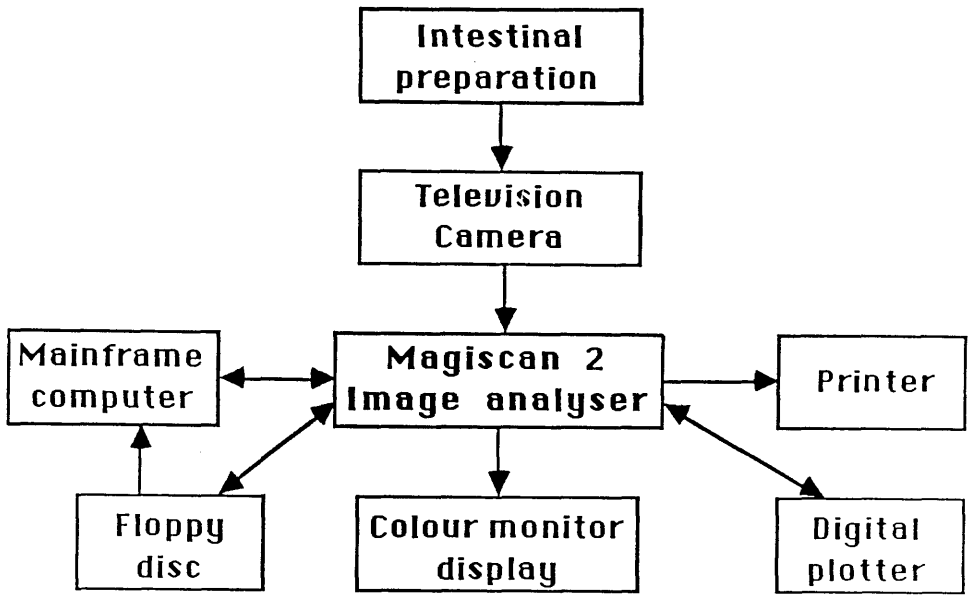


Fig. 11 Block diagram of hardware used in project.

SECTION 2.4 Image analysis and processing algorithms used

In the analysis of movement the rate of data capture must be rapid relative to the speed of movement. The period of oscillation of rabbit colon is about five seconds and therefore images must be captured not less than two per sec. The programs developed can measure from an image the average widths and lengths of the zones between bands in about 300 msec, since critical parts of the program are written in machine code (thus running ten times faster than if written in Pascal). This rate not only allows accurate analysis of movement but is also fast enough for real time image analysis.

The feature recognition routines were designed to determine the positions of the bands marked on the preparation and the positions of the edges of the tissue. The image analysis routines calculate the length, width and volume of each individual zone.

Since not all of the picture needs to be used in the analysis (the image of the preparation only filling part of the screen) two windows (regions) are selected (Fig. 12a). The first window W_1 is approximately filled by the image of the preparation, the second, larger window W_2, contains the image of the preparation and a certain amount of dark

background. The image processing uses W_1 to identify the positions of the bands. These values, along with the image within W_2, are processed to identify the positions of the edges of the preparation within consecutive bands.

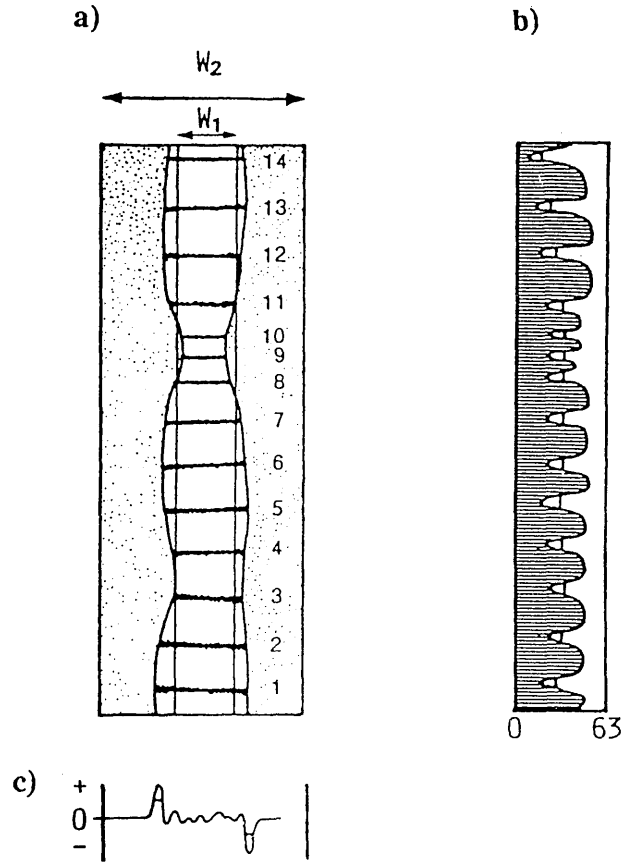


Fig. 12 (a) Diagram of preparation and windows used in analysis. W_1 is used in finding the position of the bands and W_2 is used in finding the position of edges. (b) Histogram of grey values in which the positions of minima correspond to positions of bands. (c) Graph of first differential of grey values across a single zone; the positions of the maximum and minimum turning points correspond to the mean position of the edges within that zone (c.f. Fig. 1).

This criterion is inadequate to unambiguously locate bands since other features of the lighting or the preparation may also give minima. Generally minima corresponding to bands are pronounced so some criteria (**d**,w) is chosen which will differentiate between dye bands and 'noise' (Fig. 13). Only a minimum which has a value at least **d** grey tones less than the values found w pixels on either side is considered as a 'potentially true minima'.

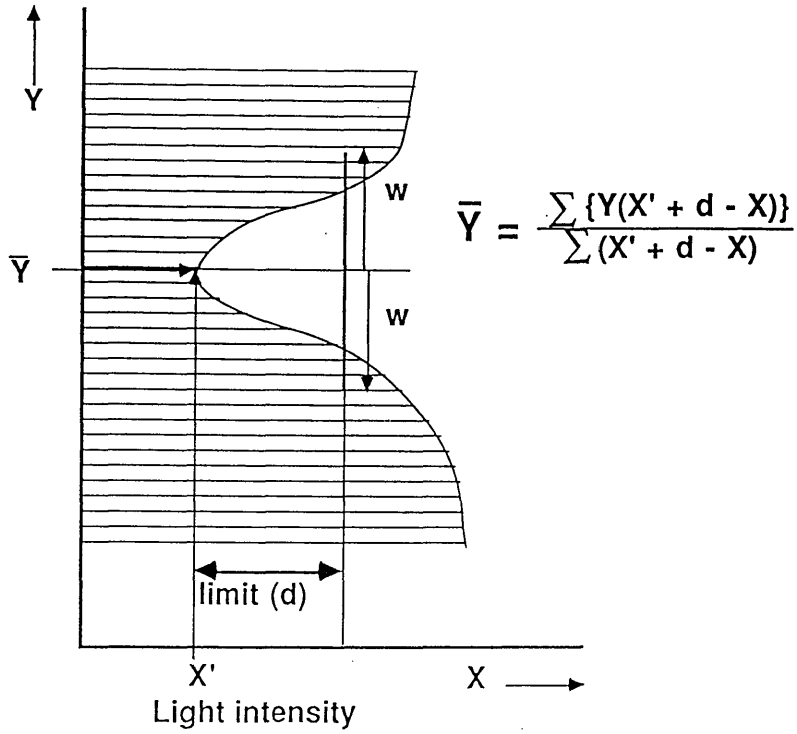


Fig. 13 Diagram showing how the position of a single minimum is calculated. The light intensity value **X'** is regarded as a potentially true minimum since the the light intensity values **w** on either side of it are more than **d** greater. The exact position of the minimum **Y** is calculated by the equation shown above.

The number of these minima is noted and the grey value sums are then smoothed, by applying the binomial formula:-

$$Y_i = {}^1/_4 (Y_{i-1} + 2 Y_i + Y_{i+1}).$$

Again the number of potentially true minima is noted and the process repeated until the number of potentially true minima converged on a value. This iterative process is carried out to remove spurious minima which, though they fulfil the **d,w** criteria, are not associated with bands. These spurious minima are relatively susceptible to erosion by cumulative smoothing. The minima remaining are regarded as true minima. Having identified these minima their precise positions are calculated.

Typically, the image of the preparation occupied most of the height of the frame, i.e. 500 pixels; such that the average length of each zone is about 35 pixels. If the measurement is accurate to ±1 pixel then the smallest observable change in length is 3%. This resolution is insufficient to resolve small movements. An algorithm is implemented, which makes use of a weighted mean formula, so that the position of a band could be interpolated to a fraction of a pixel (Fig. 13).

$$\bar{Y} = \frac{\sum Y\,(X' + d - X)}{\sum\,(X' + d - X)}$$

It is important to choose appropriate values of **w** and **d**. The parameter **w** is used only in rejecting spurious minima. The value chosen for **w** is typically half the smallest distance between bands. This means that the search routine does not overlap adjacent minima, and yet is wide enough to define the position of the minimum being tested. The value of **d** is typically half the average depth of the minima since this is a good compromise between accepting minima which are spurious and rejecting minima which are real but shallow. Provided the bands are marked clearly the value of **d** is not critical. However in interpolating the position of the minima the value of **d** must be carefully selected. If the value of **d** is too small then too few values contribute to the weighted mean for accurate interpolation. If the value of **d** is too large contributions to the mean will be accepted from outlying points which may distort the interpreted value.

In order to give the mean position of the edge in each zone of the preparation the total of the grey values for each column of the image within W_2 is calculated between consecutive dye bands. As the background of the preparation was arranged to be darker than the preparation itself, a histogram of these values indicates the position of the preparation within the image as a region of relatively low grey value compared to the background. The location of the most rapid change indicates the edges of the preparation. This is most easily found by taking the first differential of the sums across W_2. The edges of the tissue correspond to a maximum value of the differential at one edge of the tissue and a minimum at the other edge. To locate these it is sufficient to find the maximum and minimum values of the differential (Fig. 12c). (Generally the maxima and minima produced by uneven illumination of the preparation was not large enough to produce maxima and minima greater than those corresponding to the edges of the preparation.) If an edge is not exactly aligned with the analysis window the shape of the differential will change slightly, but the algorithm will still find the maximum and minimum. The position of the turning points are then extrapolated to a fraction of a pixel using the same algorithm as was used to identify the positions of the bands. The mean width of a zone is the difference in location between the position of the two edges.

The positions of the bands and edges found by the above algorithms are not changed by the intensity of illumination. This is in contrast to segmentation of a grey image by a threshold to give a binary image, which is the method commonly used to find features. Tests under most lighting conditions established that this system of algorithms would accurately and repeatedly detect the positions of the edges and the markers (Fig. 14,15).

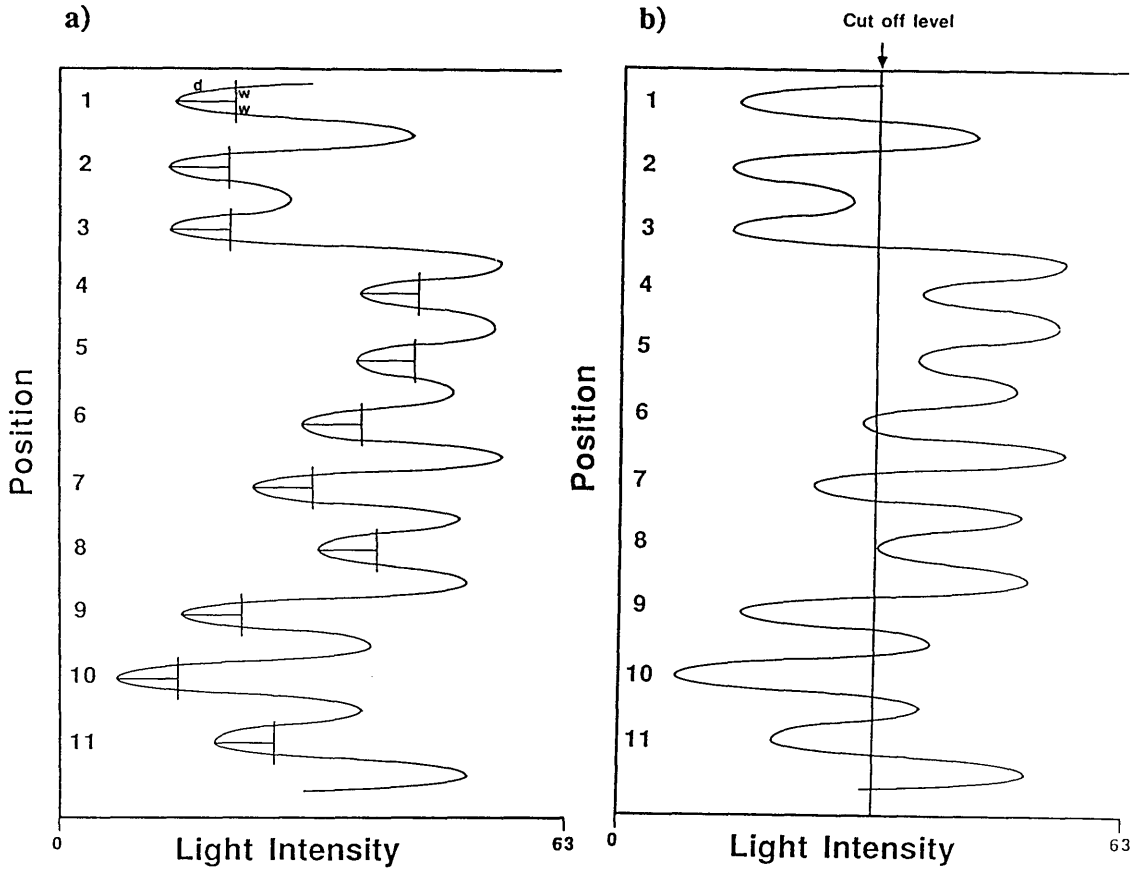


Fig. 14 Diagram comparing the two methods of identifying the 11 bands. The algorithms used in this project correctly finds all the bands (a), whereas traditional binary segmentation (b) will fail to locate all the minima correctly, whatever cutoff level is chosen.

The positions of the bands and the edges are saved in memory. By using spare image memory for this purpose it is possible to save the data from up to 25,000 images. Data can also be stored on disk.

Before displaying the results, or further analysis is undertaken, the collected data must be checked and if necessary corrected for missing, extra or spurious bands. These errors rarely occurred but can be caused by gross movement of the preparation which can lead to bands moving into or out of the analysis window. This is particularly likely to occur if pharmacological agents, such as acetylcholine, are added to the bathing fluid. Other errors could be produced by anomalies in lighting, poor tissue marking, or gas bubbles from the Krebs solution.

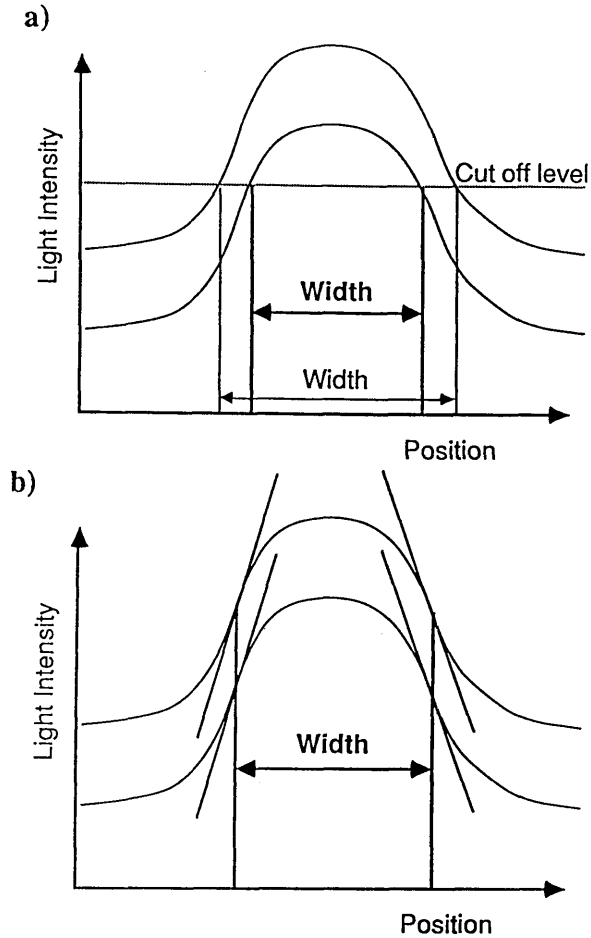


Fig. 15 Diagram comparing the effect of changing illumination intensity on the identification of edges by (a) finding the position of the inflection as used in this project and (b) binary segmentation algorithms. The widths found using binary segmentation are altered by changing light conditions whereas the algorithms used in this project resist this influence.

The program copes with these errors in the following manner. The positions of bands in a frame are compared with the positions in the previous frame. Since the sampling rate is much faster than the period of movement of the gut, there should be relatively little change in the positions of the bands between images. Working from the top of the preparation, which is fixed, the program compares the distance between bands with the distance between the bands in the previous images. A band is considered to be true if the distance between it and the previous band is within 33% of the corresponding distance in the previous image. If necessary extra, spurious bands are ignored. Bands which are missed are compensated for by interpolating from the positions of bands which are regarded as being valid. If errors need correction this is noted. The corrected data can be stored on disk (Fig. 16).

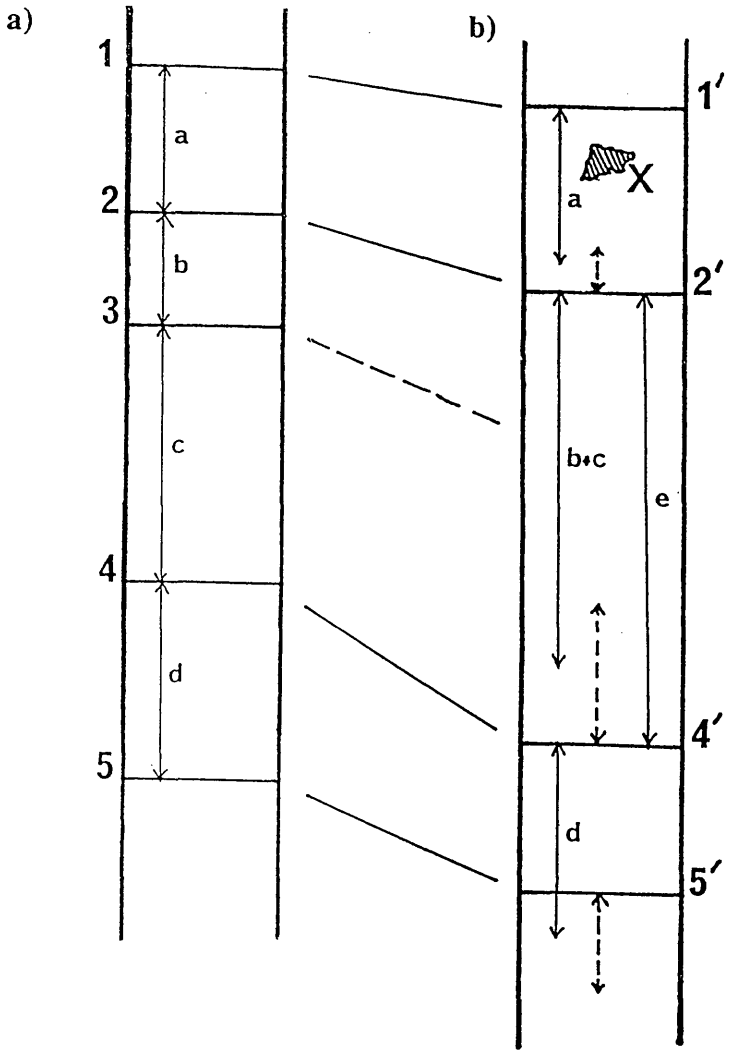


Fig. 16 Diagram showing method for correcting data. The bands on image (b) are checked by comparing them with those on the previous image in the sequence (a).

Band **1'** taken to be the same as band **1.**

Band **2'** is approximately the same distance from band **1'** as **2** is from **1,** therefore defined as being band **2.**

Band **3'** not found and not shown in diagram (possibly the band is not clear enough) therefore position is calculated using the ratio of distances measured in both directions, i.e. missing distance from **2'** = b x **e** + **(b+c)** .

Band **4'** is approximately the same distance from band **2'** as **4** is from **2,** therefore defined as being band **4** and used to calculate position of band **3'.**

Band **5'** is approximately the same distance from band **4'** as band **5** is from **4** therefore defined to be band **5.**

N.B. Object **X** in the second image (possibly some tissue debris in the waterbath) produces a minima of grey image but is not found to be band **2'** because band 2' is closer to the expected position based on band **1'** + distance **a.**

Though line **4'** is closer in position than line **5'** to the position that line **5** had the algorithm correctly associates line **5'** with line **5.**

SECTION 2.5 Magiscan Operating Procedure

The program for image analysis and processing is menu driven with many options available (Table A). A usual way through these options was as follows:-

1. Program started: live T.V. image displayed on monitor.
2. Windows set: light pen used to adjust widths and heights of analysis windows (W_1, W_2).
3. Calibration set: light pen used to indicate reference length in image.
4. Slow analysis rate selected: 1 image per 5 sec.
5. Full graphics option selected: displays histograms as in Fig. 1.
6. Maximum number of bands to be analysed chosen.
7. Set discriminators: used for band and edge detection.
8. Collect test data: if not all bands/edges found then adjust lighting and discriminators (c.f. Fig. 1)
9. Stop test data.
10. Simple graphics selected.
11. Analysis rate selected: normally 2-4 image/sec., specify number of photographs.
12. Analysis started.
13. Stop analysis: automatically on limit or manually.
14. Check data for errors: if necessary correct or reject.
15. Save data to disk file.
16. Data or graphics displayed on screen.
17. Data or graphics sent to printer/plotter for hard copy.
18. Data transferred to mainframe computer for statistical analysis.

Table A. Options available within analysis program.

A	begin/stop analysis
B	band alignment and correction
C	calibrate with scale bar or enter numerical value
D	display results graphically on monitor, graphics terminal or digital plotter
E	erase all graphs
F	fast analysis toggle (graphics/no graphics)
G	graphics toggle (full/partial graphics)
H	help menu
I	initialise system to defaults
M	set criteria for band and edge finding
N	set number of smooths
O	output toggle for pseudocolour/monochrome images
P	printer baud rate toggle 9600/1200 baud
Q	quit program
R	read data from disc file
T	time interval between pictures
V	values of data sent to console or printer
W	Window setup
Z	Colour/monochrome toggle (text and graphs)
:*	show disk directories.

CHAPTER 3. RESULTS

SECTION 3.1 Using Data from a Small Number of Experiments

The following results were derived from the limited number of specimens used to test the system. Lengths and widths of zones were measured directly and volumes were calculated assuming the preparation had a circular cross-section. In general three zones were analysed statistically for each of the twelve tissue samples. Extrapolations to the whole population made from small number of data sets always run the risk of being inaccurate (the ability to collect large data sets being one of the reasons for developing this system). The results have been included to demonstrate the viability of the system and to point the way for some further investigations.

SECTION 3.2 Graphical Presentation of Results

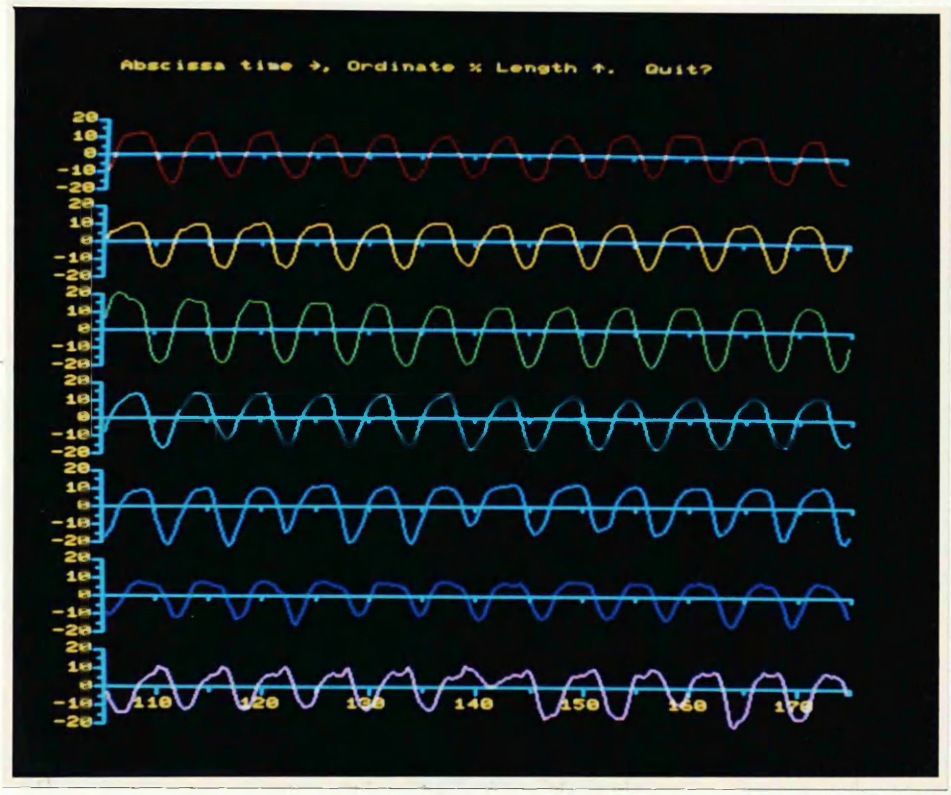
The results of an experiment can be summarised graphically in a number of ways. A graphical display on the console of the image analyser (Fig. 17) or a digital plotter shows the time course of percentage changes in length or width of each zone, compared to its mean value. The derived parameters volume or length:width ratio can also be plotted. Pseudocolour images of the results, in which the colours show the changes within individual zones, can also be displayed on the console (Fig. 18). In both these displays, selecting a particular time and viewing vertically shows the percentages changes within the preparation at that time. Following one zone across a graph shows the percentage changes with time of that zone, compared to its mean value. Contractions are shown as negative values (yellow or red) whereas relaxations are positive (blue or magenta).

SECTION 3.3 Statistical Analysis of Widths, Lengths and Volumes

The times of the cycle for the length or width movement on the twelve tissue samples were analysed statistically. The mean width period (± standard deviation) was 5·52±0·16 sec and mean length period of 5·58±0·18 sec.

The effects of pressure on the preparation were investigated over the pressure range 0–10cm H_2O. Increased pressure was associated with increased dimensions. The relationship of pressure to width was linear ($r=0{\cdot}46$, 33df, $P\approx1\%$) (Fig. 19). The influence of pressure on average length was also linear ($r=0{\cdot}70$, 33df, $P<0{\cdot}1\%$) (Fig. 20) as was the relationship of pressure to average volume ($r=0{\cdot}70$, 33df, $P<0.1\%$) (Fig.21).

a)



b)

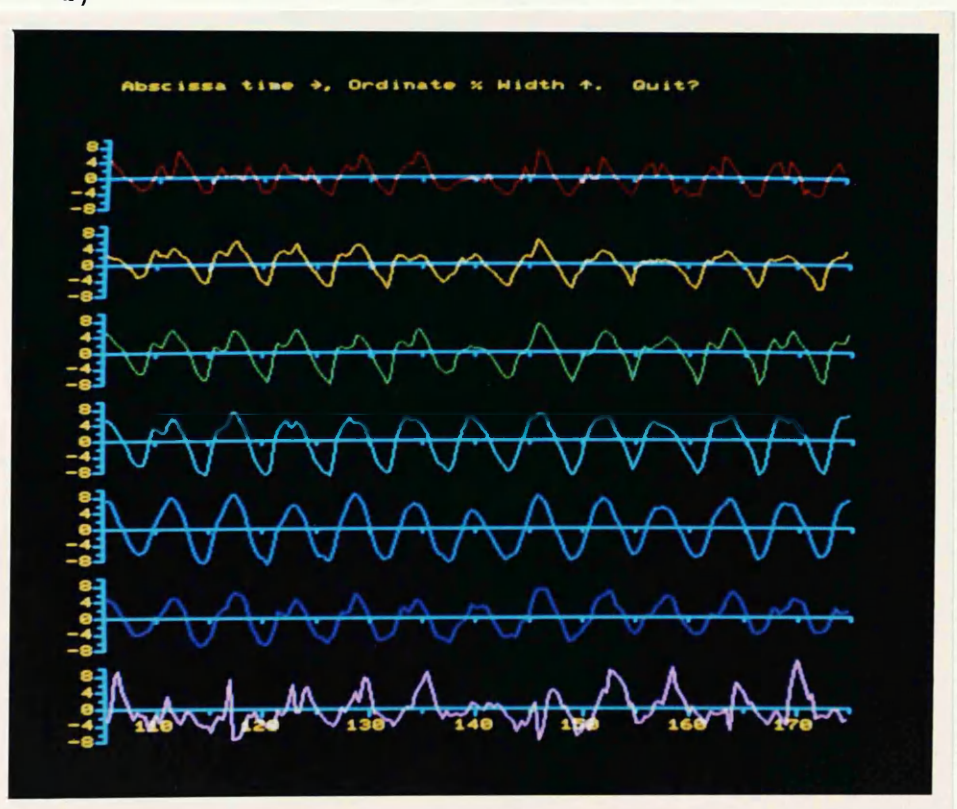
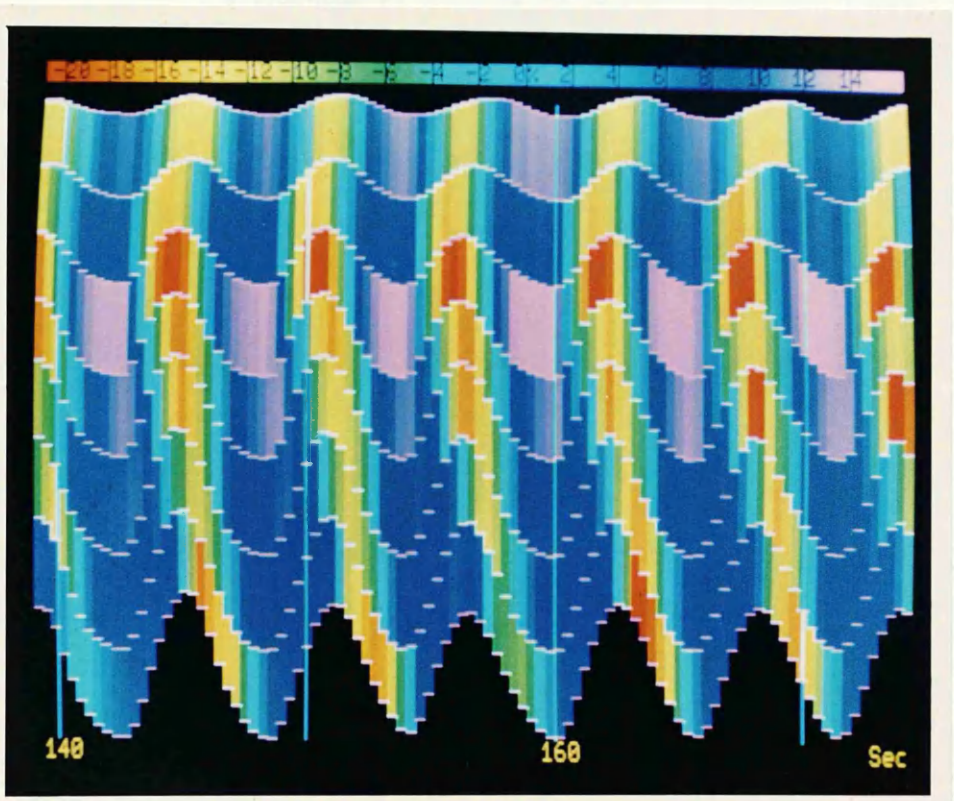


Fig. 17 Graphical display on the console showing the percentage changes in (a) length and (b) width.

a)



b)

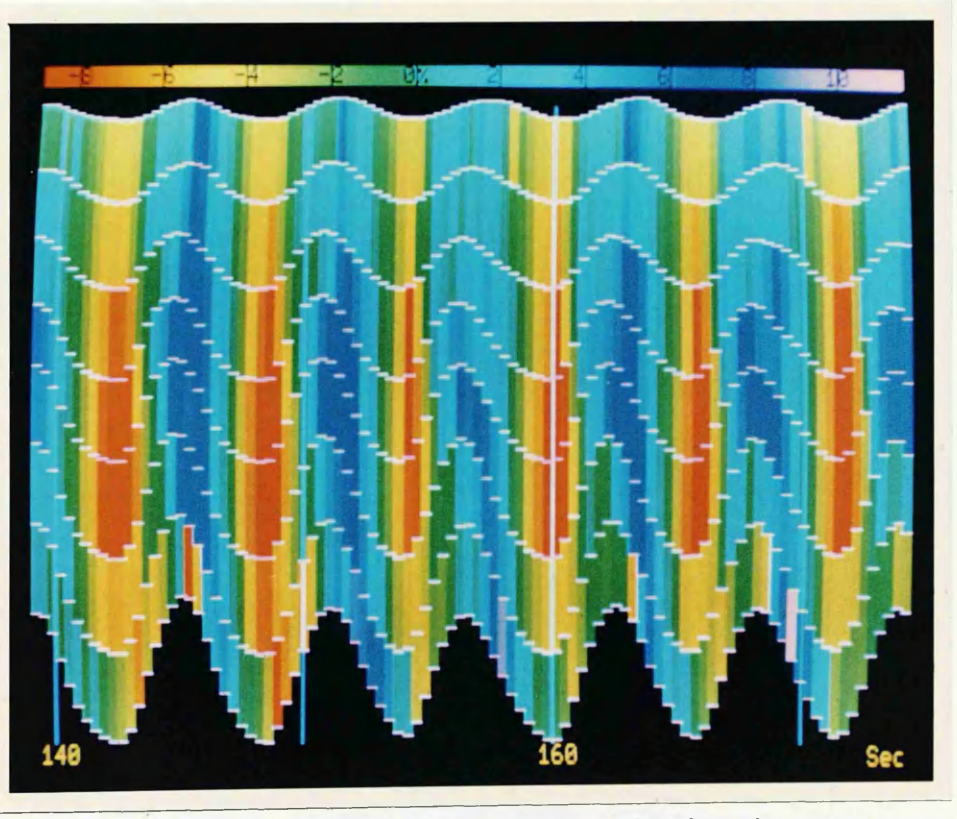


Fig. 18 Pseudocolour display on the console showing the percentage changes in (a) length and (b) width. (These show part of the data from fig. 17).

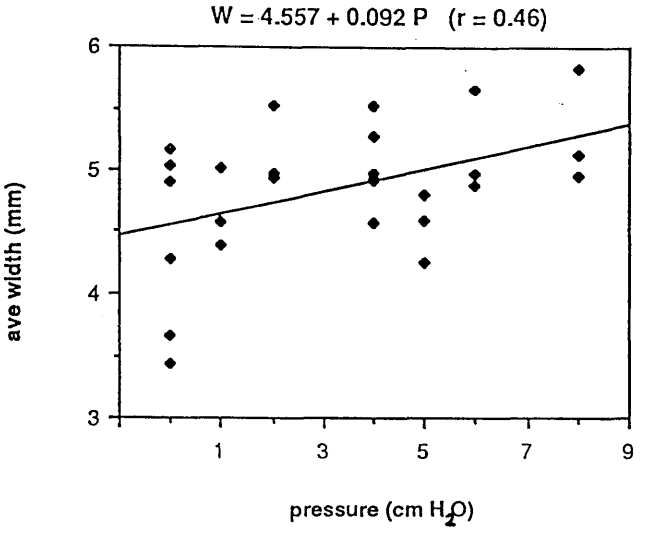


Fig. 19 Graph of average width (W) against intraluminal pressure (P).

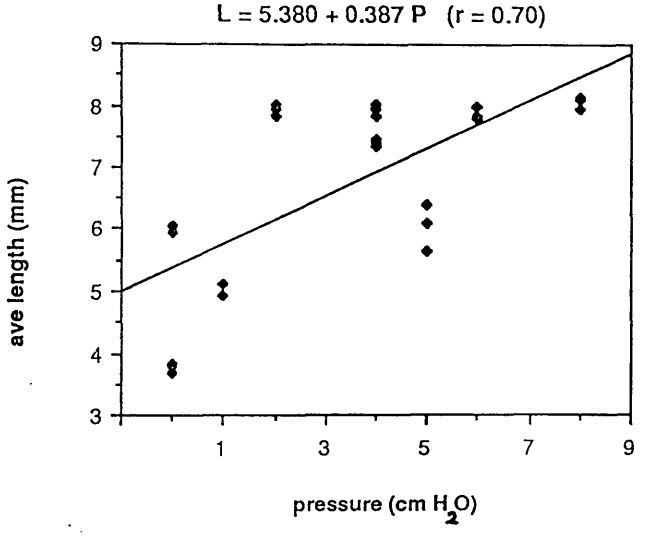


Fig. 20 Graph of average length (L) against intraluminal pressure (P).

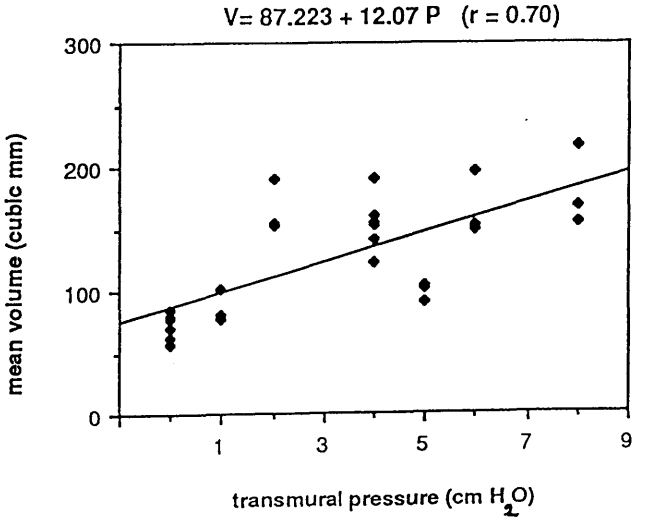


Fig. 21 Graph of average volume (V) against intraluminal pressure (P).

Using zones in which the length and width movements had the same period, the relationship between length and width was investigated. Using all the data recorded for each zone the best description from any set of data was found with a fourth order polynomial, giving the equation for this zone that:-

$$\text{Width} = 576\text{xlength} - 0{\cdot}184\text{xlength}^2 + 2{\cdot}6\text{x}10^{-4}\text{xlength}^3 - 1{\cdot}37\text{x}10^{-7}\text{xlength}^4 - 668$$

(r= 0·632) [measurements in mm].

SECTION 3.4 Analysis of Period

The period of oscillation was analysed using univariate and bivariate analyses from the Biomedical Data Processing statistics package (B.M.D.P. Statistical Software Inc.). This gave the period for the width and length movements in each zone and the phase relationship between them. These statistics are outside the capabilities of small statistical packages. In order to carry them out the data was transferred to a large mainframe computer.

The length and width periodicities were similar within most zones. (r=0·87) (Fig. 22). Some zones had the same periods of movement in length and width, some had not. A zone with the same periodicity in length and width would have a constant phase difference between its two movements, but the phase difference could vary between such zones. Obviously in a zone, where length and width movements had different periods, the movements would occur in and out of phase over a given period of time. Different zones within a single specimen could have different patterns of motility.

The influence of pressure on both length and width periods was best expressed linearly with increased pressure tending towards decreased period for both the length period (r=-0·57, 33df, P<0·1%) (Fig. 23) and width (r=-0·52, 33df, P=0·1%) (Fig. 24).

The differences in the length period and width period within a zone, expressed as the time for the movements to go out of and back into phase, ranged from the infinite (zones with length and width periods the same) through 647 sec to 15 sec. There was no clear relationship between pressure and this time (Fig. 25).

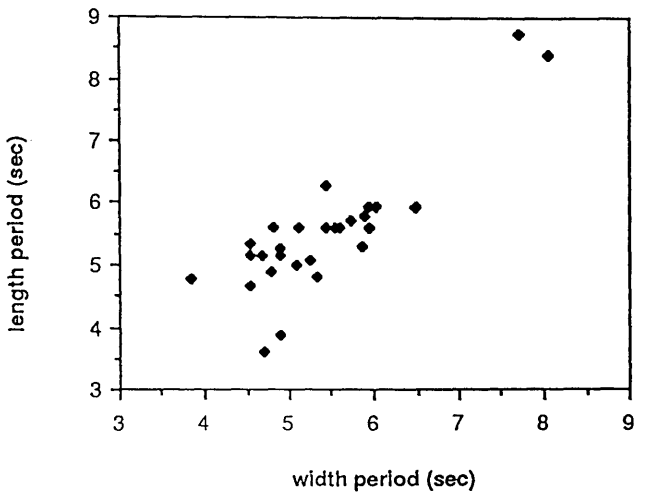


Fig. 22 Graph of length period against width period.

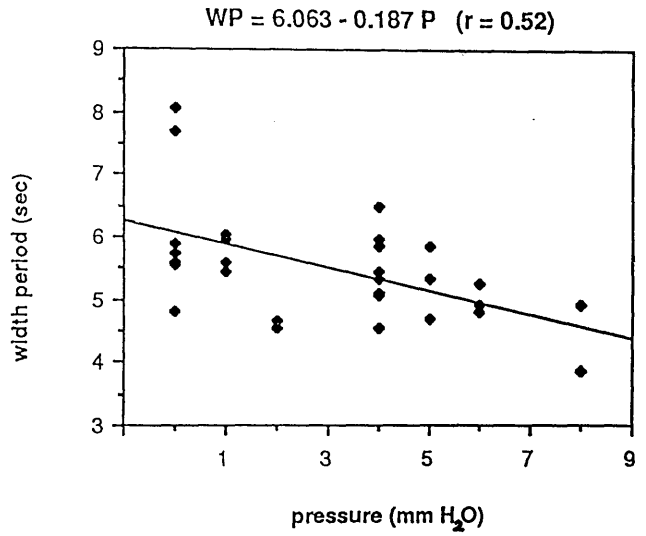


Fig. 23 Graph of width period (WP) against intraluminal pressure (P).

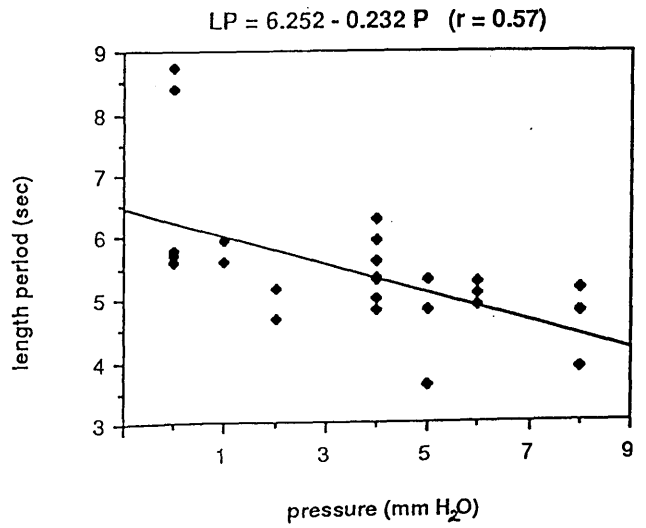


Fig. 24 Graph of length period (LP) against intraluminal pressure (P).

SECTION 3.5 Activity of Zones.

It was noticed that different specimens of tissue had different degrees of activity. In order to quantify this effect a 'coefficient of activity variation' was calculated, which was defined as the standard deviation of the measurement within a zone divided by its mean value. The activities along even a short stretch of intestine (each zone is approximately 5 mm long) varied quite considerably (Fig. 26) and there was no strong relationship between length and width coefficients (Fig. 27). Pressure over the range investigated (0–10 cm H_2O) appeared to have little effect on either length or width coefficient.

SECTION 3.6 Discussion of Results

The general agreement between the results from the image analysis system with those used by earlier workers is reassuring.

Other investigators have noted the stimulating effect of intraluminal pressure on the intestine (Bayliss & Starling, 1900; Feldberg & Lin, 1949; Kosterlitz, Pierce & Robinson; 1955, 1956; Kosterlitz, 1956; Hardcastle & Mann, 1968; McKirdy, 1968 and others) which in this project was found to reduce the period of contractions but not the 'activity coefficient'. The period of contraction of the colon measured using image analysis during the course of this project (4·8–8·8 sec) also agrees with results obtained in other studies using different methods (Bayliss & Starling, 1900; Feldberg & Lin, 1949; Eickholt *et al.*, 1967; McKirdy & Mackenna, 1972).

Using univariate, bivariate and other statistical analyses with large data sets showed some interesting phenomena. Small differences in period of length and width movements could be quantified for each short zone. Some of the zones can take over ten minutes to move out of and into phase. Since many earlier investigators took measurements over relatively short periods of time, they would find, with the accuracy of quantification available to them, that length and width movements had the same period and a constant phase difference. It is possible that this may be the explanation of the differences in results found by different investigators, but it is curious that some investigators consistently found the same phase difference. Their preparations may have suffered less degeneration than the ones tested here and had retained a greater degree of co-ordination.

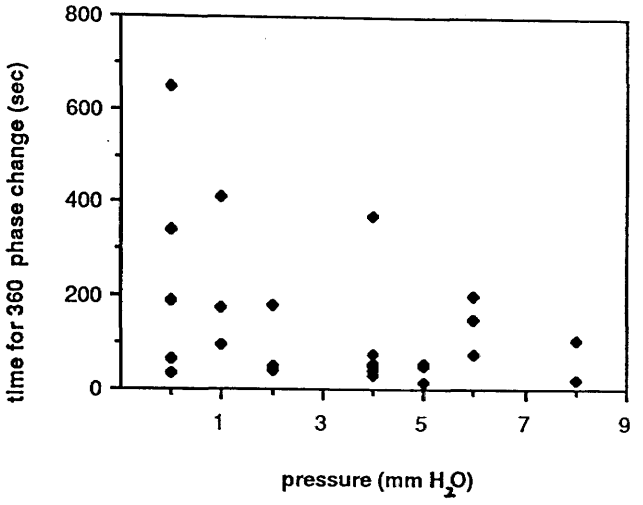


Fig. 25 Graph of time for phase shift of 360° against intraluminal pressure.

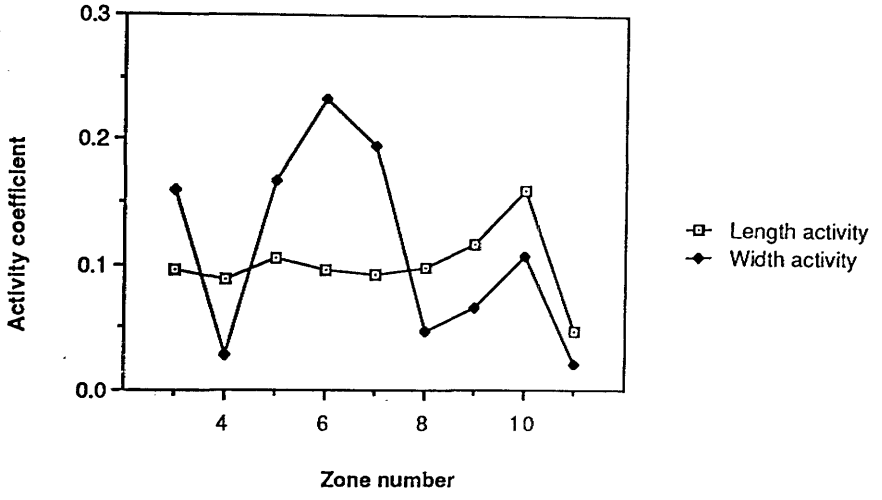


Fig. 26 Graph showing 'activity coefficient' along the length of a tissue samples. This example shows the degree of variability in the tissues.

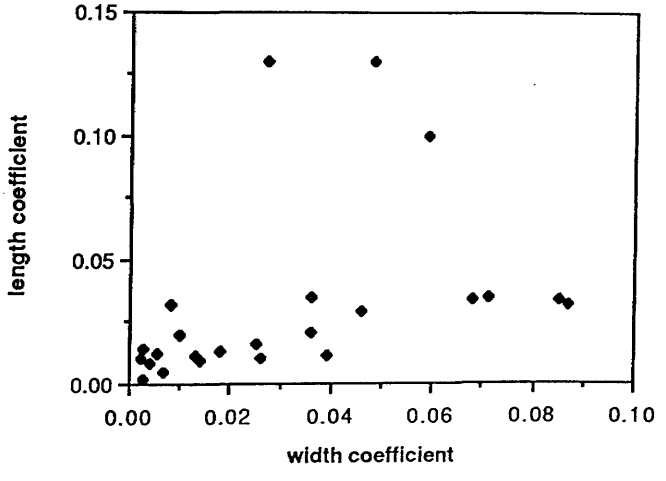


Fig. 27 Graph of length coefficient against width coefficient.

The ability of the image analysis system to analyse in detail a length of intestinal preparation as a series of contiguous zones allows the detection of non-homogeneity in the intestine. Not only have differences in period been detected within and between zones from the same tissue sample, but variation in resting, and pressure-stimulated activity has also been measured. The intestinal activity along the length of the preparation does not seem to vary systematically, in the limited number of samples studied. Active zones were sometimes located next to quiescent ones and on occasions propagated activity was detected. Further study, using this system, is required to interpret these phenomena and to attempt to determine if these are artefacts of the preparation.

CHAPTER 4: CONCLUSIONS

SECTION 4.1 Aims and Achievements of This Project.

The aim of this research was to develop a system which compared with earlier methods was an improvement in:-

1) Accuracy of measurement;
2) Speed of analysis;
3) Ease of data collection and quantitative analysis;
4) Ability to analyse over long periods of time whilst preserving temporal resolution;
5) Ability to analyse the preparation as a series of contiguous small zones;
6) Objectivity of measurement;
7) Flexibility of data handling for graphical display and statistical analysis;
8) Minimum interaction between preparation and measuring equipment;
9) Values obtained independent of lighting intensity.

As has been described in the results section all these were achieved, and in testing the system various tentative statements about the nature of colonic motility in the rabbit could be made.

SECTION 4.2 Future Development

This image analysis technique has considerable potential in the analysis of muscle movement. In addition to analysing gut movements (Clarke & Moss, 1983, 1986; Brodie *et al.*, 1987), programs using similar algorithms have been developed for the Magiscan 2 image analyser to investigate other aspects of muscular movement. Tissues studied include skeletal muscle (Bell, Boyd & Moss, 1982), *vas deferens* (Moss, 1984) and cardiac muscle (Moss, Miller & Lamont, 1986). Other smooth muscle preparations, such as esophagus or blood vessels, could be analysed by the existing program. The more complex movements of stomach and uterus could be analysed by developing the system further. To date only isolated preparations have been studied using this system, but in principle an *in vivo* analysis could be carried out.

The analysis of the locomotion of animals, such as segmented worms, could also be improved by the application of these techniques.

The program developed during this project is suitable for the study of the actions of neurotransmitters and other pharmacological agents, electrical transmural stimulation and stimulation of the extrinsic nerves on the preparation. An important aspect which could be investigated is the observation reviewed by Bennett (1968) that the addition of drugs directly into the waterbath may give different results than agents delivered intraluminally or via the vascular system or *in vivo*. An examples of this is morphia which does not effect muscle strips when added to the bath but has inhibitory effects when perfused through the vasculature. The work of Klinge (1951) and Bulbring, Lin and Schofield (1958) showed that intestinal preparations were most active if the inside of the preparation was oxygenated. If the outside (serosal) surface was oxygenated and the inside was allowed to become anoxic, the activity decreased. These phenomena would be easily investigated since in the preparation used in this project the tissue effectively formed a chamber, the inside of which was the intestinal mucosa, which could be separately perfused.

REFERENCES

Alvarez, W.C. & Zimmerman, A. (1927) The absence of inhibition ahead of peristaltic rushes. *Am. J. Physiol.* **83** 52-59.

Alvarez, W.C. & Mahoney, L.J. (1924) Peristaltic rush in the rabbit. *Am J. Physiol.* **69** 211-225.

Ballard, D.H. & Brown, C.M. (1982) 'Computer Vision', New Jersey, Prentice-Hall.

Bayliss, W.M. & Starling, E.H. (1899) The movements and innervation of the small intestine. *J. Physiol.* **24** 99-143.

Bayliss, W.M. & Starling, E.H. (1900) The movements and innervation of the large intestine. *J. Physiol.* **26** 107-118.

Bell, K., Boyd, I.A. & Moss, V.A. (1982) On-line computerised image analysis of sarcomere length of intrafusal fibres in isolated cat muscle spindles. *J. Physiol.* **329** 18-19P.

Benmair, Y., Dreyfuss, F., Fishel, B., Frei, E.H. & Gilat, T. (1977) Study of gastric emptying using a ferromagnetic tracer. *Gastroenterology* **73** 1041-1045.

Benmair, Y., Fishel, B., Frei, E.H. & Gilat, T. (1977) Evaluation of a magnetic method for measurement of small intestinal transit time. *Am. J. Gastroenterology* **68** 470-475.

Bennett, A. (1968) Relationship between *in vitro* studies of gastrointestinal muscle and motility of the intestinal tract *in vivo*. *Am. J. dig. Dis.* **13** 410-414.

Bertaccini, G. (ed) (1982) 'Mediators and drugs in gastrointestinal Motility' Vol 1. ('Handbuch der experimentellen pharmakologie' Vol 59/1), Berlin: Springer-Verlag.

Bortoff, A. (1976) Myogenic control of intestinal motility. *Physiol. Rev.* **56** 418-434.

Boyd, I.A. & Moss, V.A. (1984) On-line measurement of the changes in sarcomere length in isolated living muscle spindles using computerised image analysis. *Proc. int. symp. Feedback and motor control*, Glasgow. p134.

Bradbury, S. (1979) Microscopical image analysis: problems and approaches. *J. Microsc.* **115** 137-150.

Bradbury, S. (1983) Commercial image analysers and the characterization of microscopical images. *J. Microsc.* **131** 203-210.

Brodie, W.E., Clarke, G.S., Elder, H.Y., Moss, V.A. & Stevenson, G.S. (1987) The analysis of gut motility by image analysis. *Proc Physiol Soc.*(Glasgow, June 1987) 18P.

Brodie, W.E. (1978) 'Sequence of muscle activity in transport peristalsis in gut' B.Sc. thesis, Department of Physiology, University of Glasgow.

Bucknell, A. (1966) 'Studies on the physiology and pharmacology of the colon of man and other animals' Ph.D. Thesis, University of London.

Bulbring, E., Lin, R.C.Y. & Schofield, G. (1958) An investigation of the peristaltic reflex in relation to anatomical observation. *Quart. J. exp. Physiol.* **43** 26-37.

Burnett, F.L. (1923) The intestinal rate and form of the feces. *Am. J. Roentgenol.* **10** 599-604.

Cannon, W.B. (1902) The movements of the intestines studied by means of the Röntgen rays. *Am. J. Physiol.* **6** 251-277.

Christensen, J., Glover, J.R., Macagno, E.O., Singerman, R.B. & Weisbrodt, N.W. (1971) Statistics of contractions at a point in human duodenum. *Am. J. Physiol.* **221** 1818-1823.

Clarke, G.S. & Moss, V.A. (1983) The application of image analysis techniques to the movements of the gut. Presented to the Scottish Electro-physiological Soc. meeting, Sept 1983.

Clarke, G.S. & Moss, V.A. (1986) The coordination of the muscle layers in the isolated rabbit colon. *Proc. R. Microsc. Soc.* **21** S81.

Cole, L.G. & Einhorn, M. (1910) Radiograms of the digestive tract by inflation with air. *N. York Med. J.* **42** 705-708.

Corazziari, E. (1982) *In vivo* techniques for the study of gastrointestinal motility. In: Bertaccini, G. (ed) 'Mediators and drugs in gastrointestinal Motility' Vol 1. ('Handbuch der experimentellen pharmakologie' Vol 59/1) 181-204. Berlin: Springer-Verlag.

Currie, G.C. & Henderson, V.E. (1926) A study of the movements of the large intestine in the guinea-pig. *Am. J. Physiol.* **78** 287-298.

Davenport, H.W. (1977) 'Physiology of the digestive tract', Chicago: Year Book Publishers (4th edition) p 75.

Dixon, R.N. (1977) Delineation and Feature extraction. *Proc. SPIE* **130** 15.

Eickholt, T.H., Box, R.H. & Courville, N.D. (1967) Activity of different segments of rabbit gastrointestinal tract. *J. pharm. Sci.* **56** 1328-1330.

Elder, H.Y. & Trueman, E.R. (1980) Aspects of animal movement. *Soc. exper. Biol. Seminar Series* **5** 71-92 (fig. 4f 82-83) Camb. Univ. Press.

Eley, K.G., Bennett, A. & Stockley, H.L. (1977) The effects of prostaglandins E_{1_1} E_{2_2} F_{1c} and F_{2c} on guinea-pig ileal and colonic peristalsis. *J. Pharmac. Pharmacol.* **29** 276-280.

Elliot, T.R. & Barcley-Smith E. (1904) Antiperistalsis and other muscular activities of the colon. *J. Physiol.* **31** 272-304.

Feldberg, W. & Lin, R.C.Y. (1949) The action of local anaesthetics and d-tubocurarine on the isolated intestine of the rabbit and the guinea-pig. *Brit. J. Pharmac. Chemother.* **4** 33-44.

Frigo, G.M. & Lecchini, S. (1970) An improved method for studying the peristaltic reflex in isolated colon. *Br. J. Pharmacol.* **39** 346-356.

Garry, R.C. (1933) 'Observations on the caudal end of the large bowel' D.Sc. Thesis, University of Glasgow.

Garry, R.C. (1934) The movements of the large intestine. *Physiol. Rev.* **14** 103-132.

Gebauer, A., Lissner, J. & Schott, O. (1967) 'Roentgen television' New York, London: Grune & Stratton.

Glasser, O. (1945) 'Dr. W.C. Röntgen', Springfield Illinois: Charles. C. Thomas.

Grundy, W., Scratchard, T. & Scratchard, T.J. (1983) Computer aided analysis of gastrointestinal motility. *J. Physiol.* **342** 4P.

Hansky, Y.J. & Connell, A.M. (1962) Measurements of gastrointestinal transit using radioactive chromium. *Gut* **3** 187-188.

Hardcastle, J.D. & Mann, C.V. (1968) Study of large bowel peristalsis. *Gut* **9** 512-520.

Harris, L.D. & Pope, C.E. 2nd. (1964) 'Squeeze' Vs Resistance, an evaluation of the mechanism of sphincter competence. *J. clin. Invest.* **43** 2272-2278.

Harvey, R.F., MacKie, D.B., Brown, N.J.G., Keeling, D. & Davies, W.T. (1970) Measuring gastric emptying using a gamma camera. *Lancet* **1** 16-18.

Hightower, N.C. (1968) 'Motor action of the small bowel' in: Code, C.F. (ed) 'Alimentary Canal' (*Handbook of Physiology,* **Vol IV**, sect 6) 2001-2024 Washington D.C.: American Physiological Society.

Hinton, J.M., Lennard-Jones, J.E. & Young, A.W. (1969) A new method for studying gut transit time using radioopaque markers. *Gut* **10** 842-847.

Hollis, J.B. & Castell, D.O. (1972) Amplitude of esophageal peristalsis as measured by rapid infusion. *Gastroenterology* **63** 417-422.

Hukuhara, T. (1931) The normal movements of the small intestine investigated by means of the abdominal window and cinematography. *Physiol. Abstr.* **16** 469

Hukuhara, T. & Neya, T. (1968) The movements of the colon of rats and guinea-pigs. *Jap. J. Physiol.* **18** 551-562.

Hunter, R.D., Moss, V.A. & Elder, H.Y. (1983) Image analysis of the burrowing mechanisms of *Polyphysia crassa* and *Priapulus caudatus*. *J. Zoology* **199** 305-323.

Ishizawa, M. & Miyazaki, E. (1973a) Actions of prostaglandins on gastrointestinal motility. *Sapporo Med. J.* **42** 366-373.

Ishizawa, M. & Miyazaki, E. (1973b) Effect of prostaglandins on the movement of guinea-pig isolated intestine. *Jpn. J. smooth muscle Res.* **9** 235-237.

Ishizawa, M. & Miyazaki, E. (1975) Effect of prostaglandin $F_{2\alpha}$ on the propulsive activity of the isolated segmental colon of the guinea-pig. *Prostaglandins* **10** 759-768.

Jacoby, H.I., Bass, P. & Bennet, D.R. (1963) *In vivo* extraluminal contractile force transducer for gastrointestinal muscle. *J. Appl. Physiol.* **18** 658-665.

Jenkinson, G. (1985) Image analysis takes off. *Nature* **316** 87-88.

Johnson, I.T. (1976) An alternative method of animal sacrifice, effect of intestine *in vitro*. *Experimentia* **33** 347-348.

Klinge, F.W. (1951) Behavour of isolated intestinal sections without one or both plexuses. *Am. J. Physiol.* **164** 284-293.

Kosterlitz, H.W., Pirie, V.W. & Robinson, J.A. (1955) Contractions of longitudinal muscle of isolated guinea-pig ileum by increasing pressure in lumen. *J. Physiol.* **128** 8-9P.

Kosterlitz, H.W., Pirie, V.W. & Robinson, J.A. (1956) The mechanism of the peristaltic reflex in the isolated guinea-pig ileum. *J. Physiol.* **133** 681-694.

Langley, J.N. & Magnus, R. (1905) Some observations of the movements of the intestine before and after degenerative section of the mesenteric nerves. *J. Physiol.* **33**

Lee, C.Y. (1960) The effect of stimulation of extrinsic nerves on peristalsis and on the release of 5-Hydroxytryptamine in the large intestine of the guinea-pig and the rabbit. *J. Physiol.* **152** 405-418.

Luboshez, B.E. (1931) Cineradiogifia. *Radiol. Med.* **18** 450-457.

Mackenna, B.R. & McKirdy, H.C. (1970) A simple method for investigating the functional relationship of the longitudinal and circular layers of the muscularis externa of the rabbit bowel using a flat preparation. *J. Physiol.* **211** 18P.

Mackenna, B.R. & McKirdy, H.C. (1972) Peristalsis in the Rabbit distal colon. *J. Physiol.* **220** 33-54.

McKirdy, H.C. (1968) 'Factors influencing the movements of the distal colon of the rabbit' Ph.D. Thesis, University of Glasgow.

McKirdy, H.C. (1972) Functional relationship of Longitudinal and circular layers of *muscularis externa* of rabbit large intestine. *J. Physiol.* **227** 839-853.

Millhon, W.A., Hoffman, D.E., Jarvis, P,. Croff, J., Milhon, J.S. & Crites, N.A. (1968) Preliminary report on Millhon-Crites intraesophageal motility probe. *Am. J. dig. Dis.* **13** 929-934.

Moss, V.A. (1981) Computer-linked planimeters: some hints and algorithms. *Proc. R. Microsc. Soc.* **16** 120-124.

Moss, V.A. (1982a) Stereological measurements and image analysis using a computer-linked planimeter. *J. Physiol.* **329** 14-15P.

Moss, V.A. (1982b) Analysis of muscular movements of gut peristalsis using a computer-linked planimeter *J. Physiol.* **329** 15-16P.

Moss, V.A. (1984) The use of computerised image analysis to measure the mechanical response of the isolated rat *vas deferens*. *J. Physiol.* **355** 5P.

Moss, V.A. (1985) The use of image analysis for real-time analysis of muscle movements. The Fourth European Symposium for Stereology ed. B. Karlsson, R. Warren & J. Wasen. 443-446 (Gothenburg, ISBN 91-7032-202-3); The application of real-time image analysis to measure the mechanical response of muscle. *Acta Stereol.* **5** 267-272.

Moss, V.A., Miller, D.J. & Lamont, C. (1986) Real-time analysis of sarcomere length during activation of chemically-skinned cardiac muscle. *Proc. R. Microsc. Soc.* **21** S81.

Nelson, T.S. & Angell, J.B. (1979) Microminiature force transducers for chronic *in vivo* use. Abstracts 7th International Symposium on Gastrointestinal Motility, 55 University of Iowa, Iowa City .

Pascaud, X.B., Benton, M.J.H. & Bass, P. (1978) Miniature transducer for recording intestinal motility in unrestrained chronic rats. *Am. J. Physiol.* **235** E522-E537.

Poggio, T. (1984) Vision by man and machine. *Scientific American* **250** 68-78.

Raiford, T. & Mulinos, M.G (1934a) Intestinal activity in the exteriorised colon of the dog. *Am. J. Physiol.* **110** 123-128.

Raiford, T. & Mulinos, M.G (1934b) The myenteric reflex as exhibited by the exteriorised colon of the dog. *Am. J. Physiol.* **110** 129-136.

Ritchie, J.A. (1968) Colonic motor activity and bowel function *Gut* **9** 442-456.

Rothman, M.M., & Katz, A.B. (1964) 'Analysis of feces. Carmine or charcoal bowel motility test' in: 'Gastroenterology' Bockus, H.L. (ed), 2nd ed, Vol 2. 695-696. Philadelphia.

Roswick, R.P., Stedford, R.D. & Brooke, B.N. (1969) New methods for studying intestinal transport time. *Gut* **8** 195-196.

Sabotini, L. (1909) Nouvo metodo per osservare le intestina. *Arch. di Fisiol. Firienze.* **6** 265-268.

Schneller, F. (1925) Zur Physiologie der Dünndarmbewegungen. *Pflügers Arch. ges Physiol.* **209** 177-217.

Singer, C. (1959) 'History of Biology' 3rd Ed. London: Abelard-Schuman.

Singerman, W.B. (1970) Time and space correlation of human small bowel contractions methods of detection and computer analysis. *Physiologist* **13** 309.

Steggerda, F.R. & Gianturco, C. (1936) Visualisation of different organs in normal unanaesthetised animals. *Science* **84** 582.

Tasaki, K. & Farrar, J.T. (1969) Mechanics of small intestine muscle function in the dog. *Am. J. Physiol.* **217** 1224-1228.

Trendelenburg, P. (1917) Physiologische und pharmakologische versuche über die dünndarmperistalti. *Arch. Exp. Path. Pharmak.* **81** 55-129.

Waller, S.L. (1975) Differential measurements of small and large bowel transit times in constipation and diarrhoea, a new approach. *Gut* **16** 372-378.

Wilkinson, R. (1971) Polyethylene glycol 4000 as a continuously administered non-absorbable fecal marker for metabolic balance studies in human subjects. *Gut* **12** 644-660.

Wingate, D.L., Sondberg, R.J. & Phillips, S.F. (1972) A comparison of stable and ^{14}C-labelled polyethylene glycol or volume indicators in the human jejunum. *Gut* **13** 812-815.

Wolf, B.S. & Khilnani, M.T. (1966) Progress in gastroenterological radiology. *Gastroenterology* **51** 542-549.

Wolf, B.S., Heitman, P. & Cohen, B.R. (1968) The inferior esophageal sphincter, the manometric high pressure zone and hiatal incompetence. *Amer. J. Roentgen.* **103** 251-276.

Main Image Analysis Program.

```
Program GUT;                          { Source file GUT28.TEXT }

Uses  M2TYPES,M2IPROOT,M2SYS,REALFUNC,VIDEO,FEATURE,
        GRAPHICS,TIMER,               {System Library}
      {$U #5:S4G.CODE} S,             {User Library}
      {$U #5:G6CODE.CODE} GM;         {Microcode Library}

Type  Equiv = Record
      Case INTEGER of
        0: (R:Real);
        1: (I1,I2:Integer)
        end;

Var   I,J,K,L,N,Y,M,C,G,Baud,Max,Min,Summits,NPhoto,
          NSmooth,PLine,PWord,GLine,GWord,NPut,NGet,NChar,
          Range,VMinD,VMinW,HMinD,HMinW,MaxBand,MaxPhoto :Integer;
      G1Img,G2Img,GreyImg,TextImg,BinIm,DataImg,B3Img    :Image;
      Total,VBox,HBox,Wind                               :Pointset;
      Pt                                                 :Point;
      Title                                              :String;
      DiscName                                           :String [5];
      GOn,Anal,Colour,Slow,BandW,Plot                    :Boolean;
      REdge,LEdge,TInt,Time,Time0,Magnif,Z               :Real;
      VDist                                              :Array[0..31] of Real;
      Vert,VAns,Hori,HAns,GData,PData                    :Imhst;
      R2I                                                :Equiv;
      YMax,YMin,YStep,TMax,TMin,TStep                    :Real;
      YSpan,TSpan,XOrig,YOrig                            :Integer;
```

```
{
        ---------====--------
        |       |  |       |                          Vert.   Hori.
        |       |  |       |<-Total                   -----   -----
        |       |  |<-VBox|    box   Window     - VBox     HBox
        |       |  |       |          Data array- Vert     Hori §
        |       |__|       |          Minima     - VAns     HAns
        |       |__|<-HBox|          Real mins.- VDist    LEdge
        |       |  |       | REdge
        |       |  |       |            § 1st differential of data
        ---------====--------


        _____          _____
             .\--------/.          For a peak to be accepted
               \       / ^         it must be deep enough so
                \     /  |         that at ±MinW pixels from
                 \   /   |MinD      the lowest value Min the
           Min____\ /    v          value in the data array is
              <---><----> ±MinW     greater than Min+MinD.

Data array:-
NPhoto=Data[0],          TInt=Data[1] and [2],      Magnif=Data[3] and [4],
MaxBand=Data[5],         Line=Data[6],              VBox.height=Data[7],
VBox.width=Data[8],      HBox.width=Data[9],        VMinW=Data[10],
VMinD=Data[11],          HMinW=Data[12],            HMinD=Data[13],
NSmooth=Data[14].
}

Procedure TEST;      Forward;
Procedure ANALYSE;   Forward;

(*****************************************************************)
```

```
Procedure READKEY;

{ Read one character from keyboard }

Begin
Repeat TEST until KEYPRESS;
READ(Key);     WRITE(CHR(7)); OrdKey:=ORD(Key);
If (OrdKey=27) then
  begin
  REDIRECT ('#1:');     BAUDRATE (9600);
  DOTSIZE (1,2,-1);     EXIT(Program)
  end;
If (Key in ['a'..'z']) then OrdKey:=OrdKey-32;
Key:=CHR(OrdKey)
end;

(*********************************************************************)

Function READINT:Integer;

{ Read integer number from keyboard }

Var     Int,X,Y                 :Integer;

Begin
CURSORXY (X,Y); Int:=0; NChar:=0;
  Repeat
  READKEY;
  If (Key in ['0'..'9']) then
    begin Int:=Int*10+OrdKey-48; Nchar:=Nchar+1 end
  else if NOT (Key in[' ',Chr(13),'.']) then
    begin
    Int:=0; Nchar:=0; GOTOXY (X,Y);  WRITE('          ');
    GOTOXY (X,Y)
    end;
  until (Key in [' ',Chr(13),'.']);
READINT:=Int
end;

(*********************************************************************)

Function READREAL:Real;

{ Read real number from keyboard }

Var     I,J     :Integer;
        R       :Real;

Begin
I:=READINT;
If (Key='.') then
  begin
  R:=READINT;  For J:= 1 to NChar do R:=R*0.1
  end;
READREAL:=R+I
end;

(*********************************************************************)

Procedure PUTVAL (Val:Integer);

{ Put a value into the image memory }

Begin
PData[PWord]:=Val;  NPut:=NPut+1;   PWord:=PWord+1;
If (PWord=512) then
  begin
  PWord:=0;  PUTLINE (DataImg,PLine,PData);
  If (PLine>804) then
    if (PLine=814) then
      begin
      WRITELN (CHR(home),'******WARNING- Disc Data limit ******');
     ANALYSE
      end
    else
      WRITE (CHR(home),814-PLine,' lines free in data buffer')
```

```
  else PLine:=PLine+1
  end
end;

(*************************************************************************)

Function GETVAL:Integer;

{ Get a value from image memory.  N.B. GLine should point to NEXT line }

Begin
TEST;
If (GWord=0) then
   begin
  GETLINE (DataImg,GLine,GData);  GLine:=GLine+1
   end;
 GETVAL:=GData[GWord];  NGet:=NGet+1;  GWord:=(GWord+1) mod 512
 end;

 (*************************************************************************)

 Procedure LIVEIM;

 { Show live image }

 Begin
 LIVE (GreyImg,InTab,OutTab);  TEXTIMAGE (TextImg);
 OVLAY (G2Img,Y);   OVLAY (G1Img,M);   OVLAY (TextImg,C)
 end;

 (*************************************************************************)

 Procedure OUTPUT;

 { Switch display of live picture from B/W to Pseudocolour }

 Const Max=32767;

Var    I,J     :Integer;

Begin
Colour:=NOT Colour;
If Colour then
  begin
  With OutTab do
    begin
    For I:=0 to 255 do
      begin  redtab[I]:=0;  greentab[I]:=0;  bluetab[I]:=0  end;
    For I:=1 to 51 do
      begin  redtab[I]:=Max;  greentab[I]:=(I-1)*642  end;
    For I:=52 to 102 do
      begin  greentab[I]:=Max;   redtab[I]:=(102-I)*642  end;
    For I:=103 to 153 do
      begin  greentab[I]:=Max;   bluetab[I]:=(I-103)*642  end;
    For I:=154 to 204 do
      begin  bluetab[I]:=Max;   greentab[I]:=(204-I)*642  end;
    For I:=205 to 255 do
      begin bluetab[I]:=Max;   redtab[I]:=(I-205)*642 end;
    end
  end
else OutTab:=InTab;
LIVEIM;
If NOT Anal then
  begin
  PHOTO;  DISPLAY (GreyImg,OutTab);  DEFWINDOW (Wind,0,0,8,16);
  With Wind.origin do
     for I:=0 to 63 do begin  x:=I*8;  DRAW (Wind,GreyImg,I)  end;
  READKEY;  LIVEIM
  end
end;

(*************************************************************************)
```

```
 {$I  #5:G3G.TEXT}      { see below }

{ Subroutine DISP for Graphics display.
 G3.TEXT for Tektronix output, G3G.TEXT for Gould/H.P. output.
  N.B. Needs corresponding library S3.CODE or S3G.CODE }

(*********************************************************************)

Procedure SAVEDATA;

{ Save data in image memory on disc }

Var    FL     :FILE;

Begin
If Anal then ANALYSE;
If NOT Anal then
  begin
  WRITE ('SAVE data in file '); READLN (title);
  RESET (FL,Title); I:=IORESULT;
  If I=0 then
    begin
    WRITE ('File already exists, Overwrite? ');
   READKEY;
    If (Key='Y') then I:=1
    end;
  CLOSE(FL);
  If I<>0 then
    begin
    REWRITE (FL,Title);
    If IORESULT=0 then
      begin
      GETLINE (DataImg,0,PData);
       R2I.I1:=PData[3];  R2I.I2:=PData[4];
       If (R2I.R<>Magnif) then
         begin
          WRITELN;  WRITE (' Change Mag. from ',R2I.R:7:2,' to ',Magnif:7:2,' [Y/N] ');
         READKEY;
         If (Key='Y') then
           begin
            R2I.R:=Magnif; PData[3]:=R2I.I1; PData[4]:=R2I.I2;
           PUTLINE(DataImg,0,PData)
           end
         end;
       For J:=0 to PLine do
         begin
         GETLINE (DataImg,J,PData);
         If (BLOCKWRITE(FL,PData,2)<>2) then
           begin  WRITE (CHR(31),'Q Bad data write');  CLOSE (FL,purge)   end
         else WRITE (CHR(31),'W Line ',J:3)
         end;
       CLOSE (FL,lock)
       end
     else
       begin WRITE('Bad data file, Disc full?');  CLOSE(FL{,purge})  end
     end
   end
end;

(*********************************************************************)

Procedure READDATA;

{ Recall data from disc into image meory }

Var    FL     :FILE;

Begin
If Anal then ANALYSE;
If NOT Anal then
  begin
  If (Title='Live') then
    begin
    WRITE ('Current data not saved.   Continue?');  READKEY;
    If (Key='Y') then Title:='Saved'
    end;
```

```
If (Title<>'Live') then
  begin
 WRITE ('RECALL from file '); READLN (Title);
 RESET (FL,Title);
 If (IORESULT=0) then
    begin
   If (BLOCKREAD(FL,GData,2)=2) then
      begin
      NPhoto:=GData[0];
      R2I.I1:=GData[1];      R2I.I2:=GData[2];          TInt:=R2I.R;
      R2I.I1:=GData[3];      R2I.I2:=GData[4];          Magnif:=R2I.R;
      MaxBand:=GData[5];  GLine:=GData[6];
       VBox.height:=GData[7];              Total.height:=VBox.height;
       VBox.width:=GData[8];               HBox.width:=GData[9];
       Total.width:=HBox.width;            VMinW:=GData[10];
       VMinD:=GData[11];    HMinW:=GData[12];
       HMinD:=GData[13];    NSmooth:=GData[14];
       PUTLINE (DataImg,0,GData);
        WRITELN (NPhoto,' Photos ', TInt:5:2,'sec/photo.  Max. bands ',MaxBand);
       For I:=1 to GLine do if BLOCKREAD(FL,GData,2)=2 then
          begin
          WRITE (CHR(31),'W Line ',I:3);  PUTLINE (DataImg,I,GData)
          end
       end
     else WRITE ('BAD Data READ')
     end
    else WRITE ('File ',Title,' not found');
   CLOSE(FL)
   end
  end
end;

(*************************************************************************)

Procedure VALUES;

{ Print out values of data from image memory }

Var    I,J,N0,N1,X,Y,P1,P2        :Integer;
       LP                         :Boolean;
       R                          :Real;

Begin
If NOT Anal then
  begin
  GETLINE (DataImg,0,GData);NPhoto:=GData[0];
 {R2I.I1:=GData[1];    R2I.I2:=GData[2];      TInt:=R2I.R;
  R2I.I1:=GData[3];    R2I.I2:=GData[4];      Magnif:=R2I.R;
  MaxBand:=GData[5];   GLine:=GData[6];}
  end
else PUTLINE (DataImg,PLine,PData);
WRITE (NPhoto,' Photos- values from no. ');   N0:=READINT;
If (N0<=0) then begin N0:=1; WRITE ('1') end;
WRITE (' to ');         N1:=READINT;
If (N1<N0) or (N1>NPhoto) then begin N1:=NPhoto; WRITE (N1) end;
WRITELN;        WRITE ('Output to Printer or Console ');
READKEY;        LP:=Key='P';
If LP then REDIRECT ('#6:')
else if (N1>N0+10) then N1:=N0+10;
WRITELN;       TEST;
WRITELN (Title,'  Time step',TInt:5:2,'secs.  Magnification x',
                              Magnif:9:4,' Max. bands ',MaxBand);
If LP then WRITELN ('VH=',VBox.height,' VW=',VBox.width,
  ' HW=',HBox.width,' Min:VW=',VMinW,' VD=',VMinD,' HW=',
  HMinW,' HD=',HMinD,' Sm=',NSmooth);

NGet:=2*MaxBand;                R:=2.0*N0*MaxBand;
GLine:=TRUNC(R/512.0);          GETLINE (DataImg,GLine,GData);
GWord:=ROUND(R-512.0*GLine);    GLine:=GLine+1;
For I:=N0 to N1 do if (Key<>'X') then
  begin
  While (NGet<2*MaxBand) do J:=GETVAL;  NGet:=0;
  If (I mod 5 =0) then
    begin
    If KEYPRESS then
      begin
```

```
  READKEY; READKEY;
  {If (Key='P') then begin REDIRECT ('#6:'); LP:=true end;}
   If (Key='C') then begin REDIRECT ('#1:'); LP:=false end
   end;
 WRITELN
  end;
Summits:=GETVAL;
If (Summits>MaxBand) then
  begin
  WRITE (Summits,' No. min. ',CHR(137));
 Summits:=READINT;  WRITELN
  end;
 WRITE (I:4,' #',Summits:3,' L: ');
P1:=GETVAL;
 For J:=2 to Summits do
  begin
 CURSORXY (X,Y);
 If NOT LP and (X>58) then WRITELN;
  P2:=GETVAL;  WRITE ((P2-P1)*0.02:6:1);  P1:=P2
  end;
 If NOT LP then
  begin WRITELN; WRITE ('       '); TEXTIMAGE (G1Img) end;
 WRITE ('  W: ');
 For J:=2 to Summits do
   begin
  CURSORXY (X,Y);
  If NOT LP and (X>59) then WRITELN;
  WRITE (GETVAL*.02:5:1)
  endM
 WRITELN;    TEXTIMAGE (TextImg)
 end;
If NOT LP then READKEY;
WRITE (CHR(ff));  CLEARIM (G1Img);  REDIRECT ('#1:')
end;

(*********************************************************************)
```

```
Procedure WINDOW;

{ Set up new windows for analysis }

Var    OldPt          :Point;

Begin
Key:='Y';
If Anal then
 begin WRITE ('Adjust WINDOW- Are you sure? ');  READKEY end;
If (Key='Y') then
 begin
 WRITE (CHR(home),'Adjust origin, width or height with Lightpen. `X" to exit');
  Repeat
  Repeat TEST until LPEN(Pt) or KEYPRESS;
   Repeat
   TEST;
   If (Pt<>OldPt) and NOT KEYPRESS and (Pt.x<511) then with Total,Total.origin do
    begin
     WRITE (CHR(31),' !Total',x:4,',',y:3,' h',height:4.
     ' w',width:4,'. VBox',VBox.origin.x:4,' w',VBox.width:4,'    ');
    FRAMEDRAW (Total,G2Img,0,2);  FRAMEDRAW (VBox,G2Img,0,1);
    If (Pt.y<y+height div 3) then
      if (Pt.x<(x+VBox.origin.x) div 2) then
       begin
        VBox.origin.y:=Pt.y;
         VBox.origin.x:=VBox.origin.x+Pt.x-x;
       origin:=Pt;
      end
     else
        begin  VBox.origin:=Pt;  y:=Pt.y  end
    else
      if (Pt.y>y+2*height div 3 +10) then
       begin
        height:=Pt.y-y;  VBox.height:=height
      end
     else
        if (Pt.x>(x+width+VBox.origin.x+VBox.width) div 2)
```

```
            then width:=Pt.x-x
        else
          if (Pt.x>VBox.origin.x) then
              VBox.width:=Pt.x-VBox.origin.x;
      FRAMEDRAW (Total,G2Img,1,2);  FRAMEDRAW (VBox,G2Img,1,1);
      OldPt:=Pt
      end
    until NOT LPEN(Pt)
   until KEYPRESS;
 READKEY;     HBox:=Total
 end
end;

(*********************************************************************)

Procedure CALIBRATE;

{ Calibrate input }

Var     XX,YY:Real;
        Pt      :Point;

Begin
  Repeat
 TEST;
  WRITELN (CHR(home),'x',Magnif:9:4,' Calibrate with light pen or type value ');
  Repeat TEST until KEYPRESS or LPEN(Pt);
  If KEYPRESS then
    begin
   WRITE ('x '); XX:=READREAL;
    If (XX>0.0) then
      begin
      WRITE ('pixels/unit length');  Magnif:=XX
      end
    end
   else
    begin
    WRITE (CHR(7));  DEFWINDOW (Wind,Pt.x-1,Pt.y-1,3,3);
    DRAW (Wind,G2Img,1);  XX:=Pt.x;    YY:=Pt.y;
   Repeat TEST until NOT LPEN (Pt);
   Repeat TEST until LPEN (Pt);        WRITE (CHR(7));
    Wind.origin.x:=Pt.x-1;   Wind.origin.y:=Pt.y-1;
   DRAW (Wind,G2img,1);  XX:=XX-Pt.x; XX:=SQRT(XX*XX+YY*YY);
    WRITE (ROUND(XX),' pixels.  Ref. length ');
   YY:=READREAL;
    If (YY>0.0) then
      begin
      Magnif:=XX/YY;
       WRITE (' Mag.=',Magnif:9:4,' pixels/unit length')
      end
    end;
  WRITELN;    WRITE ('Press `X" to exit');
 READKEY
  until (Key='X') and (Magnif>0.0)
end;

(*********************************************************************)

Procedure SMOOTHER (Var LH:Integer; Var Data,Minima:Imhst;
                    Var No,MinD,MinW:Integer);

{ Repeat smoothing until number of minima constant }

Var     Pre,Stop                :Integer;

Begin
Stop:=0;      Pre:=0;
  Repeat
  No:=0;      GETMIN (LH,Data,Minima,No,MinW,MinD);
  If (Pre=No) then Stop:=Stop+1 else Stop:=0;
  Pre:=No
  until (Stop>=NSmooth)
end;

(*********************************************************************)
```

```
PROCEDURE LENGRAPH;

{ Display values for one photo }

Var    I,L     :Integer;

Begin
With Total do
  begin
  DEFWINDOW (Wind,origin.x+width+1,origin.y,510-origin.x-width,height+16);
  DRAW (Wind,TextImg,0);      DRAW (Wind,G2Img,0);
  If (Summits>0) then
    if (height div Summits<40) then DOTSIZE (1,1,-1);
  For I:=0 to Summits-1 do
    begin
    GOTOABS (origin.x+width,origin.y+ROUND(VDist[I])-6);
    WRITE (VDist[I]+Total.origin.y:6:2)
    end
  end;

DEFWINDOW (Wind,0,Total.origin.y,64,Total.height);
DRAW (Wind,G1Img,0);    DRAW (Wind,TextImg,0);
DEFWINDOW (Wind,0,0,1,1);  Min:=10000;  Max:=-Min;
For I:=0 to VBox.height-1 do
  begin
  If (Vert[I]<Min) then Min:=Vert[I];
  If (Vert[I]>Max) then Max:=Vert[I];
  Wind.width:=Vert[I] div VBox.width +1;
  Wind.origin.y:=I+VBox.origin.y;
  DRAW(Wind,G1Img,1)
  end;
WRITELN(CHR(31),' >V',Min:5,' to',Max:5);
Wind.width:=1;          Wind.height:=2*VMinW;
With Wind.origin do for I:=0 to Summits-1 do
  begin
  x:=(Vert[VAns[I*4]]+VMinD) div VBox.width +1;
  y:=VAns[I*4]+VBox.origin.y-VMinW;
  DRAW (Wind,TextImg,1)
  end
end;

(*****************************************************************)

PROCEDURE WWIND;

{ Measure the widths of each band found on one photo }

Var    I,J,K,L,M,N          :Integer;
       Wedge :Real;

  PROCEDURE WW0;

    begin
    DEFWINDOW (Wind,Total.origin.x,0,Total.width,64);
    DRAW (Wind,TextImg,0);    DRAW (Wind,G1Img,0);
    Min:=Hori[HAns[4]];       Max:=Hori[HAns[0]];
    Range:=Max-Min;           Wind.width:=1;
    If (Range<=0) then Range:=1;
    For I:=HMinW+1 to M-HMinW-1  do
      begin
      Wind.origin.X:=I+Total.origin.x+1;
      Wind.height:=ROUND((Hori[I]-Min)/Range*62.0)+2;
      DRAW (Wind,G1Img,1)
      end;
    WRITE(CHR(31),' ?H',Min-4096:5,' to',Max-4096:5);
    Wind.height:=1;   Wind.width:=2*HMinW;
    With Wind.origin do
      begin
      x:=HAns[0]+Total.origin.x+1-HMinW;
      y:=ROUND((Max-HMinD-Min)/Range*62.0)+1;
      DRAW (Wind,TextImg,1);
      x:=HAns[4]+Total.origin.x+1-HMinW;
      y:=ROUND(HMinD/Range*62.0)+1;
      DRAW (Wind,TextImg,1)
      end
    end;
```

```
Begin
If Slow then DRAW (Total,G1Img,0);
DEFWINDOW (Wind,0,0,1,1);
For N:=0 to Summits-2 do
  begin
   HBox.origin.y:=ROUND(VDist[N])+1+Total.origin.y;
  J:=ROUND(VDist[N+1]-VDist[N])-2;
  If (J>0) then
    begin
     HBox.height:=J;
    If Slow then FRAMEDRAW(HBox,G1Img,1,1);
    CROSSW(HBox,GreyImg,Hori);  M:=HBox.width-1;
    DIFFER(M,Hori);
    K:=1;       GETMIN (M,Hori,HAns,K,HMinW,HMinD);
     Z:=HAns[2]; If (Z<0.0) then Z:=65536.0-Z;
    If (Hans[3]>0) then LEdge:=HAns[1]+Z/HAns[3]
    else LEdge:=HAns[1];
     Z:=HAns[6]; If (Z<0.0) then Z:=65536.0-Z;
    If (HAns[7]>0) then REdge:=HAns[5]+Z/HAns[7]
                else REdge:=HAns[5];

    If GOn and (TInt>=2.5) then WW0;

     Wind:=HBox;      Wind.width:=1;
     If Slow then
       begin
        Wind.origin.x:=ROUND(REdge)+Total.origin.x+1;
       DRAW (Wind,G1Img,1);
        Wind.origin.x:=ROUND(LEdge)+Total.origin.x+1;
       DRAW (Wind,G1Img,1)
       end;

     If GOn then with HBox do
       begin
      TEXTIMAGE (G2Img);
        GOTOABS (origin.x+width,origin.y-6+height div 2);
        WRITE (origin.x+LEdge:6:2,origin.x+REdge:7:2,' =',
                        (REdge-LEdge)/Magnif:6:2);
      TEXTIMAGE (TextImg);
      If KEYPRESS then begin READKEY;  READKEY;
        If (Key in ['G','X']) then GOn:=false end
      end
    end;
   WEdge:=(REdge-LEdge);
   If (WEdge>655.34) or (WEdge <-655.34) then
     Begin
    WRITELN('WEdge ',WEdge,' LEdge ',LEdge);
    WEdge:=0;  READKEY
    end;
  PUTVAL (ROUND(50.0*WEdge))
  end;
DOTSIZE (1,2,-1)
end;

(*****************************************************************)

Procedure ANALYSE;

{ Start the analysis of a sequence of photos }

Begin
If Anal then
  begin
  WRITELN ('Stop data collection? ');   Key:='Y';
  If (PLine<814) and (NPhoto<MaxPhoto) then READKEY;
  If (Key='Y') then
    begin
     Anal:=false;
    PUTLINE (DataImg,PLine,PData);
    GETLINE (DataImg,PLine,PData);
    GETLINE (DataImg,0,PData);  PData[0]:=NPhoto;
    PData[6]:=PLine;            PUTLINE (DataImg,0,PData)
    end
  else WRITE ('Still collecting data')
  end
```

```
else
 begin
LIVEIM;
 WRITE ('No. of photos ');     MaxPhoto:=READINT;
 If (MaxPhoto=0) then MaxPhoto:=32767;  Title:='Live';
 FRAMEDRAW(Total,G2Img,1,2);   FRAMEDRAW(VBox,G2Img,1,1);
 GETLINE (DataImg,0,GData);    Key:='Y';
 If (GData[0]>0) then
   begin
  WRITE ('Start new data? '); READKEY
   end;
 If (Key='Y') then
   begin
   For I:=0 to 511 do PData[I]:=0;
   DRAW  (DataImg.whole,DataImg,0);
   NPhoto:=0;   PLine:=0;   PWord:=0;   NPut:=0;
   PUTVAL(0);
    R2I.R:=TInt;              PUTVAL(R2I.I1);          PUTVAL(R2I.I2);
    R2I.R:=Magnif;            PUTVAL(R2I.I1);          PUTVAL(R2I.I2);
   PUTVAL (MaxBand);          PUTVAL(0);
    PUTVAL (VBox.height);     PUTVAL (VBox.width);
    PUTVAL (HBox.width);      PUTVAL (VMinW);
    PUTVAL (VMinD);           PUTVAL (HMinW);
    PUTVAL (HMinD);           PUTVAL (NSmooth)
    end;
  Time:=0.05;   Time0:=0.0;   SETTIMER (0.0);  Anal:=true
  end
end;

(*************************************************************************)

Procedure TEST;

{ Check time for next photo }

Var    X,Y       :Integer;

Begin

If Anal and (READTIMER>Time0) then
  begin
  While (NPut<2*MaxBand) do PUTVAL(0);
  NPut:=0;     CURSORXY (X,Y);
  If (READTIMER>Time) then
          WRITE (CHR(31),'J?',READTIMER-Time:5:2)
  else if Slow then WRITE (CHR(31),'J?      ');
  Repeat until (READTIMER>Time);
  VLOAD (GreyImg);      NPhoto:=NPhoto+1;
  WRITE (CHR(31),'S?',NPhoto:4);
  Time:=Time+TInt;      Time0:=Time-0.05;
  AVWIND (VBox,GreyImg,Vert);
  SMOOTHER (VBox.height,Vert,VAns,Summits,VMinD,VMinW);
  If (Summits>MaxBand) then Summits:=MaxBand;
  PUTVAL (Summits);
  For I:=0 to Summits-1 do
    begin
    L:=I*4;  Z:=VAns[L+2];  If (Z<0.0) then Z:=65536.0-Z;
    If (Vans[L+3]>0) then VDist[I]:=VAns[L+1]+Z/VAns[L+3]
    else VDist[I]:=VAns[L+1];
   PUTVAL(ROUND(50.0*VDist[I]))
    end;
  If GOn then LENGRAPH;
  WWIND;     GOTOXY (X,Y);
  If (NPhoto>=MaxPhoto) then ANALYSE
  end
end;

(*************************************************************************)

Procedure BANDS;

{ Procedure to align bands
  NMinOld, LOld, WOld = Last (corr.) data, LOld[0] = top of VBox
  NMin,    LNew, WNew = Current (raw) data
           LAdj, WAdj = Current (corrected) data}

Var      I,J,JJ,K,KK,AbsD,NMinOld,NError,Shift,N,LL,
```

```
    NMin,Check,Dist,MaxB2          :Integer;
    LOld,WOld,LNew,WNew,LAdj,WAdj  :Array[0..63] of integer;

{=============================================================}

Procedure BANDERROR;

Begin
If (NError=NMinOld) then
 begin  LAdj:=LOld; WAdj:=WOld end     {No Bands Found}
else
 begin      {Some Bands Missing}
 J:=1;      {Bottom/screen/ Band Missing}
 While (LAdj[J]=0) do J:=J+1;  Dist:=LAdj[J]-LOld[J];
 For JJ:=1 to J-1 do
   begin
  LAdj[JJ]:=LOld[JJ]+Dist;  WAdj[JJ]:=WOld[JJ]
   end;

 N:=J;   JJ:=100;    {Middle Bands Missing}
 For K:=J+1 to NMinOld do
   begin
  If (LAdj[K]=0) then JJ:=K
  else
    begin
    If (JJ=K-1) then
      begin
    Z:=(LAdj[K]-LAdj[N])/(LOld[K]-LOld[N]);
      For JJ:=N+1 to K-1 do
        begin
      LAdj[JJ]:=LAdj[N]+ROUND((LOld[JJ]-LOld[N])*Z);
       WAdj[JJ]:=WAdj[N]
        end;
      JJ:=100
      end;
    N:=K
    end
  end;

 If (N<NMinOld) then
   begin    {End Bands Missing}
  Dist:=LAdj[N]-LOld[N];
   For JJ:=N to NMinOld do
     begin
    LAdj[JJ]:=LOld[JJ]+Dist;  WAdj[JJ]:=WOld[JJ]
     end
   end;
 WRITE ('Gaps ',NError:2);
 {For J:=1 to NMinOld do WRITE (LAdj[J]*0.02:6:1)}
 end
end;

{=============================================================}

Procedure BAND0;

Begin
WRITELN ('Aligning Bands');   MaxB2:=2*MaxBand;
GLine:=1;     GWord:=MaxB2;   NGet:=0;
PLine:=0;     PWord:=MaxB2*2; NPut:=MaxB2;
GETLINE (DataImg,0,GData);   NMinOld:=GETVAL;
 J:=VBox.origin.y+VBox.height;
LOld[0]:=J;     LNew[0]:=J;  LAdj[0]:=J;
For J:=NMinOld downto 1 do LOld[J]:=GETVAL;
For J:=NMinOld-1 downto 1  do WOld[J]:=GETVAL;
PData:=GData
end;

{=============================================================}

Begin  {BANDS}
If (NPhoto>0) then
 begin
 BAND0;
 For I:=2 to NPhoto do
   begin
```

```
 WRITE (CHR(31),'0 ',I:2);
While (NGet<MaxB2) do J:=GETVAL;
NGet:=0;   NMin:=GETVAL; WRITELN;
  If (NMin>MaxBand) or (NMin<0) then
  begin
  WRITE ('NMin=',NMin);   READKEY;
  WRITE (CHR(clearln));   NMin:=0
  end;
For J:=NMin downto 1 do LNew[J]:=GETVAL;
 LNew[NMin+1]:=-32767;
For J:=NMin-1 downto 1 do WNew[J]:=GETVAL;

 Check:=(LOld[1]-LOld[2]) div 3;   NError:=0; Shift:=0;
 N:=1;   JJ:=0;
 For J:=1 to NMinOld do
   begin
   AbsD:=32767;
   For K:=N to NMin do
     begin
     Dist:=LNew[K]-LOld[J]+Shift;
      If (Dist<0) then Dist:=-Dist;
      If (Dist<AbsD) then begin KK:=K; AbsD:=Dist end
      end;
    If (AbsD<Check) then
      begin
     LAdj[J]:=LNew[KK];      Shift:=LOld[J]-LNew[KK];
     If (KK=JJ+1) then WAdj[J]:=WNew[KK]
      else if (JJ>0) then
        begin
        Z:=0.0;   LL:=0;
        For K:=JJ to KK-1 do
          begin
         L:=LNew[K+1]-LNew[K];
          Z:=Z+1.0*WNew[K]*L;  LL:=LL+L
          end;
       WAdj[J-1]:=ROUND(Z/LL)
        end;
      JJ:=KK; N:=KK+1
        end
      else
        begin
        LAdj[J]:=0;      NError:=NError+1;
        end;
      Check:=(LOld[J]-LOld[J+1]) div 3
      end;
    If (NError>0) then BANDERROR;
    While (NPut<MaxB2) do PUTVAL (0);
    NPut:=0;   LOld:=LAdj;    WOld:=WAdj;
    PUTVAL (NMinOld);
    For J:=NMinOld downto 1 do PUTVAL (LAdj[J]);
    For J:=NMinOld-1 downto 1 do PUTVAL (WAdj[J])
    end
  end;
PUTLINE (DataImg,PLine,PData)
end;

(*********************************************************************)

Procedure  MINIMA;

{ Set new values for finding minima }

Var    I        :Integer;

Begin
Key:='Y';
If Anal then
  begin
  WRITE ('Change MINIMA- Are you sure? ');      READKEY;
  WRITE (CHR(clearln))
  end;
If (Key='Y') then
  begin
  WRITE (CHR(HOME),'VERTICAL:   Minima Width (',VMinW,')');
  I:=READINT;   TEST;      If (I>0) then VMinW:=I;
  WRITE (CHR(31),'E Depth (',VMinD,') ');
```

```
 I:=READINT;  TEST;    If (I>0) then VMinD:=I;
 WRITE (CHR(31),' !HORIZONTAL: Minima Width (',HMinW,')');
 I:=READINT;  TEST;    If (I>0) then HMinW:=I;
 WRITE (CHR(31),'E!Depth (',HMinD,') ');
 I:=READINT;  TEST;    If (I>0) then HMinD:=I;
 If NOT Anal then
   begin
   WRITE (CHR(31),' "Max. no. of bands (',MaxBand,') ');
   I:=READINT;  TEST;    If (I>8) then MaxBand:=I
   end
 end
end;

(*********************************************************************

Procedure PSEUDOCOLOUR;

{ Overlays in colour or white }

Begin
BandW:=NOT BandW;
If BandW then
  begin Y:=white;  M:=white;   C:=white; G:=white end
else
  begin Y:=yellow; M:=magenta; C:=cyan;  G:=green end;
OVLAY (G2Img,Y); OVLAY (G1Img,M); OVLAY (TextImg,C)
end;

(*********************************************************************)

Procedure BINDISP;

{ Display 1 bit plane }

Begin
OVKILL;    Square:=False;  OVLAY (BinIm,white);
  Repeat
  READKEY; WRITE(CHR(8),' ',CHR(8)); OVKILL;
  With BinIm do
   Begin
   If (Key in ['0'..'9']) then lsbit:=Ordkey-48;
   If (Key in ['A'..'F']) then lsbit:=Ordkey-55;
    Case Ordkey of
     14: origin.y:=512;
     15: origin.y:=0;
     18: origin.x:=0;
     19: origin.x:=512;
     43: DRAW (BinIm.whole,BinIm,1);
     45: CLEARIM (BinIm);
     78: PRINTIM (BinIm,False);
     80: PRINTIM (BinIm,True)
    end;
   OVLAY (BinIm,white)
   end
  until (Key='X');
OVKILL;  LIVEIM
end;

(*********************************************************************)

Procedure INIT;

{ Initialise back to defaults }

begin
If Anal then ANALYSE;
If NOT Anal then
  begin
  DEFIMAGE (TextImg,0,512,full,0,1);
  DEFIMAGE (G1Img,  0,512,full,1,1);
  DEFIMAGE (G2Img,  0,512,full,2,1);
  DEFIMAGE (GreyImg,0,512,full,8,6);
  DEFIMAGE (DataImg,512,0,full,0,16);
  DEFIMAGE (B3Img,  0,512,full,0,3);
  DEFIMAGE (Binim,  0,512,full,0,1);
 TEXTIMAGE(TextImg); CLEARIM (B3Img);
```

```
 BandW:=true;                          PSEUDOCOLOUR;
 DEFWINDOW(VBox,175,64,60,430);        FRAMEDRAW (VBox,G2Img,1,1);
 DEFWINDOW(Total,120,64,170,430);      FRAMEDRAW (Total,G2Img,1,2);
 For I:= 0 to 511 do Vert[I]:=0;
 HBox:=Total;          NSmooth:=3;          Title:='New';
 VMinW:=10;            VMinD:=50;           MaxBand:=10;
 HMinW:=10;            HMinD:=100;          Colour:=false;
 Wind.form:=wndw;      TInt:=1.0;           Magnif:=1.0;
 GOn:=false;           Slow:=true;          NPhoto:=0;
 Plot:=false;          YMin:=-5.0;          NormalPlot:=true;
 YMax:=20.0;           YStep:=5.0;          YSpan:=350;
 TMin:=0.0;            TMax:=25.0;          TStep:=2.5;
 TSpan:=3500;          DiscName:=':'
 end
end;

(*********************************************************************}

Procedure HELP;

Var    HelpImg     :Image;

{=================================================================}

Procedure HELP1;

Begin
DEFIMAGE (HelpImg,0,512,full,3,1);
TEXTIMAGE(HelpImg);  OVKILL; OVLAY(HelpImg,G);
WRITELN ('  Options for GUT program');
WRITELN ('  ------- --- --- -------'); TEST;
WRITE   ('A Analyse photos start/stop.      (',NPhoto,
                    'photos). Status:-');
If Anal then WRITELN ('Collecting. ') else WRITELN ('Ready. ');
If (NPhoto=0) then WRITELN ('      (Data storage empty)')
  else WRITELN ('     ( ',PLine+1,' lines of data)   ');
WRITELN ('B Band alignment'); TEST;
WRITELN ('C Calibrate with scale bar or enter value.');
WRITELN ('      (Mag. =',Magnif:9:4,' pixels/unit length)'); TEST;
WRITELN ('D Display results');
WRITELN ('E Erase graphs.  Type ^L to erase text.');
WRITE   ('F Fast analysis (i.e. no graphics) ');
If Slow then WRITELN ('SLOW.') else WRITELN ('FAST.');
WRITE   ('G Graphics switch for full graphs of each photo '); TEST;
If GOn then WRITELN ('ON. ') else WRITELN ('OFF.');
WRITELN ('H or ? Help.');
WRITELN ('I Initialise system back to defaults.'); TEST
end;

{=================================================================}

Procedure HELP2;

Begin
WRITELN ('M Minima- characteristics for finding bands and widths.');
WRITELN
   ('      (Vertical:   depth',VMinD:4,' width +-',VMinW:2,')'); TEST;
WRITELN
   ('      (Horizontal: depth',HMinD:4,' width +-',HMinW:2,')');
WRITELN ('      (Max. no. of bands',MaxBand:3,')'); TEST;
WRITELN ('N No. of smooths (',NSmooth,'). ');
WRITE   ('O Output in pseudocolour ');
If Colour then WRITELN ('ON. ') else WRITELN ('OFF.');
WRITELN ('P Printer baud rate 9600 or 1200. (',Baud,'baud)');    TEST;
WRITELN ('Q Quit program. `Esc" key aborts at any stage.');
WRITELN ('     (^X" is used to exit from procedures)'); TEST;
WRITELN ('R Read data from disc file.');
WRITELN ('S Save data to disc file.');
WRITELN ('T Time interval between photos. (',TInt:4:2,'secs)'); TEST;
WRITELN ('V Values written to console or printer (',Baud,'baud).')
end;

{=================================================================}

Begin
HELP1; HELP2;
```

```
WRITELN ('W Window for analysis.  Use light pen to adjust.');    TEST;
With Total,Total.origin do WRITELN ('      (Total box X=',x:3,
          '-',x+width:3,' width',width:4,' Y=',y:3,'-',
          y+height:3,' height',height:4,')'); TEST;
With VBox, VBox.origin  do WRITELN ('      (Vert. box X=',x:3,
         '-',x+width:3,' width',width:4,')'); TEST;
WRITE   ('Z monitor:- ');
If BandW then WRITELN ('B & W ') else WRITELN ('Colour');
WRITE ('   Press any key to exit.',CHR(31),'8?',CHR(131),
'V. A. Moss & G. S. Clarke (October 1984)');
READKEY; OVKILL; OVLAY (G2Img,Y); OVLAY (G1Img,M);
OVLAY (TextImg,C); TEXTIMAGE (TextImg)
end;

(*****************************************************************)

Begin   { Main program }
OVLYLOAD ('#5:G6CODE');   SINIT;
Anal:=false;    INIT;      Baud:=9600;
Repeat
 LIVEIM;      WRITELN (CHR(home),
    'Window, Minima, Calib., Time, Analyse, Values or Help');
 Repeat TEST until KEYPRESS or LPEN(Pt);
 If KEYPRESS then READKEY else Key:='W';
 WRITE (CHR(clearln),CHR(home),CHR(clearln));
 Case Key of
  'A': ANALYSE;
  'B': BANDS;
  'C': CALIBRATE;
  'D': DISP;
  'E': CLEARIM (B3Img);
  'F': begin
       Slow:=NOT Slow;
       If Slow then WRITE ('Slow') else WRITE ('Fast');
       If NOT Slow then GOn:=false
       end;
  'G': Begin
       GOn:=NOT GOn;
       WRITE ('Graphics ');
       If GOn then WRITE ('ON ') else WRITE ('OFF')
       end;
  'H','?': HELP;
  'I': INIT;
  'J': BINDISP;
  'M': MINIMA;
  'N': begin
       WRITE ('No. of smooths ');     I:=READINT;
       If (I>0) then NSmooth:=I
       end;
  'O': OUTPUT;
  'P': Begin  Baud:=10800-Baud;  WRITE (Baud,' baud');  BAUDRATE (Baud)  end;
  'Q': begin
       WRITE ('QUIT- are you sure? ');
       READKEY;      If (Key='Y') then Key:=CHR(27)
       end;
  'R': READDATA;
  'S': SAVEDATA;
  'T': begin
       Key:='Y';
       If Anal then
        begin  WRITE ('TIME step- Are you sure?');  READKEY  end;
        WRITE ('Time interval',TInt:5:2,'sec ',CHR(137));
        If (Key='Y') then
          begin
          Z:=READREAL; If (Z>0.0) then TInt:=Z ;
          If (TInt<1.0) then GOn:=false
          end
        end
       end;
  'V': VALUES;
  'W': WINDOW;
  'Z': PSEUDOCOLOUR;
   ':','*','#': begin
       DiscName[1]:=Key;
       If (Key='#') then
         begin
```

```
        READKEY;  DiscName:='#5:';  DiscName[2]:=Key
        end;
      DIRECTORY (DiscName);  DiscName:=':'
      end
  end
 until  (Key=CHR(27));
BAUDRATE (9600)
end.
```


Graphics Display Subroutines

```
Procedure  DISP;                          { Display or Plot data }

Var        Min,Max,I,J,C0,C1,N0,N1,NMin,MaxB2,PMin,PMax  :Integer;
           Percent                        :Boolean;
           R,Z,VMin,VMax,Span,Time        :Real;
           Posn                           :Array[0..63] of integer;
           Ref                            :Array[0..63] of real;
           Mag                            :Array[0..3] of real;
           PlotVar                        :0..3;
           Txt0Im,Grf1Im,Grf2Im,DispIm,EraseIm     :Image;
           PS                              :Pointset;
```

{═══}

```
Procedure CONTROLS;

{ Control values for % changes }

Begin
WRITE (CHR(31),' #Control photos ');  C0:=READINT;
If (C0<=0) then begin C0:=N0; WRITE ('1') end;
WRITE (' to ');  C1:=READINT;
If (C1<C0) or (C1>NPhoto) then begin C1:=N1; WRITE (C1) end;
For J:=0 to NMin do Ref[J]:=0.0;
NGet:=MaxB2;                R:=1.0*C0*MaxB2;
GLine:=TRUNC(R/512.0);      GETLINE (DataImg,GLine,GData);
GWord:=ROUND(R-512.0*GLine); GLine:=GLine+1;
For I:=C0 to C1 do
  begin
  WRITE (CHR(31),'[#',I);
  While (NGet<MaxB2) do J:=GETVAL;  NGet:=0;
 NMin:=GETVAL;
 If (NMin>MaxBand) then
   begin
   WRITE (NMin,' No. min. ',CHR(137));
  NMin:=READINT;  WRITELN
```

```
  end;
 For J:=1 to NMin do Posn[J]:=GETVAL;
 Case PlotVar of
  0:                {L:=Pn+1 - Pn}
     For J:=1 to NMin-1 do Ref[J]:=Ref[J]+Posn[J+1]-Posn[J];
  1: For J:=1 to NMin-1 do Ref[J]:=Ref[J]+GETVAL; {W, read P first}
  2: For J:=1 to NMin-1 do
       begin
      K:=GETVAL;     {V=π*W*W*L/4/50/50/50}
        Ref[J]:=Ref[J]+6.283186E-6*K*K*(Posn[J+1]-Posn[J])
       end;
  3: For J:=1 to NMin-1 do
       begin
      K:=GETVAL;     {Ratio L/W}
       Ref[J]:=Ref[J]+(Posn[J+1]-Posn[J])/K
       end
  end
 end;
Z:=1.0/(C1-C0+1);  If (PlotVar<2) then Z:=Z*0.02;
For J:=1 to NMin-1 do Ref[J]:=Ref[J]*Z;
WRITELN (CHR(31),'S#Ref. values:-');
For J:=1 to NMin-1 do
  begin
  WRITE (Ref[J]/Mag[PlotVar]:7:2);
  If (J mod 9 =0) then WRITELN
  end
end;

{=============================================================}

Procedure LIMITS;

{ Data limits for plotting }

{-------------------------------------------------}

  Procedure XLIMITS;

Begin
Case PlotVar of
 0: For J:=1 to NMin-1 do   {L:=Pn+1 - Pn}
     begin
     K:=Posn[J+1]-Posn[J];
     If Percent then
       begin
       Z:=K/Ref[J];
       If (Z<VMin) then VMin:=Z;
       If (Z>VMax) then VMax:=Z
       end
     else
       begin
       If (K<Min) then Min:=K;
       If (K>Max) then Max:=K
       end;
     K:=L
     end;
 1: For J:=1 to NMin-1 do   {W, read Pi first}
     begin
     K:=GETVAL;
     If Percent then
       begin
       Z:=K/Ref[J];
       If (Z<VMin) then VMin:=Z;
       If (Z>VMax) then VMax:=Z
       end
     else
       begin
       If (K>Max) then Max:=K;
       If (K<Min) then Min:=K
       end
     end;
 2: For J:=1 to NMin-1 do
     begin
     K:=GETVAL;     {V=π*W*W*L/4/50/50/50}
       Z:=6.283186E-6*K*K*(Posn[J+1]-Posn[J]);
     If Percent then Z:=Z/Ref[J];
```

```
      If (Z<VMin) then VMin:=Z;
      If (Z>VMax) then VMax:=Z
    end;
  3: For J:=1 to NMin-1 do
      begin
     K:=GETVAL;    {R=L/W}
      Z:=(Posn[J+1]-Posn[J])/K;
      If Percent then Z:=Z/Ref[J];
      If (Z<VMin) then VMin:=Z;
      If (Z>VMax) then VMax:=Z
      end
   end
 end;
{------------------------------------------------------- ---}
begin
Min:=32767;  Max:=-32767;  VMin:=1.0E20;  VMax:=-VMin;
NGet:=MaxB2;                R:=1.0*N0*MaxB2;
GLine:=TRUNC(R/512.0);        GETLINE (DataImg,GLine,GData);
GWord:=ROUND(R-512.0*GLine);   GLine:=GLine+1;
PMin:=5000;      PMax:=20000;
For I:=N0 to N1 do
  begin
  WRITE (CHR(31),'[$',I);
  While (NGet<MaxB2) do J:=GETVAL;  NGet:=0;  NMin:=GETVAL;
  If (NMin>MaxBand) then
    begin
    WRITE (NMin,' Mins ? ',CHR(137));
   NMin:=READINT;  WRITELN
    end;
   For J:=1 to NMin do Posn[J]:=GETVAL;
   If (PMin>Posn[1]) then PMin:=Posn[1];
   If (PMax<Posn[NMin]) then PMax:=Posn[NMin];
  XLIMITS
   end;
 If (PlotVar<2) then if Percent then
     begin VMin:=VMin*0.02; VMax:=VMax*0.02 end
   else
     begin VMin:=Min*0.02;  VMax:=Max*0.02 end;
If Percent then
  begin VMax:=100.0*(VMax-1.0);  VMin:=100.0*(VMin-1.0)  end
else
  begin VMin:=VMin/Mag[PlotVar]; VMax:=VMax/Mag[PlotVar] end;
WRITELN;  WRITELN ('Limits ',VMin:7:2,' to ',VMax:7:2)
end;

{=============================================================}

Procedure COLOURPLOT;

{ Colour plot on RGB monitor }

Var  Scale  :Real;
     K50,YShift :Integer;

{-----------------------------------------------------------}

  Procedure PLOTBOX;

  Var  ZZ     :Real;

  Begin
  With PS do
    begin
    origin.y:=Posn[J] div K50 +YShift;
   ZZ:=(Z-VMin)*Span;
    If (ZZ>32766.0) or (ZZ<-32768.0) then
      begin
      WRITE (CHR(home),' Error Z=',Z,' VMin=',VMin,' Span=',Span);
     READKEY;  ZZ:=0.0
      end;
    K:=ROUND(ZZ)+1;  height:=1;
    If (I>=C0) and (I<=C1) then DRAW (PS,Grf1Im,1) else DRAW (PS,Grf2Im,1);
    height:=(Posn[J+1]-Posn[J]) div K50 +1;  DRAW (PS,DispIm,K)
   end
  end;
```

```
{-------------------------------------------------------}

Procedure GRAPH0;
Var  Tick   :Integer;
Begin
OVLAY (Grf2Im,C); OVLAY (Grf1Im,M); OVLAY (Txt0Im,Y);
TEXTIMAGE (Txt0Im); DEFWINDOW (PS,0,16,1,464);
Scale:=511.0/(N1-N0+1); I:=N1-N0; Tick:=1; {Plot time scale}
If (I>10)  then Tick:=2;
If (I>40)  then Tick:=5;
If (I>80)  then Tick:=10;
If (I>160) then Tick:=20;
If (I>400) then Tick:=50;
If (I>800) then Tick:=100;
If (I>1600) then Tick:=200;
J:=TRUNC((N0-1)*TInt);
For I:=J div Tick to TRUNC((N1-1)*TInt) div Tick do
  begin
  K:=ROUND((I*Tick-J)*511.0/(N1-N0+1)/TInt);
  PS.origin.x:=K;  DRAW (PS,Grf2Im,1);
  If (I mod 2 =0) then
    begin
    If K<8 then K:=8; GOTOABS (K-8,0); WRITE (I*Tick)
    end
  end;
 GOTOABS (488,0); WRITE ('Sec');
 DEFWINDOW (PS,0,496,2,16);   {Plot Key}
 For I:=1 to 255 dobegin PS.origin.x:=2*I-2; DRAW (PS,DispIm,I) end;
 Span:=VMax-Vmin; Z:=1.0; R:=(3*VMax-Vmin)*0.5;
 If (R>20.0)  then Z:=2.0;
 If (R>50.0)  then Z:=5.0;
 If (R>100.0) then Z:=10.0;
 If (R>200.0) then Z:=20.0;
 If (R>500.0) then Z:=50.0;
 PS.width:=1;
 For I:=TRUNC(VMin/Z) to TRUNC(VMax/Z) do
   begin
   J:=ROUND((I*Z-VMin)*504.0/Span);
   GOTOABS (J-7,496); WRITE (ROUND(I*Z));
   If (I=0) then WRITE ('%');
   PS.origin.x:=J;    DRAW (PS,DispIm,0)
   end;
 K50:=(PMax-PMin) div 480;  YShift:=16-PMin div K50
end;

{-------------------------------------------------------}

begin {COLOURPLOT}
GRAPH0;  Span:=254.0/Span;
DEFWINDOW (PS,0,0,TRUNC(Scale+0.999),1);
NGet:=MaxB2;  R:=1.0*N0*MaxB2;
GLine:=TRUNC(R/512.0); GETLINE (DataImg,GLine,GData);
GWord:=ROUND(R-512.0*GLine); GLine:=GLine+1; Key:=' ';
With PS do for I:=N0 to N1 do if (Key<>'X') then
  begin
  origin.x:=ROUND((I-N0)*Scale);
 While (NGet<MaxB2) do J:=GETVAL; NGet:=0; NMin:=GETVAL;
 If (NMin>MaxBand) then
   begin
   WRITE (NMin,' No. min. ',CHR(137)); NMin:=READINT; WRITELN
   end;
 For J:=1 to NMin do Posn[J]:=GETVAL;
 Case PlotVar of
   0: begin       "    {L:=Pn+1 - Pn}
      For J:=1 to NMin-1 do
        begin
        Z:=0.02*(Posn[J+1]-Posn[J]);
        If Percent then Z:=100.0*(Z/Ref[J]-1.0) else Z:=Z/Magnif;
       PLOTBOX
       end
     end;
   1: begin          {W}
      For J:=1 to NMin-1 do
        begin
       Z:=0.02*GETVAL;
        If Percent then Z:=100.0*(Z/Ref[J]-1.0) else Z:=Z/Magnif;
```

```
    PLOTBOX
     end
    end;
  2: begin
    For J:=1 to NMin-1 do
     begin
    K:=GETVAL;    {V=π*W*W*L/4/50/50/50}
      Z:=6.283186E-6*K*K*(Posn[J+1]-Posn[J]);
     If Percent then Z:=100.0*(Z/Ref[J]-1) else Z:=Z/Mag[2];
    PLOTBOX
     end
    end;
  3: begin
    For J:=1 to NMin-1 do
     begin
    K:=GETVAL; Z:=(Posn[J+1]-Posn[J])/K;  {R=L/W}
     If Percent then Z:=100.0*(Z/Ref[J]-1);
    PLOTBOX
     end
    end
  end;
 height:=1; origin.y:=Posn[NMin] div K50 +YShift;
 If (I>=C0) and (I<=C1) then DRAW (PS,Grf1Im,1)
                        else DRAW (PS,Grf2Im,1);
 If KEYPRESS then READKEY
 end;
WRITE(CHR(31),' ')
end;

{=============================================================}

Procedure DIGIPLOT;

{ Digital plot on T 4010, T 4662, Gould or HP plotter }

Var   XPos,YPos,NPlot,XX,YY          :Integer;
      YScale,TScale                  :Real;
```



```
{-------------------------------------------------------------}

Procedure PLOTAXES;

Begin
If Plotter then
 begin REDIRECT ('#6:'); PENCOLOUR (1) end
else GraphIm:=Grf2Im;
YScale:=YSpan/(YMax-YMin);  TScale:=TSpan/(TMax-TMin);
MOVETO (XOrig,YOrig+YSpan); DRAWTO (XOrig,YOrig);
For K:=TRUNC(YMin/YStep) to TRUNC(YMax/YStep) do
  begin
  YPos:=ROUND((K*YStep-YMin)*YScale)+YOrig;
  If (K mod 2=0) then
    begin
    MOVETO (XOrig-320,YPos-30);  If Plotter then WRITE ('LB');
    WRITE (K*YStep:5:1);  If Plotter then WRITE (CHR(3));
    MOVETO (XOrig-40,YPos)
    end
  else MOVETO (XOrig-20,YPos);
  DRAWTO (XOrig,YPos)
  end;
YPos:=ROUND(-YMin*YScale)+YOrig;
If (YPos<YOrig) then YPos:=YOrig;
MOVETO (XOrig,YPos);     DRAWTO (XOrig+TSpan,YPos);
For K:=TRUNC(TMax/TStep) downto TRUNC(TMin/TStep)+1 do
  begin
  XPos:=ROUND((K*TStep-TMin)*TScale)+XOrig;
  If (J=1) and (K mod 2 =0) then
    begin
    MOVETO (XPos-196,YPos-128);  If Plotter then WRITE ('LB');
    WRITE (K*TStep:5:1);  If Plotter then WRITE (CHR(3));
    MOVETO (XPos,YPos-40)
    end
  else MOVETO (XPos,YPos-20);
  DRAWTO (XPos,YPos)
  end;
 If Plotter then
```

```
 begin
 PENCOLOUR (PlotVar+2);
 If Percent then
   begin
   MOVETO (XOrig+400*PlotVar,YOrig+YSpan);
   WRITE ('LB',Ref[J]/Mag[PlotVar]:6:2,CHR(3))
   end;
 REDIRECT ('#1:');
 end
else GraphIm:=Grf1Im
end;

{-------------------------------------------------------------}

 Procedure PLOTDATA (B:Integer);

 Var  P0,P1                :Integer;
      YYMin,YYMax,TVal     :Real;
      Jump                 :Boolean;

 Begin
 Jump:=true;
 If Plotter then REDIRECT ('#6:');
 P0:=ROUND (TMin/TInt);        If (P0<N0) then P0:=N0;
 P1:=ROUND (TMax/TInt)+1;      If (P1>N1) then P1:=N1;
 YYMin:=1.1*YMin-0.1*YMax;     YYMax:=1.1*YMax-0.1*YMin;
 NGet:=MaxB2;                  R:=1.0*P0*MaxB2;
 GLine:=TRUNC(R/512.0);        GETLINE (DataImg,GLine,GData);
 GWord:=ROUND(R-512.0*GLine);  GLine:=GLine+1;
 For I:=P0 to P1 do
   begin
   While (NGet<MaxB2) do L:=GETVAL;  NGet:=0;
   NMin:=GETVAL;
   If (NMin>MaxBand) then
     begin
     REDIRECT ('#1:'); WRITE (NMin,' No. min. ',CHR(137));
     NMin:=READINT;   WRITELN;  REDIRECT ('#6:')
     end;
 For L:=1 to NMin do Posn[L]:=GETVAL;
 Case PlotVar of
  0: begin          {L}
     Z:=0.02*(Posn[B+1]-Posn[B]);
     If Percent then Z:=100.0*(Z/Ref[B]-1.0) else Z:=Z/Magnif
     end;
  1: begin          {W}
     For L:=1 to B do K:=GETVAL;
     If Percent then Z:=2.0*K/Ref[B]-100.0 else Z:=0.02*K/Magnif
     end;
  2: begin          {V}
     For L:=1 to B do K:=GETVAL;
     Z:=6.283186E-6*K*K*(Posn[B+1]-Posn[B]);
     If Percent then Z:=100.0*(Z/Ref[B]-1) else Z:=Z/Mag[2]
     end;
  3: begin          {R}
     For L:=1 to B do K:=GETVAL;
     Z:=(Posn[B+1]-Posn[B])/K;
     If Percent then Z:=100.0*(Z/Ref[B]-1)
     end
 end;
 TVal:=TInt*(I-1);
 If (TVal>=TMin) and (TVal<=TMax)
                 and (Z>=YYMin) and (Z<=YYMax) then
   begin
   XPos:=XOrig+ROUND((TVal-TMin)*TScale);
   YPos:=YOrig+ROUND((Z-YMin)*YScale);
   If Jump then MOVETO (XPos,YPos) else DRAWTO (XPos,YPos);
   Jump:=false;
   end
 else Jump:=true
end;
REDIRECT ('#1:')
end;

{-------------------------------------------------------------}

Procedure NEWAXES;
```

Micro Code suboutines and changes to P-Code interpreter

```
        .TITL GM

.PROC AVWIND <PROCEDURE AVWIND (VAR WIND:POINTSET;
                                VAR GREYIM:IMAGE; VAR DATA:IMHST);>

; Procedure to get the average for each y-value of a window

AVWIND: NOP                     :JSR ACPOP      ;Start of data array
        MOV AC,PUTPTR           :JSR VPOPIM     ;Image
        NOP                     :JSR RDMSK
        MOV AC,RMSK             :JSR VPOPPS     ;Pointset
        NOP                     :JSR FINITPS
        NOP             :SR                     ;Get 1st point

ALOOP:  MOV PS.VF1,AC                           ;Width
        MOV AC,CNTR1
        ZER TEMP1
ALOOP1: MOV IM(RS),AC                           ;Get grey value
        ADD AC,TEMP1            :JSR NXTPT      ;Add to previous
        DEC CNTR1       :SR     :JMP ALOOP2 NZ  ;Exit if no pts.
        NOP                     :JMP ALOOP1 NZ  ;All x values
        MOV TEMP1,AC            :JSR MMPUT      ;Save total
        NOP                     :JMP ALOOP
ALOOP2: NOP                     :JMP ENDIPC

.PROC GETMIN <PROCEDURE GETMIN(NDATA:INTEGER; VAR DATA,
        MINARR:IMHST; VAR NMIN:INTEGER; MINW,MIND:INTEGER);>

; Procedure to smooth an array  X_i = (X_i-1 + 2*X_i + X_i+1) / 4
; The result will have X_1 = X_2 and Xl = X_n-1
; The smoothed array is then searched for minima

GETMIN: NOP                     :JSR ACPOP      ;Depth of Min.
        MOV AC,MIND             :JSR ACPOP      ;Width of Min.
        MOV AC,MINW             :JSR ACPOP      ;No. min. locn.
        MOV AC,NMIN             :JSR ACPOP      ;Min array start
        MOV AC,MINPTR           :JSR ACPOP      ;Data array start
        MOV AC,PUTPTR                           ;Save for PUTs
        INC AC,GETPTR,+AC       ;Dec. in DLOOP
        MOV AC,DATAST           :JSR ACPOP      ;Offset for mins.
        DEC AC,AC                               ;No. data = n
        DEC AC,CNTR1,+AC        ;n-2 smooths
        MOV AC,CNTR2                            ;and save it

; Evaluate for each element  X_i = (X_i + 2*X_i+1 + X_i+2) / 4

DLOOP:  DEC GETPTR              :JSR MMGET      ;X_i Adj. GETPTR
        MOV AC,TEMP1            :JSR MMGET      ;2*X_i+1
        ADD AC(1L),TEMP1  :JSR MMGET            ;X_i+2
        ADD AC,TEMP1,AC                         ;Total
        AND AC(2R),%3FFF,AC     :JSR MMPUT      ;Mean
        DEC CNTR1                               ;Finished yet?
        DEC GETPTR              :JMP DLOOP NZ   ;Adj. GETPTR

; Move array sideways           X_i+1 = X_i
; Initially PUTPTR points to Xn-1 and GETPTR to Xn
; Make X_n = X_n-1 in the smoothed array i.e. X_n = X_n-2 as calc.
        DEC GETPTR
        DEC GETPTR                              ;Points at n-2
        MOV GETPTR,MAF                          ;i.e. X_n-2
        MOV MM,AC
        INC PUTPTR,MA                           ;i.e. X_n
        MOV AC,MM                               ;X_n = X_n-2
        MOV CNTR2,AC                            ;n-2 shifts
        MOV AC,CNTR1
BLOOP:  MOV GETPTR,MAF                          ;Points at X_i-1
        DEC GETPTR                              ;Backwards
        MOV MM,AC                               ;Get value
        MOV PUTPTR,MA                           ;Points X_i
        MOV AC,MM                               ;Copy forward
        DEC CNTR1                               ;Finished yet?
        DEC PUTPTR      :JMP BLOOP NZ           ;Backwards
```

```
; The last move will make  X1 = X2
; Now find the minima i.e X1 < X1-1 and X1 <= X1+1
; and check if real min.  X1 < X1-minw - mind and X1 < X1+minw -mind
; GETPTR points to X1 and PUTPTR to X2

        MOV MINPTR,AC                       ;O/p to Min array
        MOV AC,PUTPTR
        ZER CNTR1                           ;No. of min.
        MOV GETPTR,AC                       ;Ignore X1 to Xminw
        ADD AC,MINW,AC
        MOV AC,GETPTR
        MOV MINW,AC
        SUB AC(1L),CNTR2,+AC                ;n-2.MINW tests
        MOV NMIN,MAF                        ;Branch if differential
        MOV MM,#                            ;i.e. No.Min<>0
        NOP                 :JMP DIFF NZ

; Routine to find minima in array

        ADD AC,GETPTR,AC
        MOV AC,CNTR2
MLOOP:  NOP                 :JSR MMGET      ;Get X1-1
        MOV AC,TEMP1        :JSR MMGET      ;Get X1
        SBR AC,TEMP1                        ;? X1-1 > X1
        MOV AC,TEMP1        :JMP MLOOP1 PR  ;Points to i+1
        NOP                 :JSR MMGET      ;Get X1+1
        SBR AC,TEMP1,#                      ;? X1-1 >= X1
        DEC GETPTR,+AC      :JMP MLOOP1 NR  ;Points to i+1
        DEC AC,AC
        SBR AC,MINW,MAF
        SBR MM,MIND,AC
        SBR AC,TEMP1,#
        DEC GETPTR,AC       :JMP MLOOP1 NR
        ADD AC,MINW,MAF
        SBR MM,MIND,AC
        SBR AC,TEMP1,#
        MOV GETPTR,AC       :JMP MLOOP1 NR  ;Save array no. i
        SBR AC,DATAST,AC    :JSR MMPUT
        INC CNTR1                           ;and count mins.
        MOV MIND,AC                         ;Find the ave. min.
        ADD AC,TEMP1
        DEC GETPTR,AC
MLOOP2: DEC AC,MAF                          ;Hunt back for start
        SUB MM,TEMP1,#
        DEC AC,AC           :JMP MLOOP2 PR
        INC AC,GETPTR,+AC
        SBR AC,DATAST,AC
        INC AC,AC           :JSR MMPUT      ;Save offset
        SET PTR                             ;Now sum across peak
        ZER DMM
        ZER DMMXMA
MLOOP3: INC PTR             :JSR MMGET
        SUB AC,TEMP1,AC
        MPY AC,PTR          :JMP MLOOP4 NR
        ADD AC,DMM
        ADD PR,DMMXMA       :JMP MLOOP3
MLOOP4: MOV DMMXMA,AC       :JSR MMPUT      ;Save increment ratio
        MOV DMM,AC          :JSR MMPUT
MLOOP1: MOV GETPTR,AC
        SUB AC,CNTR2,#
        DEC GETPTR          :JMP MLOOP PR   ;Points to i
        MOV NMIN,MA                         ;Save no. of min.
        MOV CNTR1,MM        :JMP ENDIPC

; Routine to find max and min of differential array

DIFF:   MOV GETPTR,AC
        MOV AC,LMIN
        MOV AC,LMAX         :JSR MMGET
        MOV AC,MIN
        MOV AC,MAX
DIFF1:  NOP                 :JSR MMGET
        SUB AC,MIN,#
        NOP                 :JMP DIFF2 NR
        MOV AC,MIN
        MOV GETPTR,AC
```

```
        MOV AC,LMIN             :JMP DIFF3
DIFF2:  SBR AC,MAX,#
        NOP                     :JMP DIFF3 NR
        MOV AC,MAX
        MOV GETPTR,AC
        MOV AC,LMAX
DIFF3:  DEC CNTR2
        NOP                     :JMP DIFF1 NZ
        MOV LMAX,AC
        SBR AC,DATAST,AC        :JSR MMPUT
        MOV MAX,AC
        SBR AC,MIND,AC
        MOV AC,TEMP1
        MOV LMAX,AC
DIFF4:  DEC AC,MAF
        SBR MM,TEMP1,#
        DEC AC,AC               :JMP DIFF4 PR
        INC AC,GETPTR,+AC
        SBR AC,DATAST,AC
        INC AC,AC               :JSR MMPUT
        SET PTR
        ZER DMM
        ZER DMMXMA
DIFF5:  INC PTR                 :JSR MMGET
        SBR AC,TEMP1,AC
        MPY AC,PTR              :JMP DIFF6 NR
        ADD AC,DMM
        ADD PR,DMMXMA           :JMP DIFF5
DIFF6:  MOV DMMXMA,AC           :JSR MMPUT
        MOV DMM,AC              :JSR MMPUT
        MOV LMIN,AC
        SBR AC,DATAST,AC        :JSR MMPUT
        MOV MIN,AC
        ADD AC,MIND,AC
        MOV AC,TEMP1
        MOV LMIN,AC
DIFF7:  DEC AC,MAF
        SUB MM,TEMP1,#
        DEC AC,AC               :JMP DIFF7 PR
        INC AC,GETPTR,+AC
        SBR AC,DATAST,AC
        INC AC,AC               :JSR MMPUT
        SET PTR
        ZER DMM
        ZER DMMXMA
DIFF8:  INC PTR                 :JSR MMGET
        SUB AC,TEMP1,AC
        MPY AC,PTR              :JMP DIFF9 NR
        ADD AC,DMM
        ADD PR,DMMXMA           :JMP DIFF8
DIFF9:  MOV DMMXMA,AC           :JSR MMPUT
        MOV DMM,AC              :JSR MMPUT
        NOP                     :JMP ENDIPC

.PROC CROSSW <PROCEDURE CROSSW(VAR WIND:POINTSET;
                                VAR GREYIM:IMAGE; VAR DATA:IMHST);>
; Sum values across window

CROSSW: NOP                     :JSR ACPOP      ;St. data array
        MOV AC,PUTPTR           :JSR VPOPIM     ;Image
        NOP                     :JSR RDMSK
        MOV AC,RMSK             :JSR VPOPPS     ;Pointset
        NOP                     :JSR FINITPS
        NOP             :SR                     ;Get 1st point
CLOOP:  ZER TEMP1
CLOOP1: ADD IM(RS),TEMP1 :DY+1                  ;Get grey value
        DEC PS.VF3       :SR                    ;Y count-1
        MOV MAP,AC       :Y     :JMP CLOOP1 PR
        SBR AC,PS.VF2,MAP :Y                    ;Reset Y
        MOV TEMP1,AC     :DX+1  :JSR MMPUT
        MOV PS.VF2,AC    :SR                    ;Get height
        DEC PS.XCNT      :SR
        DEC AC,PS.VF3           :JMP CLOOP PR   ;Ycount=height
        NOP                     :JMP ENDIPC
```

```
.PROC DIFFER <PROCEDURE DIFFER(NUMB:INTEGER;VAR ORL:IMHST);>

; First differential of array

DIFFER:  NOP                          :JSR ACPOP
         MOV AC,PUTPTR
         MOV AC,GETPTR                :JSR ACPOP
         SBR AC,3,AC
         MOV AC,CNTRG
         NOP                          :JSR MMGET
         MOV AC,T2                    :JSR MMGET
         MOV AC,T3
LOOP1:   MOV T2,AC
         MOV AC,T1
         MOV T3,AC
         MOV AC,T2                    :JSR MMGET
         MOV AC,T3
         SBR AC,T1,AC
         ADD AC,%1000,AC              :JSR MMPUT
         DEC CNTRG
         MOV %1000,AC                 :JMP LOOP1 PR
         NOP                          :JSR MMPUT
         NOP                          :JSR MMPUT
         NOP                          :JMP ENDIPC

.PROC GETLINE <PROCEDURE GETLINE(IM:IMAGE; LINE:INTEGER;
                                                    DATA:IMHST);>
; Procedure to get 1 line from an image.

GETLINE: NOP                          :JSR ACPOP
         MOV AC,PUTPTR                :JSR ACPOP
         MOV AC,LINE
         ZER AC                       :JSR VPOPIM
         MOV IM.ORGX,MAP  :X
         MOV IM.ORGY,AC
         ADD AC,LINE,MAP  :Y          :JSR RDMSK
         MOV AC,RMSK      :SR
         MOV C511,AC      :DX+1,SR
         MOV AC,CNTR1
GETL1:   MOV IM(RS),AC    :DX+1,SR :JSR MMPUT
         DEC CNTR1
         NOP                          :JMP GETL1 PR
         NOP                          :JMP ENDIPC

.PROC PUTLINE <PROCEDURE PUTLINE(IM:IMAGE; LINE:INTEGER;
                                                    DATA:IMHST);>
; Procedure to put 1 line into an image.

PUTLINE: NOP                          :JSR ACPOP
         MOV AC,GETPTR                :JSR ACPOP
         MOV AC,LINE
         ZER AC                       :JSR VPOPIM
         MOV IM.ORGX,MAP  :X
         MOV IM.ORGY,AC
         ADD AC,LINE,MAP  :Y          :JSR WRMSK
         MOV AC,WMSK
         MOV C511,AC
         MOV AC,CNTR1     :DX-1
PUTL1:   NOP                          :JSR MMGET
         DEC CNTR1
         MOV AC(WS),IM    :DX+1,SW:JMP PUTL1 PR
         NOP                          :JMP ENDIPC

; Mods. to P-Code interpreter
.LOC     PCHAR
         AND AC,C127,AC
         ADD AC(3L),CHRBAS,AC
         MOV AC,EPADR

.LOC     TNC
         MOV SP,MAF
         AND MM(7R),C255,AC           :JSR FIXTNC
         MOV MM,FLAG                  :JMP RND NR

.LOC     529
FIXTNC:  SBR AC,%0067,#               :RET
```

Appendix B: Composition of Krebs solution

Stock solutions

Sodium chloride	90·0 g/l	(10x isotonic)
Potassium chloride	11·5 g/l	(isotonic)
Sodium hydrogen carbonate	12·9 g/l	(isotonic, gassed with CO_2)
Potassium dihydrogen phosphate	21·0 g/l	(isotonic)
Magnesium sulphate ($7H_2O$)	38·0 g/l	(isotonic)
Calcium chloride (1 M solution)	110 ml/l	(isotonic)

Krebs solution

80 ml of NaCl stock diluted to 800ml with distilled water.

Add 32 ml KCl

8 ml KH_2PO_4

168 ml $NaHCO_3$

Gas for 15 min. with 5% CO_2–95% O_2

Add 24 ml $CaCl_2$

8 ml $MgSO_4$

21 g glucose

Make up to 1litre with water, warm to 37°C, gas with 5% CO_2–95% O_2.