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A Study of the Effects of Orally
Administered Female Hormones on
the Volume and Composition of
Lacrimal Fluid related to the
Toleration of Corneal Contact
Lenses.

A Thesis Submitted for the
Degree of Doctor of Philosophy.

January 1974

SUMMARY

This study was designed to evaluate the effects of certain orally active contraceptive steroids on the eye, related to the tolerance of a corneal contact lens.

An oestrogen, ethinyloestradiol BP. 0.05 mg, a progestogen, norethisterone acetate BP. 2.50 mg and a control tablet (vitamin C, 50 mg) were utilised. The effect of these preparations on corneal curvature, lacrimal fluid volume and protein composition and directly on corneal lens tolerance was monitored in a group of 23 volunteer patients.

The progestogen was found to produce a significant ($P \geq 0.05$) decrease in tear volume as measured by a 3 minute Schirmer test. A smaller volume reduction was observed with ethinyloestradiol.

A normal cornea appears unaffected, within the measurement limits available, by the use of either hormone. However, in the presence of a corneal lens, oestrogen was found to induce substantial corneal steepening, indicative of tissue oedema, during the initial 2-3 weeks of medication. Progestogen occasionally produced a similar effect, which could recur with either hormone shortly after the end of the treatment period.

A new method of acrylamide gel electrophoresis was developed for examination of the protein concentration and composition of lacrimal fluid. This allowed much greater resolution of microquantities of unconcentrated fluid than anything previously reported.

Quantitation by densitometry has permitted the recording of medication and lens-induced changes in the protein pattern.

Tear albumin has been shown to differ from serum albumin and to consist of up to 3 subfractions, 7 further protein fractions may also be resolved. The concentration and probable origin of these proteins have been established and the overall effects of hormone administration described. Individual idiosyncratic responses are also discussed.

The study has established the nature of some effects of contraceptive steroids on the anterior eye, and the probable reasons for resultant corneal lens intolerance.

Acknowledgements.

My thanks are due to my supervisor Mr. A. G. Sabell and
to Dr. K. Dickinson and his colleagues at the University
of Birmingham Health Centre for the provision of
facilities for this work. In particular, to Dr. S. Pexman
for carrying out the medical examination of project
subjects, to Mr. F. Lane and his staff in the University
of Aston Physics Workshop for their work on a prototype
scanning densitometer and to Mrs. E. Boorman for undertaking
the typing of this thesis.

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1.   INTRODUCTION

A connection between orally administered contraceptive steroids and the eye, specifically with regard to contact lens intolerance, has been proposed periodically since the early days of oral contraceptive use in the mid-1950s.

Various ocular disorders have been reported as being associated with the use of oral contraceptives.[113]   These include:

Haemorrhage

Migraine and visual symptoms

Lateral Rectus Palsy

Cyclitis

Papilloedema

Retinal vascular disorders

Optic and Retrobulbar neuritis

It is almost impossible however to prove a causal relationship between a general medication and the type of occurrence listed above, since each disorder mentioned may occur quite spontaneously.

Some large scale studies have been undertaken, designed to evaluate any possible link between ocular disease and oral contraceptive use. Faust & Tyler[22] comparing seven different types of contraceptive found no evidence of ocular anomaly which might not be expected in any random sample of women.

Connell & Kelman[7] found no evidence to support a causal relationship between oral contraceptive medication and

ocular pathology, a conclusion based on a three year study of 611 women, half forming a control group and half using an oral contraceptive.

Nevertheless, reports of induced contact lens intolerance as opposed to ocular disease continue to occur with sufficient frequency for the British Family Planning Association for example, to include a question about contact lenses on their preliminary questionnaire.

No controlled study has ever been performed however, and the mechanism of an induced intolerance is unclear, as is the reason why this induced effect troubles only a small proportion of women wearing contact lenses.

Published work on the subject is generally in the form of clinical observation. Koetting[51] states that the most common side effect of 19-nor steroid therapy is increased sodium retention and accompanying oedema. Most trouble is found to occur in the first cycle, the incidence of complaints falling rapidly in succeeding cycles. In a small random sample of contact lens wearers using an O.C. Koetting reports that a fairly significant number of women experience difficulty, apparently due to oedema of the cornea or lids. Other complaints include excess mucus formation and reduced tear flow.

Ruben[96] points out that all the changes attributed to oral contraceptives may often occur quite normally in contact lens wearers, but does suggest that the "quality of tears" may be adversely affected by contraceptive therapy making them less effective lubricators.

Wolfe[116] considered lens discomfort to be due to lid oedema, but that such problems could be corrected by changing to another formulation contraceptive.

The relationship between tear secretion and contact lens wear has been described and emphasised in many papers and articles, e.g. Halberg,[33] Yonenaga,[117] Koetting.[52] De Rötth links the hypofunction of the lacrimal gland as found in Sjögnens syndrome with hormonal disturbance, and oestrogen has been found to decrease aqueous humour production in rabbit eyes.[58]

Goldberg[29] lists lid and deep epithelial oedema, dryness, photophobia, superficial epithelial abrasion and general discomfort occurring in previously adapted patients following the use of an oral contraceptive.

Peter & Parsons[86] describe prolonged abrasion - healing times for lens wearers using oral contraceptives, and a tendency for persistent oedema despite lens modification.

Aston University Ophthalmic Optics Department, with the co-operation and resources of Birmingham University Health Centre, was well placed to carry out the sort of controlled study that is lacking on this topic. This present work is a result of a preliminary investigation by Sabell.[97]

Sabell monitored a group of non-contact lens wearers before and after the start of contraceptive medication to try and demonstrate some regularly occurring ocular change which might help explain lens intolerance. The study

included -

(1) Measurement of corneal thickness, curvature, bedewing and stain.

(2) Measurement of the volume and consideration of the general character of the pre-corneal film.

Provisional conclusions drawn from this study were that no regular effect on corneal dimension was demonstrable, but that despite a large variation in successive readings, a reduction in the volume of tears did seem to result in a fair proportion of cases. Electrophoresis was proposed as an area for future investigation.

The question of tear volume alteration was investigated further by the author for an M.Sc. Project. Tear volume, estimated by means of a Schirmer test, was monitored in a group of volunteer female students. These were divided into contraceptive and non-contraceptive taking groups. The aim was to establish whether there existed a variation in tear volume related to the menstrual cycle and if so, to what extent this was affected by oral contraceptive use. No cyclic variation of this nature could be demonstrated, but the mean level of tear volume was found to be slightly lower in the group under medication.

Two additional subjects were monitored for 2 cycles prior to, and 2 cycles immediately after the start of oral contraceptive use. An appreciable drop in tear volume was apparent within a few days of the commencement of hormone use. (See fig. 1).

Schirmer tests were performed three times each week and the circumstances prevailing enabled the sample taking to be carried out under controlled temperature and surrounding atmosphere conditions. Measurements were taken at lunchtime after all subjects had been in the same building for the entire morning. This enabled a considerable degree of reliance to be placed on the results.

It was noted however that if a subject had been outside the building immediately before having the tear sample taken, then the results could differ considerably from those previously considered typical for that person. This point is of considerable significance in view of the unavoidable siting of the present study at the University of Birmingham Health Centre. Subjects must make their way to the Health Centre from all parts of the campus, making control of the atmospheric environment impossible, and introducing a major uncontrolled variable into the tear volume and composition sections of the work.

This M.Sc. project therefore confirmed the relationship between the lacrimal and hormonal systems. It was necessary then to expand the investigation into a "contact lens wearing" situation and to consider in addition possible variation in the composition of the lacrimal fluid since this will have a direct bearing on the physiology of the cornea.

Lacrimal fluid in common with all body fluids is a complex solution of dissolved protein, amino-acid, inorganic salts, oxygen and waste gases, with a fair amount of

Figure 1. Graph of tear flow related to the menstrual cycle obtained during the preliminary MSc project. [128]

_____ Average of 3 consecutive menstrual cycles prior to the use of an oral contraceptive.

_ _ _ _ Average of the initial 2 cycles following the start of 'Gynovlar' oral contraceptive medication.

Tear Flow(mm)

Day of menstrual cycle.
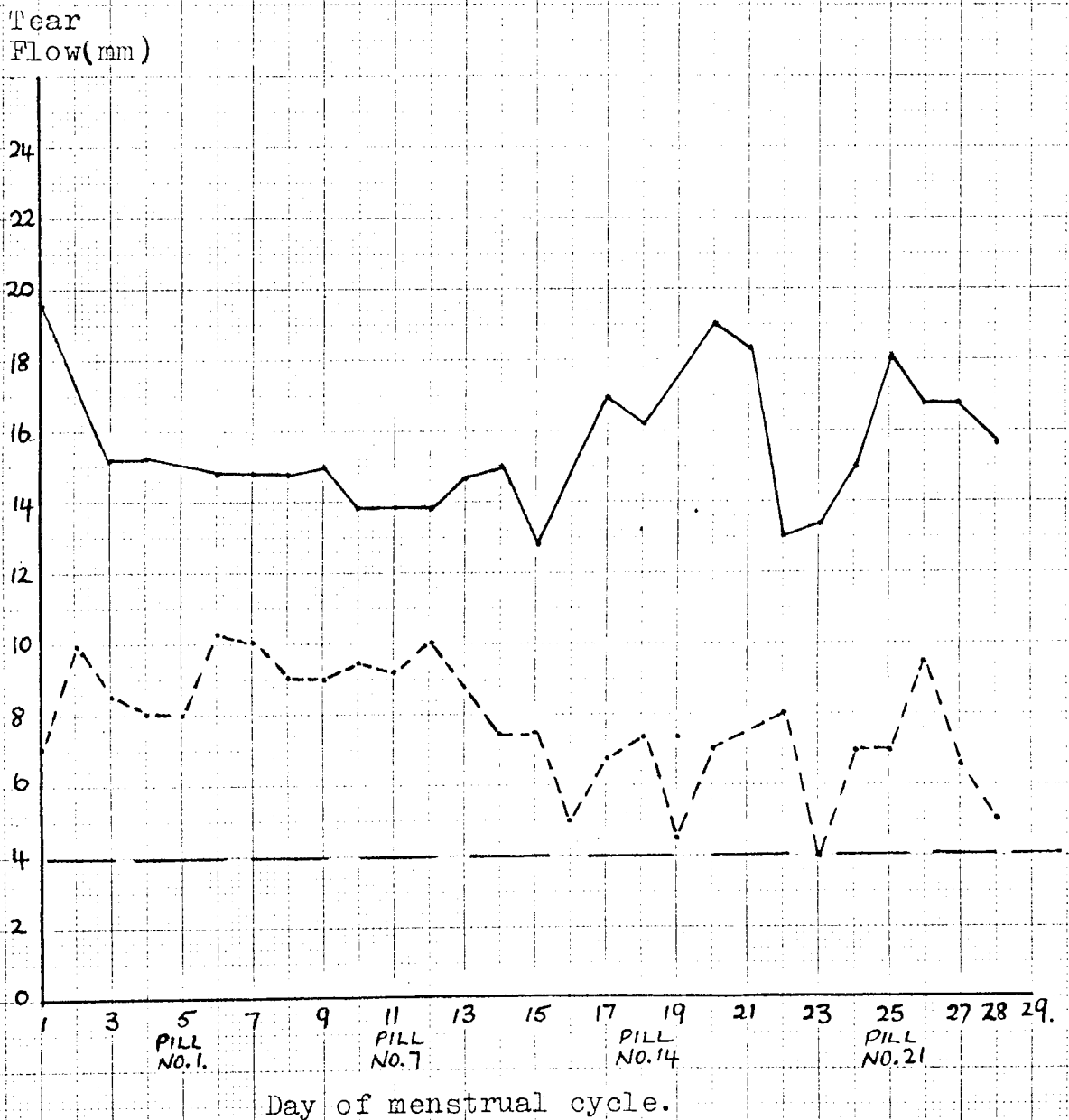
cellular and other solid material suspended in it.

Analysis has always posed problems due to the extremely dilute nature of the solution, 0.4% protein, the very small volumes available and the difficulty of obtaining normal tear fluid unaffected by reflex lacrimation induced by the sample collection procedure.

Tear proteins have been investigated by various methods since the early immunological work of Flemming.[24] A comprehensive summary may be found in Krause.[56] More recently, electrophoretic techniques predominantly on filter paper have been utilised. McEwen et al[69,70] found 5 protein fractions and stated that lysozyme is absent in cases of Keratoconjunctivitis sicca. Removal of the lacrimal gland also results in an absence of lysozyme and albumin-type components.

Erickson[18] also described the lysozyme component decreasing with decreasing rate of tear flow.

Brunish[2] using pooled samples from many subjects found six fractions with an average protein concentration of 0.4-0.6%. Krause[56] investigated tear samples both from normal subjects and from patients suffering from a large range of pathological eye conditions. The pathological protein pattern was found to be characterised by a relative increase in the size of the albumin-type component and a relative decrease in the lysozyme fraction. A maximum of 6 bands could be produced, although 4 or 5 was more usual. Krause characterised them by numbers considering that positive identification was unjustified on the basis of mobility alone.

One attempt at Agar gel electrophoresis of human tears, Francois & Rabaey[25] has proved unsatisfactory. Lysozyme being adversely affected by the gel support medium.

Lacrimal fluid itself, i.e. the secretion of the lacrimal gland, forms only a portion of the pre-corneal film on which the normal integrity of the cornea depends. Secretion from the Meibomian glands of the lids and goblet cells of the conjunctiva being also of fundamental importance. All components of the pre-corneal film are mixed and spread across the eye during blinking, and a knowledge of the variation of all constituents, not merely those of the lacrimal gland, is necessary for a true picture of any induced change.

The method of obtaining samples finally decided upon in this study, namely, filter paper discs, has meant that material from all sources is present in the sample used for the electrophoretic separation. This is felt to be an advantage over the alternative method of microcapillary liquid collection of stimulated tears[2,25,56] for in this latter case the sample relates predominantly to the output of the lacrimal gland.

For convenience however, the terms "lacrimal fluid" and "tear fluid" as used in this study may be taken, unless otherwise indicated, to refer not merely to the lacrimal gland secretion but to the combined secretion from all relevant glands of the external eye.

2. PROJECT DESIGN

(i) Factors Selected for Study

This project was designed to evaluate the nature and degree of influence of selected orally administered contraceptive steroids on the external eye and the potential effect of such compounds on contact lens tolerance.

The factors selected for study were as follows:

(1) Tear fluid volume by means of a modified 3 minute Schirmer test.

(2) Tear protein composition by means of zone electrophoresis.

(3) Corneal curvature variation by means of a Bausch and Lomb Keratometer.

(4) Corneal oedema and staining by means of visual observation with a Zeiss Jena Slit Lamp.

It was thought that corneal sensibility might be a further factor liable to variation under the influence of contraceptive steroids. It was felt, however, that a nylon thread aesthesiometer was not sufficiently precise to detect small changes in sensibility. For this reason, routine aesthesiometry was not included in the study.

The four areas of investigation selected were considered to cover all other likely points of contact between the ocular, contact lens and hormonal systems.

(ii) Outline of Project Timetable

The three years available for the project were allocated as follows:

Year 1.

Background literature survey.

Development of electrophoresis technique.

Year 2.

Term 1:   Patient selection and preliminary contact
          lens fitting.

Term 2:   Regular tear volume, electrophoresis, keratometry
          and slit lamp readings to establish the normal
          levels of these factors for each patient.

Term 3:   Readings as above with patients taking an
          allocated medication.

Year 3.

Term 1:   Initial 2 weeks of readings to confirm normal
          levels of all factors, followed by readings
          during a 4 week lens adaptation period and for
          8 weeks following the re-introduction of medi-
          cation.

Term 2:   Termination of medication and continued monitoring
          of patients.
          End of clinical section of the project.

Term 3:   Preparation of thesis.

Adherence to a strict timetable of this nature was
essential to ensure adequate time for all aspects of the
study, especially in view of the fact that the clinical
sections had to conform to the 10 week academic term. In
addition it was necessary that the first period of medication
precede the long summer vacation. Sufficient time could then
elapse to ensure that, on resumption of the project readings,
any residual hormonal effect was negligible.

(iii) <u>Steroid Selection</u>

The choice of suitable steroids for use in the project was made by reference to the current list (Summer 1971) of oral contraceptive preparations available in Great Britain.

The oestrogenic component of all oral contraceptives was found to be either ethinyloestradiol 0.05 mg. or mestranol 0.05 mg. 0.075 mg. or 0.1 mg. The progestational compound was more variable. Norethisterone 1 mg., norethisterone acetate 1 - 4 mg. and norethynodrel 2.5 mg. or 5 mg. were marginally the most common, with ethynodiol diacetate 1 mg., megestrol acetate 4 mg. and lynestrenol 2.5 mg. also being used (see p. 70).

It had been intended to form six groups of subjects, i.e. a control group, two groups taking an oestrogen and three a progestogen. This would have required a minimum of sixty patients to allow for some patient loss during the project.

Eventually, however, only 24 patients were available. Three groups only were therefore realistic, an oestrogen, a progestogen and a control group. Both synthetic oestrogens are similar in structure and properties. Ethinyloestradiol 0.05 mg. was selected because it was more readily available than mestranol.

Norethynodrel and norethisterone on the other hand are said by some authors to have different characteristics resulting from a structural difference.[14] Both are primarily progestational but norethynodrel has some oestrogenic

properties while norethisterone is an anti-oestrogen
with some anabolic properties. Norethisterone was selected
as offering the greater contrast to the oestrogen group.

Final preparations obtained were:-

Oestrogen: Ethinyloestradiol B.P. 0.05 mg.

              'Lynoral' manufactured by Organon Ltd.

Progestogen: Norethisterone acetate B.P. 2.5 mg.

              'Norlutin - A' manufactured by Parke, Davis.

Control: Vitamin C. 50 mg.

              Boots Drug Co.

(iv) Patient Selection

In view of the rather exacting requirements of the
project it was not felt that sufficient subjects could be
obtained from the small female student numbers at Aston
University. It was therefore arranged that the clinical
section of the study should be based at Birmingham University
Health Centre.

Individual letters outlining the project were sent
to all first and second year female students who had been
listed as constant spectacle wearers at the time of their
medical screening on entering University. In return for
participation in the project, contact lenses were to be
provided free of charge. There would be an additional small
financial payment.

Volunteers were given a full eye examination and
preliminary contact lens fitting. No limiting refractive
criteria were applied, and the final refractive range of
the 24 patients accepted lay between + 2.00D and -9.00D

with varying amounts of astigmatism. Two patients had a longstanding monocular amblyopia and one a small angle alternating divergent strabismus. They were therefore felt to be a typical cross section of the type of patient encountered in any contact lens practice.

Lens fitting criteria had to be more precise and only people felt to have a good chance of success with 8.50 mm diameter tricurve lenses were accepted. On the basis of very good tolerance trials this finally included two girls requiring prism-ballast lenses for residual astigmatism.

Medical examinations were arranged for all selected subjects with Dr.S.F.Pexman of the Health Centre Staff.

(v) <u>Allocation to medication groups and patient loss</u>
     <u>during the project.</u>

At the end of the first 10 week period of tear volume, keratometry and slit lamp readings, patients were arranged as far as possible into three matched groups, and a medication allocated randomly to each. Groups were matched on the basis of:

> Average tear volume
>
> Refractive error
>
> Corneal curvature
>
> Degree of corneal astigmatism
>
> Prognosis of contact lens wear
>
> Reliability as a project subject.

Tear protein results were not available at this stage. Of the 24 subjects 18 had very good lens tolerance trial

reactions, while the remaining 6 were only moderately good.
This group were all keen to wear lenses however and were
accepted in view of the difficulty in obtaining patients.

Two of these six subjects were allocated to each group.
In the control group, one dropped out at an early stage
while the other adapted well to lenses. In the progestogen
group, both experienced great difficulty with the lenses,
but completed the project, one achieving all-day wear. The
two allocated to the oestrogen group finally withdrew after
failing to adapt to lenses despite three months perseverance.

All other subjects completed the project although
other factors intervened to disrupt the timetable in a
number of cases. Additionally, one girl who developed a
thyroid gland abnormality had to be transferred from the
oestrogen to the control group after the first half of the
project since the administration of oestrogen was interfering
with thyroid function tests.

(vi) Weekly examination routine.

Prior to the supply of contact lenses, subjects were
required to attend the Health Centre twice weekly. A
Schirmer test was performed at each visit and a keratometry
and slit lamp examination once a week. The Schirmer test
was invariably carried out before any other examination to
avoid influencing the volume results in any way.

The Schirmer test was performed on the right eye only.
Strips 5 x 50 mm were cut from Whatman No.1.Filter Paper
and stored dry at room temperature. With the aid of paper
tissues, thus avoiding direct contact with the filter paper

strip, the end 4 mm were bent over and inserted behind the lower lid, one-third of the way in from the outer canthus.

The patient had previously been instructed to look "up and to the left" and told that they might blink but must not change their fixation point. The strip was left in position for 3 minutes, at which point the end of the moistened section was marked, the strip removed and suspended from a piece of selotape allowed to air-dry.

These filter paper strips formed the sample for the protein electrophoresis. All separations were carried out on the day the sample had been obtained, maximum delay being in the region of 5 hours.

After contact lenses had been supplied, this general routine was modified to include a full after-care examination every two weeks. In the intervening weeks the routine as outlined above was again adhered to.

3. <u>CLINICAL ELECTROPHORESIS</u>

(i) <u>Introduction and Survey of support media available</u>

Electrophoresis is an analytical method based on the principle that a charged molecule or particle in solution will migrate towards one electrode in an electric field. A complex solution containing a mixture of particles of differing electrical charge may therefore be separated by this system.

All electrophoresis systems must contain charged particles, placed in or on a conducting support medium exposed to an electric field. The movement of particles through or along this conducting medium is termed 'electrophoretic mobility'. The electrophoresis system may be diagrammatically represented as:-

Support medium to which sample is applied.

Electrode in buffer solution.
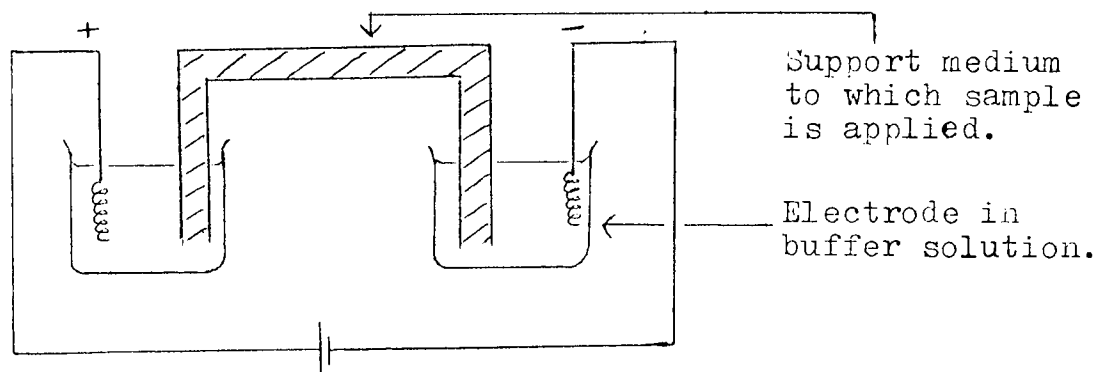
Electrophoresis is subdivided into moving boundary and zone electrophoresis. Moving boundary electrophoresis was initially introduced at the end of the last century, but was refined by Tiselius in 1937 and is now often called Tiselius' method. It utilises a fluid support medium, the sample particles are suspended in it and separate directly under the influence of the electric current.

This method however requires complex apparatus, has several technical disadvantages, and was not considered for this project owing to its need for a large quantity of sample protein.

Zone electrophoresis is a much more commonly used clinical technique. Its major difference lies in the use of a solid conducting medium to which the sample is applied. After separation into component parts, the protein may be fixed and stained on the support medium, providing a permanent record capable of quantitation.

The original support medium was filter paper and all clinical work prior to 1957 utilises this material. In 1957, Kohn introduced cellulose acetate membrane (C.A.M.) strips (manufactured by Oxoid Ltd., London) which greatly simplified and improved the technique.[53,54] Although more expensive, quality control of C.A.M. is very much better than that possible with filter paper, permitting more reproducible results to be obtained. The time required for an electrophoresis run is radically reduced on C.A.M. and greater resolution may be obtained from smaller quantities of sample.

Gel electrophoresis was the next development. First Agar Gel - a polysaccharide made from the cell walls of red sea algae, then starch, Smithies,[104] and most recently acrylamide gel, Raymond & Weintraub.[89]

Agar gel in a sense bridges the gap between paper or C.A.M. techniques and starch or acrylamide gel techniques.[80,90] The pore size of Agar gel, filter paper and C.A.M. is large

compared to the molecular size of the normal sample particles. In these media therefore, separation is on the basis of electric charge alone, there is no physical barrier to the passage of the sample molecules. Agar gel produces the same degree of resolution as C.A.M., but has the advantage of being the ideal medium for immunoelectro-phoresis - an additional technique which may be applied to proteins after their initial separation by zone electrophoresis.

Starch gel, when introduced in 1955 was found to possess much greater resolving powers than anything then available, so that it quickly became the preferred medium for analytical research work, although the substantial difficluties involved in the handling and processing the material has always limited its application to routine clinical quantitative work. Its major drawbacks concern variability between manufacturing batches and the fact that the gel must be sliced before staining to obtain good patterns. Slicing a gel into exactly equal portions is a very tricky operation. A further drawback is that it lacks transparency which makes densitometry difficult.

The vastly improved resolution of starch gel - 20 bands from a normal serum sample - may now also be achieved with acrylamide gel. The patterns produced are very similar to those obtained with starch, but the acrylamide gels have certain characteristics which render them preferable and more easily handled than starch.

These advantages are that acrylamide gel may be more

easily varied in concentration, permitting a range of
pore sizes to be produced; it is transparent which allows
ready densitometry; it does not require slicing; and it
has better keeping qualities.

Acrylamide and starch gels have relatively small
pore sizes, such that a 'sieving' effect is produced on
the protein molecules. Smaller molecules are therefore
passed preferentially through the gel matrix. Different
sized molecules with the same electric charge will therefore
be separated in either of these two media, whereas they
would migrate as a single unit on C.A.M. or Agar.

(ii) Choice of Medium

As may be seen from this preliminary outline, the
choice of support medium determines the type of electro-
phoresis to be undertaken and the degree of resolution
to be expected. Virtually all existing work on the
electrophoresis of tear fluid has been performed using
filter paper as the support medium. However, the time
required for each separation is considerable, e.g.
Erickson et al,[18] 6 hours, and Krause[56] 8 hours, and the
resolution achieved with normal unconcentrated tear fluid
may be restricted to the three major groups, lysozyme,
albumin and the grouped globulins.

It is possible to obtain greater resolution on filter
paper, 4 or 5 bands plus lysozyme, but only by concentrating
large volumes of tear fluid. For both these reasons paper
electrophoresis was ruled out.

The use of C.A.M. strips overcomes the time factor problem, a complete run being practicable in $1\frac{1}{2}$-2 hours. Separation into 3 fractions plus lysozyme is possible with small volumes of unconcentrated tear fluid. It was found by experiment however, that although separation was achieved following sample application in the form of a narrow strip of filter paper (as would be obtained from a Schirmer test), very much better resolution resulted from a liquid sample - and this is not always easy to obtain from relatively dry-eyed subjects.

Agar gel had to my knowledge been used once for human tear electrophoresis, Francois & Rabaey[25]. They found, however, that lysozyme does not migrate normally in this medium; interaction takes place between the enzyme and the gel such that its mobility is reversed and it is gradually precipitated in the process. These authors also found no fraction corresponding to the globulin fraction of paper or C.A.M. electrophoresis and were unable to explain their results.

Some preliminary work with Agar gel was nevertheless carried out, but was abandoned after some time due to difficulties with the actual production of the gel. It did not appear to be worth pursuing in view of the findings outlined above, and the fact that the potential resolution does not exceed that of C.A.M. strips.

Acrylamide and starch gel were then considered. Starch did not seem to offer any potential advantages over acrylamide, and is more difficult to prepare and use.

Acrylamide gel, with its much greater potential resolving power was therefore decided upon as the material to be developed if possible into a clinical technique for lacrimal fluid electrophoresis. Should the substance be found to be unsuitable, then C.A.M. strips were felt to offer the best alternative.

(iii) <u>Selection of electrophoresis apparatus design</u>

Within a few years of the introduction of acrylamide as an electrophoretic medium several experimental models had appeared for its use. Descriptions and papers on all variations are to be found in the proceedings of the Gel Electrophoresis Symposium of the New York Academy of Science 1964.

Raymond, co-author of the original paper introducing acrylamide, uses a vertical slab technique, in which liquid samples are introduced into wells in the top edge of the gel slab.[90] The slabs are of a homogeneous composition throughout.

At about the same time another group of workers led by Ornstein and Davis,[9,84] introduced the 'Disc' electrophoresis system. This uses acrylamide in the form of cylindrical rods formed within glass tubing. The term 'disc' used in the title is an abbreviation of 'discontinuous', and refers to the fact that the gel column is composed of three layers, (a) sample gel - large pore gel containing the sample, (b) spacer gel - also large pore and, finally, (c) running gel - small pore gel in which the separation actually takes place. Electrophoresis is carried out with

the gels in a vertical position, each end immersed in the appropriate buffer reservoir and connected to the power supply by carbon or platinum electrodes.

The need for a discontinuous system is disputed by Raymond, who considers it an added complication which in fact interferes with the initial phases of the electrophoresis. He also disagrees with much of Ornstein's mathematical description of the forces exerted on the molecules during the electrophoretic process.[84]

A simplified 'disc' electrophoresis has therefore been suggested[4,43] in which the cylindrical gel form is used without the sample and spacer gels. The liquid sample, made more viscous by the addition of urea or sucrose if necessary, is simply layered onto the running gel surface beneath the buffer solution, see Fig.2.

The apparatus required for the gel slab technique of Raymond is rather more complex than that of the disc system, so for this reason no work was attempted with this method, all attention being directed towards developing the disc or simplified system for use with lacrimal fluid.

(iv) Apparatus.   See plate 1 & 2.

Apparatus was constructed as follows, based on the description by Davis.[9]

Buffer reservoirs were prepared from transparent rigid plastic sandwich boxes, 17 cm x 11 cm x 3 cm deep. 8 holes 10.5 mm diameter were drilled at equal intervals round the circumference of a circle centred on the mid point of one box, and at matching points on the lid of a second box.

Fig.2. Acrylamide Gel Column Apparatus.

(a) Disc Electrophoresis:Ornstein and Davis[33]

Power
Supply

Electrode vessel
with buffer.

Sample gel.

Spacer gel.

Running gel.

Electrode vessel
with buffer.
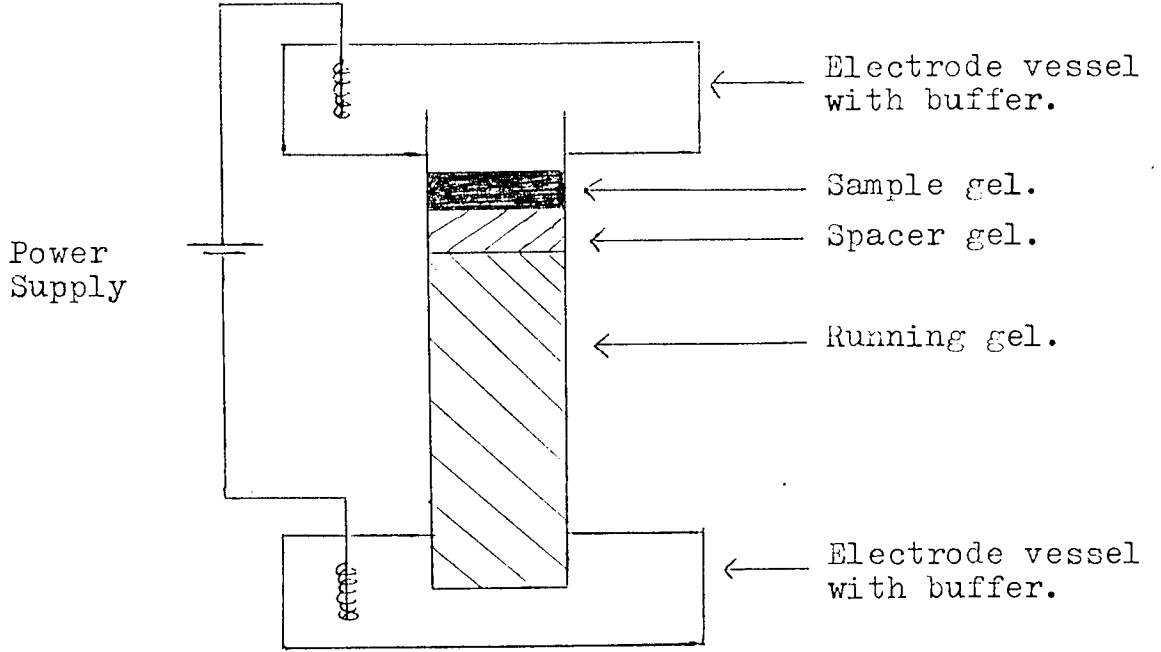
(b) Simplified Column Electrophoresis:Clarke[5]

Power
Supply

Electrode vessel
with buffer.

Liquid sample
layer.

Running gel.

Electrode vessel
with buffer.
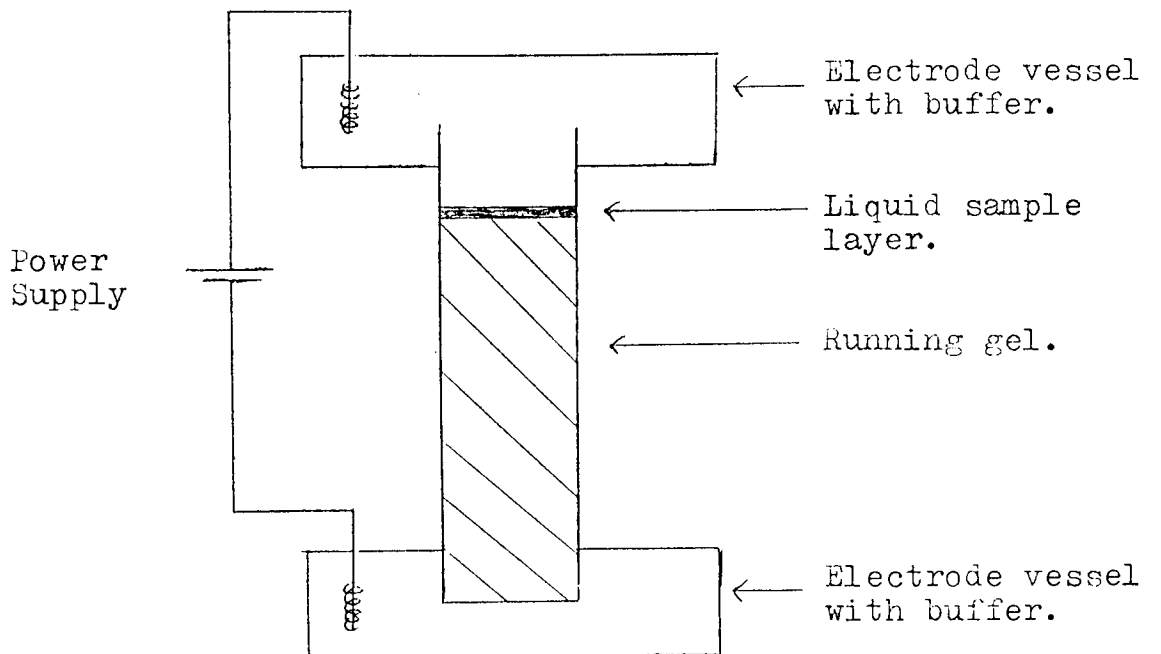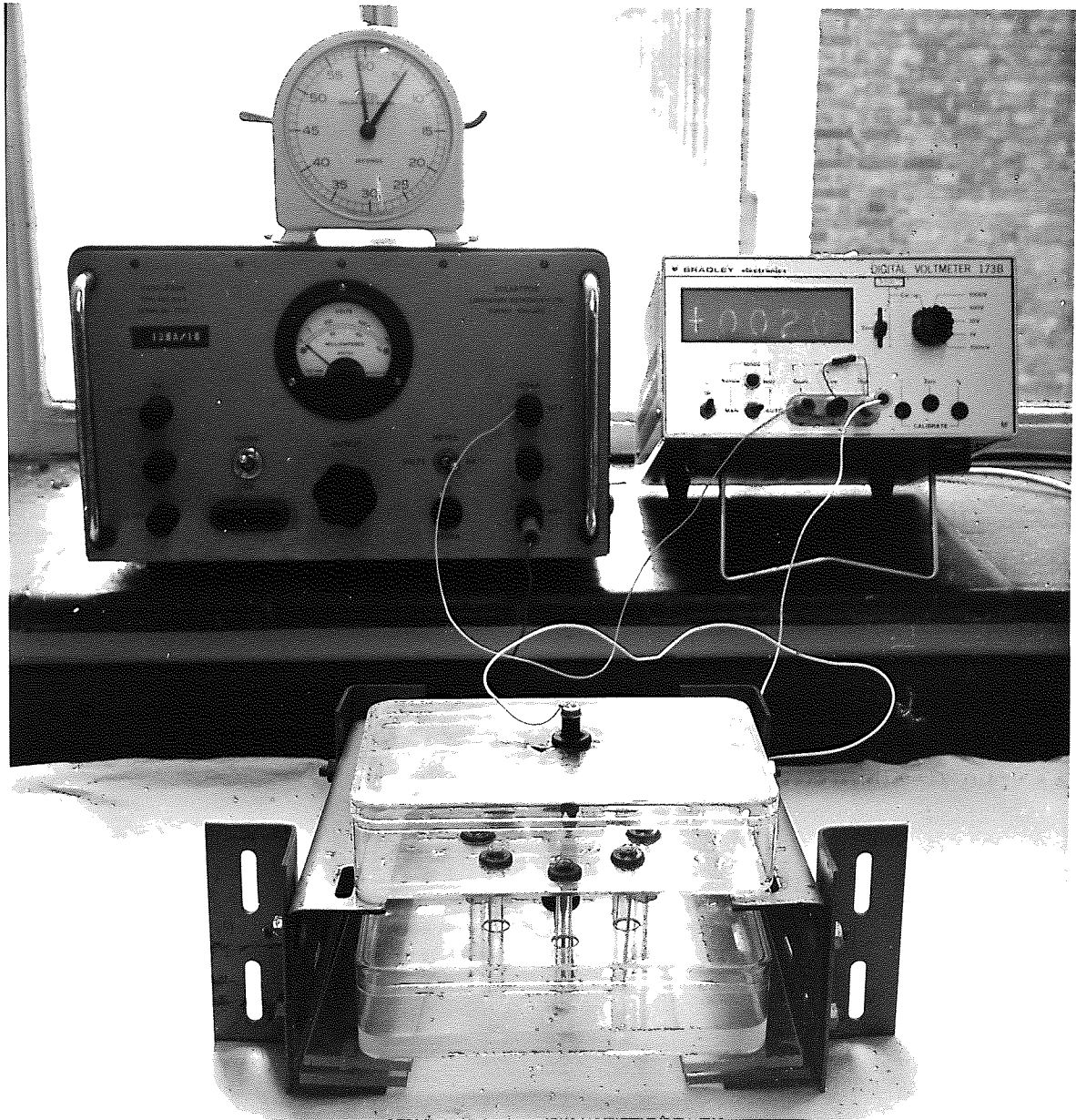
Plate 1. Vertical Column Electrophoresis Apparatus designed for use with Polyacrylamide Gel.

A hole 12.0 mm diameter was then drilled in the exact centre of both lids to carry the electrodes.

Rubber grommets of appropriate size were then inserted round the two electrode holes and the 8 holes in the box forming the upper reservoir.

Electrodes consisted of graphite rods removed from U2 Ever-ready batteries, cut down in length and with their caps soldered to wires connected to a Solartron Vari-Pac power supply. The rod forming the anode was found to require replacing at fairly regular intervals since it decomposed while in use.

Glass tubes 7 cm long were cut from one continuous length of tubing, internal diameter 5 mm, and then had both ends smoothed. When in use these tubes were inserted through the rubber grommets of the upper reservoir and through the corresponding holes in the lid of the lower reservoir. This second set of holes was found to help keep the tubes vertical as well as the lid forming a convenient carrier for the electrode.

Whatever construction is decided upon, it is essential to have all the tubes equidistant from each other and from both electrodes to ensure even voltage drop overall.

The whole structure was supported by handy-angle as shown.

A stabilised power supply should ideally be used, but as this was not available a digital voltmeter was connected across the circuit to monitor the current accurately and the power supply adjusted manually as

necessary. Considerable heat is generated during an electrophoretic run and this may damage the protein. An electric fan was therefore positioned in front of the apparatus to assist cooling.

<u>Destainer</u>.

A similar apparatus was constructed to destain the gels at the end of the electrophoresis programme. It is possible to destain gels fixed and stained with amido black simply by washing in dilute acetic acid, but this may take several days. The same result may be achieved in about an hour by immersing the gels in dilute acetic acid and passing an electric current through it. This is possible because the dye particles bind irreversibly to the protein in the gel, but in a reversible, still charged complex with the gel matrix itself. The electric current may therefore remove the background stain, while leaving the protein-stained areas intact.

This was most conveniently done in an apparatus very similar to the basic electrophoresis apparatus, but using glass tubing of greater internal diameter to allow the acid to surround the gel. The lower end of the destaining tubes were constricted slightly by firing, and then had a 'plug' of gel solidified in them. Both buffer tanks were filled with 7% acetic acid, the lower tank forming the anode, and current passed at 5mA/gel until the gel background was clear.

Having constructed a suitable apparatus it was necessary to establish the best formulation for gel and

buffer solutions; and this required an appreciation of the basic principles involved.

(v) Principles of Electrophoresis[3,63,80]

The presence of lysozyme in tear fluid prevents the straightforward use of gel systems designed for blood serum and other body fluids as may be seen from a consideration of the electrophoretic mobilities of the molecules involved.

The basic principle of electrophoresis is that, under the influence of an electric current, a charged particle in solution will migrate towards the electrode carrying the opposite charge. The charge on a particle is not constant however, it depends on the balance between negative and positively charged molecular groups within the particle. A protein is amphoteric, meaning that it can have positive, negative or zero charge depending on the pH of the surrounding medium. For a given protein, the point at which the positive and negative charges cancel out is termed its isoelectric point. At this pH, clearly the protein does not migrate. Altering the buffer pH of a system alters the charge on the sample particles and their electrophoretic characteristics.

This may be represented as follows:-

isoelectric point

pH 1	pH 7	pH 14
ACID		ALKALINE
H^+		OH^-

$$NH_3^+ - R - COOH$$

$$NH_2 - R - COO^-$$
$$+ H_2O$$

$$NH_3^+ - R - COO^-$$

In this hypothetical example the protein is represented as having an equal number of amine (NH_3^+) and carboxyl (COO^-) groups, with an isoelectric point therefore at pH7. However different proteins have differing ratios of amine and carboxyl groups such that the isoelectric point may be anywhere along the pH scale.

From the preceding description of the physical design of the system, i.e. sample application to the top of the gel, it is clear that all particles involved must migrate in the same direction - downwards into the gel.

The buffer characteristics must therefore be such as to introduce the same basic charge onto all proteins involved. They must be all negatively or all positively charged. Serum proteins have isoelectric points ranging from 4.7 - 8.2, and are usually separated in buffer systems of pH around 9.0. Lysozyme however, has an isoelectric point at pH 10.8, quite outside the serum protein range and at pH9 it migrates in the opposite direction to all other proteins.

This is quite acceptable on filter paper, C.A.M. or Agar plates where the sample may be applied at the mid-point of the support medium to allow migration in both directions. It is useless in a gel system where the lysozyme would be lost into the upper buffer reservoir.

The tear fluid electrophoresis system therefore must be based on a buffer more alkaline than 10.8 or more acid than 4.7.

The adsorption, i.e. the tendency of the molecules to adhere to the surfaces of solid bodies or liquids with

which they are in contact, of proteins increases with the lowering of the pH. Adsorption may distort a true electrophoretic pattern. Above pH7, adsorption is generally minimal, but below this level it may become significant. For this reason an alkaline buffer is to be preferred to an acid one.

Other buffer characteristics influence the electrophoresis process. Variation of the components of the solution may affect the protein pattern, although the mechanism of this inter-relationship is not understood and it is not possible to predict the effect theoretically. Each solution must be tried out individually.[23]

The ionic strength of the buffer is a considerable factor. High ionic strength produces sharper bands, but higher heat production and lower migration rates. Heat production should be avoided as far as possible so some increased diffusion of protein bands may be accepted with the use of lower ionic strength in the interests of heat reduction.

The final decision regarding running parameters is inevitably a compromise. For example, the distance of movement of a particle is directly proportional to the voltage applied and the time of electrophoresis. However a long electrophoresis run produces some radial diffusion of the protein and blurred bands. To get this amount of separation with sharper bands one increases the voltage - which generates more heat. A lower ionic strength buffer would reduce the heat but tend to blur the bands!

The formulation of the gel itself is clearly fundamental to the separation obtained.[42]   Several 'recipes' have been published both for the full disc process and the simplified version, and these were experimented with as appropriate. The length of the gel influences the distance of movement of the particles. The distance being inversely proportional to the length of the gel.

(vi) <u>Alkaline Electrophoresis</u>.

Since alkaline media are theoretically preferable, initial experimental work was conducted in a buffer pH12.

Acrylamide is a white crystalline substance which as used for electrophoresis is a mixture of two organic monomers, methylenebisacrylamide and acrylamide itself. It may be obtained as separate chemicals, but is most conveniently used as a compound, trade name Cyanogum - 41, which is a mixture in the proportions methylenebisacrylamide 5% and acrylamide 95%.

Although very soluble in water, it requires catalysing to form a gel, which varies from a soft material when made up as a 5% gel to a very stiff substance at 30% concentration.

Gels were made up as a single small pore gel in the proportions indicated by Nerenberg:-[80]

For a 10% gel -

Buffer Vol.	Cyanogum 41	Ammonium Persulphate	D.M.A.P.N.
100 ml.	10 gm.	0.25 qm.	0.465 ml.

Other gel concentrations are achieved by simple proportion of acrylamide. Ammonium persulphate and D.M.A.P.N. (β- dimethylaminopropionitrile) are the catalysts to the reaction. D.M.A.P.N. may be mixed with the acrylamide stock solution, but addition of the ammonium persulphate triggers the gelling process rapidly and must not be added until the last moment. Gel formation is impeded in the presence of air. The glass sample tubes were therefore positioned upright, supported in plasticine. They were filled to within 2-3 mm of the top with gel solution, and air excluded by layering a little distilled water over the gel surface. A fine drawn pipette was used for the water layering and any tubes in which the solutions mixed were discarded. The gel may be seen to have formed when a sharp interface becomes visible between the two substances.

Tear samples were obtained at first by means of 4 ul disposable micro-pipettes inserted into the lower formix. This is not a very satisfactory method however, since several attempts may be required before sufficient fluid is obtained.

The electrophoresis apparatus having been set up, the lacrimal fluid containing dissolved sucrose to increase viscosity and a bromophenol blue crystal as a marker, was layered onto the surface of the gel beneath the buffer solution.

Since there was no suggestion in the literature of very alkaline buffers being utilised with acrylamide gel, two routine chemical buffers were prepared.[80]

(a) 36 ml.   5.0M NaCl

     15.2 ml. 1.0M Glycine/1.0M NaCl

     12.4 ml. 2.0N Na OH

     dilute to 2 l   pH 12.00, I 0.1

(b) 375 ml.   0.25M NaOH

     120 ml.   1.0M Na_2HPO_4

     dilute to 1 l   pH 11.90

Numerous electrophoresis runs were carried out in order to compare the two buffer solutions, gel concentrations and current variation.

After electrophoresis the glass tubes were immersed in iced water for approximately 10 seconds, a No.1. hypodermic needle attached to a 5 ml syringe full of water was then inserted carefully between the gel and the tube wall at the lower end. The tube was rotated while depressing the plunger, such that the lubricated gel slid out of the tube. Gels were stained in 0.5% amido black dissolved in 7% acetic acid for an hour and then destained with the aid of the destainer or by repeated washings in 7% acetic acid.

As a result of these experiments, the glycine buffer (a) was found to produce marginally sharper results. Gels of 5%, 7.5% and 10% concentration were compared. The 7.5% and 10% gels were found to give a similar degree of resolution, superior to the 5% gel. The 7.5% gel was easier to remove from the tube however and therefore less prone to damage. This was selected as the optimum concentration.

A current of 5mA/tube for 2 hours was found to give good separation. Under these running conditions it was

possible to obtain six distinct protein bands within the gel.

It was then discovered that equally good separation could be obtained if the tear sample were applied on a filter paper disc, instead of by layering. A 5 mm disc could be punched out of the end of a filter paper strip which had been used for a Schirmer test, placed on the surface of the slightly dried gel, the top reservoir filled with buffer solution and the electrophoresis carried out as normal. This greatly simplified the sample taking procedure.

Having provisionally established the running conditions, human albumin and hen egg-white lysozyme were obtained to assist identification of the tear protein bands. Lacrimal fluid is said to contain 0.4% protein in approximately equal amounts albumin: globulin: lysozyme. The two chemicals were therefore made up as 0.13% solutions and run alongside tear fluid samples.

At this concentration the purified albumin formed two bands, the major one corresponding well with a major band on the tear fluid sample. The gels carrying the lysozyme samples, however, remained completely unstained despite repeated experiments using dilute and relatively concentrated (1%) enzyme solutions. A different approach was therefore attempted. Lysozyme was layered onto the gel and left overnight to diffuse into it. The top 5 mm of gel were then found to stain densely, showing that the lysozyme will stain if present. It had to be concluded therefore

that the very alkaline buffer was denaturing the lysozyme and preventing its normal behaviour during electrophoresis.

It was not found possible to overcome this problem and since lysozyme is one of the major lacrimal fluid components under investigation, this line of development had to be abandoned.

(vii) <u>Cationic, Low pH, electrophoresis</u>

A simplified technique was first investigated using the same basic gel formulation as used in the anionic system, but making the gel up in a buffer of pH 4.0. The buffer composition was:

> 2.0 gm   NaOH
>
> 288 ml   M. acetic acid
>
> dilute to 1 l. pH 4.00 I 0.05

Electrophoresis was carried out in 5% and 7.5% gels, particles now being positively charged, flowing towards the cathode.

At this acid pH the gels did not set easily and had to be heated, which distorted the upper surface. A maximum of 4 bands was all that could be obtained. As this line of approach did not seem very promising an attempt was made to utilise the full disc cationic system as devised by Reisfeld, Lewis and Williams.[92]

This requires a running, spacer and sample gel to be polymerised one above the other in the glass tubes in three separate operations - a much more time consuming process.

Full chemical details may be found for this method in Reisfeld et al[92] and a theoretical discussion of the requirements of such a system in Williams & Reisfeld.[115]

The small pore running solution was found to gel easily, unlike the large pore spacer and sample solutions in which it proved difficult to achieve the correct balance of catalysts to induce polymerisation.

Hen egg-white lysozyme and human albumin samples were used initially to try out the system. Both substances migrated into the running gel, the lysozyme demonstrating much greater mobility as would be expected in view of the charge differences existing between these two proteins.

Because of production difficulties, the spacer and sample gels were eventually discarded and the sample absorbed on filter paper or layered directly onto the running gel.

Optimum separation on 7.5% gels appeared to be 5 bands. 15% gels were found to offer no improvement and were much more difficult to handle.

It was felt that the dilute nature of tear fluid was possibly a limiting factor to further development of the technique. Albumin, for example, at the tear fluid physiological concentration of 0.13% solution, produces only a single stained band although even the most purified albumin available is known to be a mixture of substances. That this single band was a dilution effect, not due to an inherent flaw in the apparatus, was demonstrated by running a concentrated sample under the same experimental conditions. This concentrated sample was found to separate into six distinct bands of varying intensity, thus demonstrating the interdependence between the potential resolution of the system and the concentration of the applied sample.

At this stage however the best acrylamide system was producing only marginally better separation than the C.A.M. system - 5 bands as opposed to 4 - and was much more time consuming.

However, just before the end of the time available for this initial experimental phase of the project, two further papers were discovered, Fambrough et al[20] and Joice et al.[47] These workers described a modification of the Reisfeld cationic system, designed to operate with only the main small pore gel. With further minor modifications a combination of these two formulations were tried and resulted immediately in an improvement in resolution, some gels showing as many as 10 bands, with an average of 7 or 8 bands.

Gels of 5%, 7.5%, 15%, 20% and 25% were tried but the 7.5% again gave optimum results.

It was necessary at this point to start the clinical section of the project. Further developmental work on the electrophoresis technique was no longer feasible, so the decision was taken to use this final gel formulation as it stood. Quantitation and identification of the protein bands was carried out at a later stage in the project when a densitometer became available.

(viii) <u>Final Formulation and Procedure.</u>

Stock solutions for 7.5% gels.

<u>Solution A.</u> 48 ml 1N KOH
17.2 ml Glacial Acetic Acid
4   ml N'N'N'N' tetramethylethylenediamine
(TEMED)

deionised water to 100 ml.

Solution B.   30 gm Cyanogum-41
deionised water to 100 ml.

Solution C.   0.2% w/v. ammonium persulphate in 10M
Aqueous urea solution.

Gel Mixture.

1 vol. Solution A + 2 vol. Solution B +
5 vol. Solution C.

Buffer.
  pH 3.9   3 · 12 gm β- Alanine
1 · 10 ml glacial acetic acid
distilled water to 1 l.

Protein Stain.

0.5 gm amido-black dissolved in 100 ml
7% acetic acid and filtered.

Destaining Solution.

7% acetic acid.

Procedure.

1.   Sample tubes are cleaned, rinsed in distilled water
containing a little 'Photoflo' (approx. 1:200) and
allowed to dry. The 'Photoflo' coats the inside of
the tube and assists both water layering and the
final removal of the gel.

2.   Tubes are then positioned vertically in a strip of
plasticine to seal the lower end, and filled with
gel solution to within 3 mm of the top. Air is
excluded by layering a small quantity of distilled
water across the gel solution surface. Any tubes in
which the solutions are seen to mix must be discarded.

3.   When set, the water is shaken out of the tubes which
are then immersed in buffer solution until required.

4. Before use the sample tubes again have any solution shaken out of the top end, and are then inserted into the rubber grommets of the upper, anode, tank. All the tubes must be vertical and protrude an equal distance through the grommets. A hanging drop of buffer is placed on the bottom of each gel tube to prevent trapping air bubbles. The upper reservoir is then lowered until the bottom of the gel tubes are immersed about $\frac{1}{4}$ inch into the buffer of the lower, cathode, reservoir.

5. A paper punch is used to cut a 5 mm disc from the end of the sample Schirmer paper strip. This disc is inserted into the neck of the gel tube with forceps and pressed down so that it makes contact evenly over the gel surface.

6. The remaining space in the tube and the upper reservoir is then filled with buffer solution. The anode and cathode are inserted and the power supply turned on.

7. The current is adjusted to 2mA/tube for 5 minutes and is then increased to 4mA/tube for 85 minutes. An electric fan is positioned in front of the apparatus to assist cooling.

8. When the electrophoresis is complete, the cathode buffer is discarded since its pH is materially altered by gel catalyst substances which migrate into the cathodic tank during electrophoresis[9] The anodic buffer may be reused in the cathode tank on a subsequent occasion, after which it is in turn discarded.

In this way the tear sample is always in contact with fresh buffer solution in the upper tank, but the total volume of buffer utilised in a large series of experiments is considerably reduced.

9.   The gel tubes are removed individually from the upper reservoir and immersed in iced water for 10 seconds. A No.1 hypodermic needle attached to a 10 ml. syringe full of water is slowly introduced between the gel and the tube wall at the lower end. The tube is rotated and water from the syringe lubricates the gel so that it slides smoothly from the tube. Insertion of the needle at the top end of the gel should be avoided because of the risk of surface damage which would interfere with later densitometry.

10.   The gel is immersed in a test-tube full of dye for a minimum of one hour. It is then rinsed in water and transferred to the running tubes of the destaining apparatus. The gels are destained in 7% acetic acid at 5mA/ gel.   A preliminary soak in dilute acetic acid speeds up the destaining process.

11.   Cleared gels may be allowed to dehydrate for storage or may be maintained in a hydrated form in 3% acetic acid.

Note.

1.   The absence of a densitometer during the developmental part of the project prevented any evaluation being made of the variation between fresh and stored gels. All gels in this project, therefore, were made and used on the same day.

2.   Acrylamide gel reacts normally to the Periodic Acid -
     Schiff (P.A.S.) reagent for location of protein bound
     carbohydrates[4]. This staining procedure may therefore
     be performed to aid identification of separated bands
     as an alternative to the amido-black general protein
     stain.

4. A DISCUSSION OF THE RESPONSE OF THE ANTERIOR SEGMENT
   OF THE EYE TO THE PRESENCE OF A CORNEAL CONTACT LENS

(i) Introduction: Initial Fitting and Patient Response.

Experience has shown that the majority of eyes will
tolerate, without too obvious signs of distress, the presence
of a contact lens fitted by means of widely differing clinical
criteria.

In many patients, lenses of varying diameter, thickness
and corneal curvature relationship can all appear equally
acceptable, while in a few patients a small modification to
any of these variables may succeed in producing a comfortable
fit from one which was previously rejected.

Mandell[64a] states that "the physiological requisites for
successful lens wear are usually satisfied by three general
fitting rules: tears must circulate freely between the lens
and the cornea, excessive lens weight pressure must not be
borne on small corneal areas and the lens must not move
excessively."

These requirements are now generally achieved by fitting
a multicurve lens - i.e. one with a three or four curve back
surface - such that there is "adequate" central clearance
combined with "sufficient" peripheral stand-off. These two
factors are related to the diameter of the lens, which in
turn is related to the overall corneal diameter. It is not
possible therefore to assign exact numerical values to the
central clearance and edge stand-off. In general terms,
a large lens is fitted with a central curve parallel to the
corneal surface as far as possible, but with a wide peripheral

stand-off to take into account the limbal corneal flattening.
A small lens which is designed to remain always on the steeper
central corneal areas requires a central curve of shorter
radius than that of the cornea, thus preventing heavy central
pressure, but with a narrow peripheral stand-off to minimise
lid irritation.

A normal and acceptable reaction to lenses in a well
motivated patient follows a fairly regular pattern. Initial
lens insertion produces a "foreign-body" type reaction -
profuse lacrimation, conjunctival injection, rapid blinking
and possibly mild blepharospasm. These extreme reactions
subside within approximately 15 minutes of lens wear and the
blink rate may in fact become slower than normal if that is
found to be more comfortable. During the early stages of an
adaptation schedule this same foreign-body reaction will be
encountered each time lenses are inserted, getting progressively
less severe each day as wearing time and adaptation improve.
At the end of 3-4 weeks wear, if all day wear is achieved,
lens insertion produces no apparent reaction and the lenses
are no longer "felt" by the wearer.

In addition to lacrimation, some patients experience
photophobia and many are troubled by so-called "spectacle
blur", i.e. the inability to see clearly through existing
spectacles after removing their contact lenses.

This ease of adaptation is in fact rather remarkable
when one considers that a large piece of plastic is being
placed in very close contact with the anterior surface of
the eye and then compares this with the violence of a normal
ocular response to a foreign body.

The immediate physical criteria necessary to allow this
adequate initial comfort and therefore allow a patient to
embark on a daily wearing and adaptation schedule have been
outlined above. The developing adaptation process involves
the cornea, the pre-corneal film and lacrimal system, and
the lids and lid margins. It may also affect the conjunctiva
and occasionally the anterior angle, aqueous veins and intra-
ocular pressure.

The inter-relationship of the corneal lens with these
structures will be considered in detail.

(ii) Corneal Structure and Metabolism.

The cornea is generally considered to be a 5-layered
structure. The majority of its thickness is connective
tissue, stroma, bounded by a membrane on both surfaces,
with beyond this on one side stratified squamous epithelium
and on the other a single layer of endothelial cells.

"The chemical composition and biochemical reactions
of these various layers do not appear to differ in any major
respect from other similar tissues in the body, although
there are some differences. The mucopolysaccharide
composition of the connective tissue has a distinctive
pattern and a transport system is present in the endothelium
which is thought to control hydration of the tissue."[32]
The fact that the cornea is exposed also means that reactions
must take place at temperatures lower than that of the blood.
The structure is also avascular and this poses specialised
problems regarding nutritional supply.

Metabolites enter the cornea by three routes. From tear fluid across the epithelium, from limbal blood vessels and from the aqueous across the endothelium. Movement of substances within the cornea is by diffusion.[37] There is no circulation of lymph or other fluid and resistance to water flow in the normal stroma is high, although pathological or very oedematous corneas may form clefts or channels.[6]

The distribution and site of origin of a substance in the cornea may be calculated by reference to the laws of diffusion.[67] This indicates that the cornea is almost in equilibrium with the aqueous regarding small-medium molecular weight substances and ions, and fat insoluble material, i.e. there is free diffusion across the endothelium for these substances and therefore only the very peripheral cornea need draw any supply from the limbal blood vessels.

A large molecule, e.g. serum albumin, diffuses right to the centre of the cornea from the blood supply. Glucose, although large, has a special mechanism of rapid exchange across the endothelium and oxygen diffuses freely across both epithelial and endothelial surfaces.[6, 32] This ease of diffusion of ions across the endothelium has the effect of keeping the ionic composition of the cornea fairly constant.

The finer details of corneal metabolism are still to be clarified, mainly because of the difficulty of studying individual layers without totally disrupting the normal situation. It would seem, however, that the 3 major layers are basically self-sufficient in their metabolism.

The epithelial and endothelial carbohydrate metabolism is thought to be aerobic glycolysis by means of the Embden-Meyerhof pathway ($\simeq$ 65%) and the Pentose Phosphate shunt ($\simeq$ 35%), with final aerobic degradation of pyruvic acid to water and carbon dioxide by the citric acid cycle.[32]

The stroma is felt to carry out anaerobic glycolysis only (by the Embden-Meyerhof pathway) with a consequent high production of lactic acid as a final reduction product of pyruvic acid.[32]

In the epithelium the anaerobic glycolytic pathways (i.e. those reducing glucose to pyruvic and then to lactic acid) are more efficient than the aerobic citric acid cycle. Therefore there is some build up of lactic acid in this structure as well, since the lactic acid production is more rapid than is the final aerobic degradation to water and carbon dioxide.[49]

The function of these katabolic reactions is the liberation of energy, to be then captured in the form of high energy chemical bonds. The chemical compound adenosine triphosphate (A.T.P.) contains 2 high energy bonds and is the major energy carrier of the metabolic system. Synthesis reactions and secretory or muscular activity in the body are anabolic activities and require energy. These processes however, cannot utilise free energy and must have it supplied in the form of high energy bonds.

Anaerobic glycolysis of glycogen to lactic acid produces only 2 A.T.P. molecules, so that of the total 686,000 cals. in a glucose molecule only 50,000 cals. are captured. The remaining energy is in the lactic acid molecule which is a

waste product. The presence of oxygen and the citric acid cycle however produces another 36 A.T.P. molecules by oxidation of pyruvate to water and CO_2. Thus the full aerobic process produces a total of 38 A.T.P. molecules.

Any reduction or disruption of the oxygen supply to the epithelium will cause a drastic reduction in the amount of energy available to the tissue with consequent impairment of its function.

(iii) Hydration considerations

The intact cornea is a relatively dehydrated structure. Isolated corneal tissue placed in any water-based solution swells appreciably.[85] The intact cornea must therefore have a means of maintaining dehydration since water intake causes swelling of the polysaccharide ground substance and dislocation of the stromal latticework of fibres with consequent loss of transparency.

There are various factors involved. The epithelium and endothelium must act both as a physical barrier to the inflow of water and as a membrane allowing transport or osmosis of water out of the structure. It must be remembered that in addition to being bounded on both surfaces by fluid, the cornea is actively producing water as a waste product.

Some water loss occurs across the epithelium although the structure has a high resistance to the movement of all ionic substances and is only one-third as permeable as the endothelium.[5,13,67] Evaporation of the tear film causes concentration of this bathing solution with consequent osmotic water flow out of the cornea. If the limiting effect of the external oily layer on the tear film is

removed, evaporation of tears and consequent corneal fluid loss is greatly exaggerated. The rabbit cornea for example will dry to half its normal thickness in 2 hours.[75a]

Major control of hydration in a normal intact eye rests with the active transport mechanism of the endothelium. The exact mechanics of this transport of fluid against the concentration gradient are not clear, although the energy requirements appear to be satisfied by the metabolism of the endothelium itself on a self-supporting basis, utilising oxygen from the aqueous humour.

It is clear therefore that normal functioning of the cornea depends on the correct supply of metabolites facilitating adequate energy production for such things as cell division and formation, active transport of ions and water for maintaining transparency etc. What change then does a corneal lens produce?

(iv) Oedema

Clinically, especially during the early stages of wear, a contact lens induces epithelial oedema. This is manifest subjectively by an increased relative myopia when the person removes the contact lenses, i.e. between - 0.5D to - 2.00D needs to be added to the previous spectacle correction to produce an optimum visual acuity. In some cases this optimum visual acuity may be lower than that which was normal with spectacles prior to contact lens use, indicative of a surface distortion of the epithelium, or a stromal disturbance. The duration of this 'spectacle blur' varies between a few minutes to several days and is generally more severe when small diameter, steep fitting lenses are used.

Oedema may be noted objectively with a slit lamp and a keratometer. With a slit lamp, using the techniques of scleral scatter on low magnification and retro-illumination and high magnification, one may see areas of greyness and distortion of the corneal layers, which may also be sometimes seen to be thicker than the surrounding non-oedematous tissues. In gross oedema (see patient 9PK) the permeability of the cornea may be so affected that fluorescein penetrates through the epithelium into the stroma and thence into the anterior chamber.

Routine keratometry readings detect oedema of the central cornea by means of steeper corneal radii. If the swelling is regular the instrument mires are reflected accurately, however, if the epithelial surface is irregular, the mire reflection is distorted.

The spectacle correction, slit lamp and keratometry results thus act as supporting evidence for each other, but frequently do not correspond exactly, e.g. change in corneal power may not be equal to the refractive change, or no oedema may be visible on the slit lamp despite refractive or radii changes.

The reason for lens-induced oedema rests probably in a combination of the "osmotic theory" of Mandell and co-workers,[36,64b,65,88] and oxygen deprivation considerations.[21,87]

Taking the osmotic theory first. Corneal thickness has been shown to be related linearly to hydration.[38] It is felt that the 'foreign body' reaction of a new lens wearer which gives rise to excess lacrimation, results in

a more dilute, lower tonicity, bathing solution over the epithelium with a consequent osmotic fluid flow into the cornea.[65] If an unadapted wearer has a lens placed on one eye, an increased thickness of up to 30% is stated to occur in the other eye, which can only be due to the altered tear flow.[36,64b] If the experiment is repeated in an adapted wearer, the effect does not occur. The implication is that the adapted wearer has reverted to a normal lacrimation pattern.

In the normal state the fluid component of the pre-corneal film is concentrated by some evaporation to a slightly hypertonic level relative to the cornea, thus encouraging fluid flow out of the cornea.[75a,75b]

However during sleep the cornea thickens[93] and this fact is incorporated into the osmotic theory by assuming that the closed eye is in contact with fresh unevaporated tear fluid of lower tonicity. Therefore it swells.

The osmotic theory certainly conforms to the clinical finding of substantial corneal steepening in the early stages of lens wear, subsiding over the 3-4 week adaptation period as lens tolerance improves, but I cannot accept Mandell's claim that "tear tonicity is the primary mechanism of the adaptation process" and that oxygen deprivation is only involved in extreme cases.

(v) Corneal Steepening as an index of Oedema

In this study the average corneal steepening after 2 weeks wear (2 x 4 hours per day) was + 0.145 mm. No corneas flattened. After 4 weeks, when most had achieved

all-day wear the average steepening was + 0.102 mm and
after six months wear, it was still average + 0.070 mm.
Mandell considers 4% increase in corneal thickness to be
due to osmotic tear tonicity effects. 4% corresponds
very approximately to betweem 0.02 and 0.07 mm steepening
in keratometry reading (calculation: Appendix 1) depending
on the basic corneal curvature. At an average corneal
radius of 7.50 mm, 4% thickness change ≡ 0.05 mm radius
change. The degree of corneal steepening encountered in
this study would therefore appear to be considerably in
excess of the level explicable on the basis of tear tonicity
alone. The difference between the steepening after 2 weeks
and after six months however may be argued to represent the
subsiding osmotic induced steepening, which at 0.075 mm is
in line with the Mandell thickness results.

Hill & Fatt[39] have shown that a corneal contact lens
can interfere with movement of oxygen from the air to the
cornea under normal wearing conditions. Under a stationary
lens the movement of oxygen to the cornea is proportional
to the tear volume contained between the lens and the cornea,
and this is exhausted in only a few minutes even under a
steep lens.[40] Replenishment of oxygen must therefore occur
by tear exchange under the lens during blinking.

That oxygen deficiency causes disruption to the
epithelial metabolism is shown conclusively by Smelser[102]
who passed nitrogen through airtight goggles and produced
corneal hydration and Sattlers' Veil phenomenon. This
experiment was repeated by Mandell et al[65] who found 6-7%

swelling with oxygen-free nitrogen in 2 hours. Thickness measurements were made with a modified Haag Streit thickness device. A patient fitted with a very large tight lens produced a swelling curve almost identical to that from the anoxic environment. The lens also gave rise to severe discomfort, photophobia and blurred vision. They found experimentally[87] that oxygen pressures down to approximately 10% of normal (11-19 mm Hg) produced no hydration, although some disturbance of metabolism must have occurred as the oxygen pressure reduced towards this level before it became manifest at any particular numerical value. (It is clear also that the calculated oxygen pressure available to the cornea from the conjunctival blood vessels during sleep (i.e. 55 mm Hg) is well in excess of what is required).

Clinically it is found that a small steep fitting lens produces greater oedema and spectacle blur than an alignment fit. The less mobile lens tends to 'seal' onto the cornea making tear fluid circulation much more difficult. The provision of a fenestration hole in this type of lens appears to release the suction effect, facilitating fluid movement with consequent improvement in corneal reaction (see patient 9PK).

It must not be forgotten however that as stated earlier, the bulk of hydration control in a normal cornea rests with the endothelium and its active transport mechanism. This mechanism depends on metabolic energy, which itself requires oxygen, but this is obtained exclusively from the aqueous humour and is independent of the epithelium. Thus the

presence of a contact lens can have no direct effect on the energy supply to the active transport mechanism of hydration control.

Its effect must therefore be to limit the oxygen, and thus the energy, available to the epithelium. This in turn will interfere with the ability of the layer to maintain its cellular structure, leading to a breakdown of its ionic barrier effect and increased permeability. This would seem to confirm the clinical finding of epithelial oedema with contact lenses, not generalised oedema throughout the whole cornea as would be expected if the active transport mechanism had broken down.

A reduced energy supply in the epithelium is also likely to affect the active transport of sodium which is known to occur normally against the concentration gradient into the tissue from the tear film.[32] What effect a reduction in sodium concentration would be likely to have has not been investigated. It is an ion, however, which is intimately involved in both the water balance of cells and, after diffusion across the stroma, in the active transport mechanism of the endothelium. It is also involved in neural transmission of nerve fibres.

The more anaerobic metabolism will also give rise to greater lactic acid concentration in the tissue.[78,103] A normal epithelium is impervious to lactic acid, but the disruption of its normal permeability by a contact lens might allow it to diffuse out into the tear film where it could be slightly irritant.

It would seem clear then that although both osmotic and oxygen deprivation considerations are almost certainly involved during the early stages of lens wear, the long term effects of altered oxygen supply are of much greater significance.

Neither principle, however, provides any explanation of the 'adaptation' of a person to a contact lens. They merely help explain the clinical finding of epithelial oedema. The osmotic theory assumes that as 'adaptation' proceeds, the eye's foreign body reaction diminishes so the excess lacrimation returns to normal. This then implies that some form of neural adaptation of the cornea and lid margin has occurred so that the lens is no longer 'felt'.

On the question of oedema also, mild oedema is quite symptom free, while severe oedema can cause considerable discomfort, often without any evidence of microscopic surface corneal damage. A patient suffering an overwear syndrome for example, seen several hours after onset of the symptoms but still suffering severe pain, lacrimation and blepharo-spasm frequently shows gross oedema but very minor corneal stain, quite out of proportion to the prolonged symptoms.

(vi) <u>Sensory System Effects</u>

What then does happen to the sensory system during normal and abnormal lens wear?

Corneal sensibility is normally measured with a nylon thread Aesthesiometer or a modification of the same principle which is a rather crude method very subject to operator variables. There is on the subject a dearth of controlled

histological or neurological study and a large quantity of conflicting clinical observation.

Major work on normal human and animal corneas with comprehensive literature surveys has been done by Zander & Weddell[118] on corneal innervation and Lele & Weddell[59] on sensibility.

It has been classically taught that only free nerve endings are present in the cornea and that pain is the only sensation that can be aroused from the cornea proper. This former point would seem to be confirmed by recent electron-microscopic studies, but the latter has been disputed almost since it was first asserted by Von Frey in 1894.

The overriding weight of the evidence[59,118] would seem to show that the cornea is also sensitive to touch with a pain threshold approximately 10x the touch threshold.[74]

Surgical procedures involving section of the descending root of the 5th cranial nerve to relieve trigeminal neuralgia frequently leave patients aware of a sensation of touch on corneal stimulation although the pain threshold is abolished.[31]

Lele & Weddell[59] in one of the most controlled experiments to date are quite firm in their conclusions that in addition to touch and pain, sensations described as heat and cold are consistently experienced by untrained unbiased subjects in response to heated or cooled air jets or copper rods. These authors conclude that all four sensations of pain, touch, heat and cold are mediated by free nerve endings and that the whole concept of specific nervous energies is due for revision.

The following description of corneal innervation is taken from Zander & Weddell.[118] The corneal nerves are derived from the ophthalmic division of the 5th nerve through the ciliary nerves and enter the sclera a short distance behind the limbus from where the majority enter the corneal stroma in the form of 70-80 large bundles arranged in a radial manner. The myelin sheath is lost within 2 mm of the limbus and the bundles sub-divide to form a plexiform aggregation of fibres in up to five layers throughout the stroma, denser towards the epithelium. Schwann elements accompany nerve bundles, but not single axons. Some axons may be traced up to three-quarters of the way across the cornea, others pursue complicated courses and give rise to many daughter axons by axis cylinder division. Within the stroma nerve bundles are flattened antero-posteriorly and fibres lie side by side rather than in a cable formation.

A certain proportion of fibres terminate in the stroma. Axons leave the larger nerve bundles as naked fibres, pursue a winding course, giving off many side branches which themselves sub-divide, thus setting up a network often covering a quadrant of the cornea.

Fibres leave the plexiform arrangement in the stroma and penetrate Bowmans membrane perpendicularly, apparently through definite apertures, to reach the epithelium, where they shed their Schwann cells and continue entirely naked, lying in direct relation to the cells composing the structure. Fibres run between the basal layer cells where many terminate. Others sub-divide anything up to 15 times and pass into the more superficial layers of the epithelium, either vertically

or obliquely between the cells. The fibres are beaded and
terminate throughout the layers even among the most super-
ficial cells which they approach at all angles. All nerve
endings are between cells, not within the cellular cytoplasm.

As a final afterthought almost, these authors suggest
tentatively that the beaded free nerve endings in the
epithelium might subserve pain, while those in the stroma
might subserve touch - an idea based on the progressive
abolition of pain and then touch sensation by increasing
concentrations of local anaesthetic.

Weddell[109] is also quoted as describing a gross
variability from week to week in the number of clusters
of corneal naked nerve endings simulating in appearance a
Krause's end bulb - an observation which would indicate that
the normal nerve endings may be in a constantly changing
dynamic state.

Electrical transmission along a nerve is mediated by
an ACh/ protein enzyme system sited in the surface membrane
structure, which alters the conductance of the membrane in
response to a stimulus, allowing free flow of sodium and
potassium ions across it. This ion flow then acts as the
stimulus to trigger the depolarisation of the next section
of the nerve.[79] The reaction time of the nerve fibre and
enzymic reaction is of the order of 30-40 usec. A nerve
may therefore potentially transmit many thousand impulses
per second.

The role of any receptor or sensory unit is to record
the state of, or change in, the chemical or physical
environment by initiation of an impulse which is then

conducted by the primary afferent fibre to the C.N.S.

Receptors tend to be sensitive to one particular form of energy and ignore others. Pain receptors are the exception to this and have a low sensitivity to all forms of energy but react to any energy form which reaches damaging or near damaging levels.[109] Since corneal nerves are considered to be pain receptors, they may potentially react to any stimulus, i.e. internal or external pressure, chemical change, temperature change, or physical damage to nerve endings.

Studies of corneal sensibility to touch stimuli in normal eyes compared with contact lens wearers give rise to conflicting results. It would seem fairly universally accepted that sensibility, as measured with an aesthesiometer, is reduced during the early stages of lens wear to quite anaesthetic levels.[1,11,81] Dixon[11] calibrated a nylon thread aesthesiometer in terms of the weight in milligrams of the pressure required to bend the thread at different lengths. He then measured corneal and lid sensitivity before lens fitting and again when fully adapted, and found that the pressure to the central cornea had to be increased from the equivalent of 1 mg (the pre-fitting normal) up to 13 mg. before it was detected by the adapted wearer. Lid margin sensitivity was also affected from an average of 13 mg pre-fitting to 50 mg when adapted. Lowther & Hill[61] also measured the lower lid margin sensitivity threshold. They found it dropped slightly during the first 7 days of lens wear and then very substantially, until by day 21 the instrument nylon thread could not be detected at all.

Dixon continued his experiment by removing one lens from an adapted wearer. The non-wearing eye took 5 days to return to normal, while the other remained at its adapted level. He makes other observations. Within a large group of adapted patients he finds some with no sensory reduction of the cornea or lids at all. Others are so insensitive that a Schiøtz tonometry may be performed without any local anaesthetic, and in these patients there is no recovery of sensibility overnight. Sensory measurements are equal first thing in the morning and after 8 hours lens wear.

Kraar & Cummings[55] compared a group of successful and unsuccessful lens wearers, all examined after 8-10 hours without lenses. They found a significant difference in the central corneal sensitivity with no significant difference on the limbal areas.

The contact lens effects on corneal sensitivity would seem to fall into several distinct categories. Firstly, there is the rapidly diminishing reaction on initially inserting a lens. Secondly, the semi permanent (i.e.taking several days to return to normal) reduction in sensitivity which develops during the adaptation period and is then commonly - though not invariably - present in established wearers.

There is then the question of oedema produced pain or discomfort accompanying an overwear syndrome or resulting from poor lens design. In this latter case, however, the discomfort tends to build up over a few hours of lens wear, but then will often subside if the lenses are left in place. (see patient 8JH).

The initial reduction in response to a new lens is
the easiest to explain if one may visualise it as a
conventional steady stimulus response, i.e. a reduction
in the rate of firing of the sensory nerve impulses as the
stimulus is maintained. At this stage, however, the lens
is still "felt" by the person although less dramatically
than in the first few minutes.

A major complication in the evaluation of this whole
question is the psychology involved in the situation.
A book by Melzack[72] appropriately entitled "The Puzzle of
Pain" states "there is much evidence that pain is not simply
a function of the amount of bodily damage alone. Rather,
the amount and quality of pain we feel are also determined
by our previous experience, by our ability to understand
the cause of the pain and to grasp its consequence ....
pain is much more variable and modifiable than has been
believed in the past and differs from person to person and
from culture to culture." (p.21)

Considering touch as a similar but less intense stimulus
than pain, one can see that it becomes very difficult to
ascertain what proportion of the so-called sensory adaptation
to contact lenses is in fact due to localised corneal effect
and what is attributable to a psychological acceptance of,
and familiarity with, this new experience. Undoubtedly an
insufficiently motivated person will never succeed in wearing
lenses, no matter how skilled the practitioner.

Thus the initial rapid improvement in ocular reaction
may well be partly due to reduced sensory input, but it is

almost certainly due to the considerable relaxation of
tension on the part of the patient as the fitting session
progresses.

The long term reduction in sensitivity is much more
difficult to explain. In those comfortable all-day wearers
with normally registering corneas, some form of psychological
or central sensory suppression must be occurring. In those
wearers with manifestly reduced corneal sensitivity it is
still possible that a central mechanism is involved, although
localised corneal level changes also seem likely.

The nature of such localised change is totally
uninvestigated. One can therefore only speculate:

(a) direct mechanical damage to the nerve endings by
    pressure or friction from the lens;

(b) interference by the lens with the normal energy
    producing metabolism of the neural system -
    O_2; temp.,excess lactic acid in surrounding
    tissue etc.

(c) interference with the sodium active transport
    system into epithelium producing a deficiency of
    ions necessary for neural transmission.

The reduction in sensitivity of the lower lid margin[11,61]
is presumably due to continual mechanical impact of the
lid on the edge of the lens. Again it is unknown if one
is dealing with localised receptor damage, or central
suppression of a repeated peripheral imput.

The origin of the discomfort induced by oedema is
also uncertain. It tends to build up gradually during

the day, mild attacks often subsiding if the lenses are left in place. Alternatively, the eyes may be quite comfortable with the lenses in place, and only start to hurt some time after lens removal. The oedema (visible with a slit lamp) may be generalised or localised to a discrete small section of the epithelium. In very acute instances vesicles may form within the epithelial layers (Patient 9PK). The epithelial surface, however, usually appears to be quite unbroken. The stimulus firing the nerves therefore would seem to be related directly to the free nerve endings position within the tissue, i.e. either physical dislocation of the fibre as the structure swells, or the swelling pressure of the tissues or the change in the chemical environment of the nerve ending, in terms of absence of oxygen and a locatised appreciable build up in lactic acid.

The whole question of neurophysiological adaptation to contact lenses clearly requires a great deal of work to be done on it before convincing answers are likely to be available. In particular, histological study is required, although this will still leave the problem of extrapolating from animal to human eyes.

The foregoing sections have reviewed the cornea or epithelium as a whole. The superficial epithelial layer however requires separate consideration due to its dependence on the pre-corneal film for functional and mechanical integrity, and in view of its very close physical proximity to a contact lens.

(vii) Superficial Epithelium and Pre-Corneal Film

Disregarding contact lens wearers, for normality the superficial epithelium requires a 'normal' pre-corneal film.

Lemp et al[60] state - "The corneal epithelial surface is composed largely of lipophilic material and would be expected to be relatively hyrophobic. It would be difficult to wet this surface with an aqueous solution unless the solution contained an effective surfectant which would lower its surface tension and/or the epithelial surface became less hydrophobic through adsorption of a suitable hydrophilic-hydrophobic type material."

Further, this surface cellular layer is not optically regular. It is a mass of small indentations and covered by fine microvilli.[16] Refraction through such a surface would give rise to a very poor quality image. In reality however, the cornea may be seen to wet smoothly and has an excellent optical quality, both effects being mediated by the pre-corneal film.

The film itself is considered to consist of 3 layers. An inner mucoidal layer derived from the goblet cells of the conjunctiva and comprising predominantly glycoprotein whose chemical nature suggests considerable surface activity.[60] The middle fluid layer is derived from the lacrimal and accessory glands and is a dilute solution of protein, inorganic salts, oxygen, etc. The outer oily layer comes from the Meibomian glands of the lid margins[75a] and is a lipid and phospholipid secretion.[16,105] Each layer plays a distinct role in the defence and functioning of the eye.

Taking the question of wetting and optical quality first, Adler[119] and McEwan[70] have suggested that it is the protein dissolved in tears that reduces surface tension and improves corneal wetting. Ehlers[16] feels it is the phospholipids rubbed onto the corneal surface which produce a "surface active" layer, while Mishima[77] has shown that wiping the corneal surface does remove something essential to the maintenance of the tear film.

Lemp et al[60] and Holly et al[45] demonstrate fairly convincingly that the wetting mechanism is mediated by conjunctival glycoprotein and aqueous protein and that Meibomian gland secretion has no effect. They consider the glycoprotein plus dissolved protein lowers the surface tension of the tear film and that the glycoprotein being spread over the corneal surface by lid action and absorbed onto the corneal epithelium produces a hydrophilic surface and complete wetting. They further feel that the mucus acts as a demulcent and fills in the surface irregularities to produce a good optical quality.

Turning to the aqueous layer, there is a continuous low level secretion of 1-2 μl/min.[128] in the unstimulated eye, and it is this component which is affected following 5th nerve stimulation, e.g. by a contact lens. An estimated volume of 6.2 μl $\pm$ 2 μl is normal, although the maximum capacity of the cul de sac when in an upright position is calculated to be approximately 30 μl.[77]

It is difficult to establish the normal characteristics of this layer, since some reflex stimulation almost invariably accompanies sample collection. The protein concentration has

been variously put at between 0.2 and 0.67%, although Erickson[17] using filter paper electrophoresis from Schirmer strips (including the portion in contact with the lids) finds 2% protein concentration.

High concentration of the antibacterial enzyme-protein lysozyme - first identified and documented by Flemming[24] - are found in the lacrimal gland secretion. Tears remain sterile indefinitely and an exposed, warm moist surface such as the anterior eye would be very prone to infection without an active defence system.

More recently, however, Thompson & Galardo[111] showed that lysozyme is not the only antibacterial substance in tears and their findings have been confirmed and elaborated by Friedland et al.[27] They have separated a non-lysozyme antibacterial factor with activity some 200x that of lysozyme. It has a molecular weight between 5000-7500, and exhibits activity against a wide range of pathogens, whereas lysozyme even in high concentrations does not. On C.A.M. electrophoresis it migrates with the globulin-type fractions in the opposite direction to lysozyme.

Lysozyme itself has been the subject of quite extensive study. Its concentration is said to be decreased in diseases associated with excessive tearing or if a pathological condition involves destruction of lacrimal gland tissue[91]. On the other hand it is reported to be the first protein to decrease as the rate of flow decreases, and that the albumin/lysozyme ratio in normals is 1.5 (filter paper electrophoresis). A ratio of greater than 3 is said to give rise to a burning sensation[17]

Brunish[2] found that in irritant induced tears the
albumin content fell, while the lysozyme concentration rose
(Filter paper electrophoresis). Erickson[18] in an industrial
tear study of workers exposed to atmospheric irritants and
complaining of ocular discomfort, states that reduced
lysozome gives rise to this discomfort and also signifi-
cantly alters tear pH.

Protein and lysozyme in terms of their absolute or
relative concentration in tear fluid would thus seem to
influence general ocular comfort in addition to their
bacteriocidal aspects.

With regard to inorganic constituents of this aqueous
layer, Thaysen & Thorn[110] investigated sodium, potassium,
chlorine and urea concentrations in plasma and human tears.
They conclude that sodium and urea undergo simple diffusion
through the lacrimal gland since the amounts in plasma and
lacrimal fluid are equal over a wide range of concentrations
and secretion rates. Potassium and chlorine on the other
hand are present in significantly greater concentration in
tears than in plasma, indicating the presence of an active
secretory mechanism. Potassium concentration is also greatest
in an initial sample. Later, stimulated samples containing
much lower concentrations. The chlorine and sodium
concentrations varied together, but sodium and potassium
were independent, indicating that the amount of circulating
sodium influences the secretion of chlorine but not potassium
by the lacrimal gland.

The other major inorganic components are oxygen and
carbon dioxide. As discussed previously, during waking hours

the epithelium derives all its oxygen requirements from
this aqueous layer. During sleep oxygen is assumed to
diffuse into the fluid from the overlying blood vessels
of the lids.

The outer layer of the normal pre-corneal film is
formed by the Meibomian glands of the lids which produce
an oily surface to the film.[16,75]   This is clearly visible
in most people on examination with a slit lamp microscope,
along with wide individual variation in quantity of oil
apparently present. Its function is to limit evaporation,
since a thin liquid film on a warm surface would otherwise
rapidly evaporate. Unsuppressed evaporation from the cornea
would draw water out of the structure and cause it to
dehydrate.[75]

The pre-corneal film then is most important, and the
presence of a corneal lens may adversely affect it. The
most obvious effect is the stimulation of reflex lacrimation.
This increased tearing is cosmetically and visually annoying
to a patient, but may also have physiological effects.
Miller et al[62,73] have attempted to relate sodium and
potassium levels to lacrimation rates and ease of adaptation
to contact lenses. They have only a very small sample
(6 patients) and incomplete results but it does suggest
that those patients whose tear sodium and potassium levels
remained constant experienced very little reflex lacrimation
and minimal adaptive problems.

Those having difficulty adapting showed reflex
lacrimation and reduced sodium and potassium concentrations

(and therefore reduced osmotic pressure[66]) which reverted to pre-fitting levels as the lenses settled down. It is uncertain however, which is the underlying causitive factor in the problem. The altered ionic concentration may simply be a reflection of the excess reflex lacrimation. To further confuse the issue, Hill & Uniacke[41] measured osmotic pressure of tears during adaptation to lenses in 2 patients only. They found a significantly raised osmotic pressure in one and a significantly lowered osmotic pressure in the other, both of which returned to normal after approximately 3 weeks.

Lowered tear tonicity does produce a pre-disposition to corneal oedema however, which combined with oxygen deprivation does make the possibility of metabolic disturbance even more likely. Carbon dioxide removal may also be interrupted by a lens.

Meibomian gland secretion may also be stimulated - hypothetically due to a "massaging" effect of the lens against the lids - producing an abnormally oily tear film and greased up lenses. This may clear up spontaneously as adaptation proceeds (see patient 18GS) but is a very difficult problem to eradicate if it develops in an established wearer (see patient 19VH).

The most significant and most long term effect of a lens on the pre-corneal film concerns the inner mucoidal layer. Since the mucopolyssacharide secretion originates outside the cornea, from the conjunctival goblet cells, it must be spread across the eye by lid action, and the presence of a contact lens obviously interferes with this mechanism.

Figure 3. Corneal disturbance resulting from disruption
of the normal pre-corneal film.
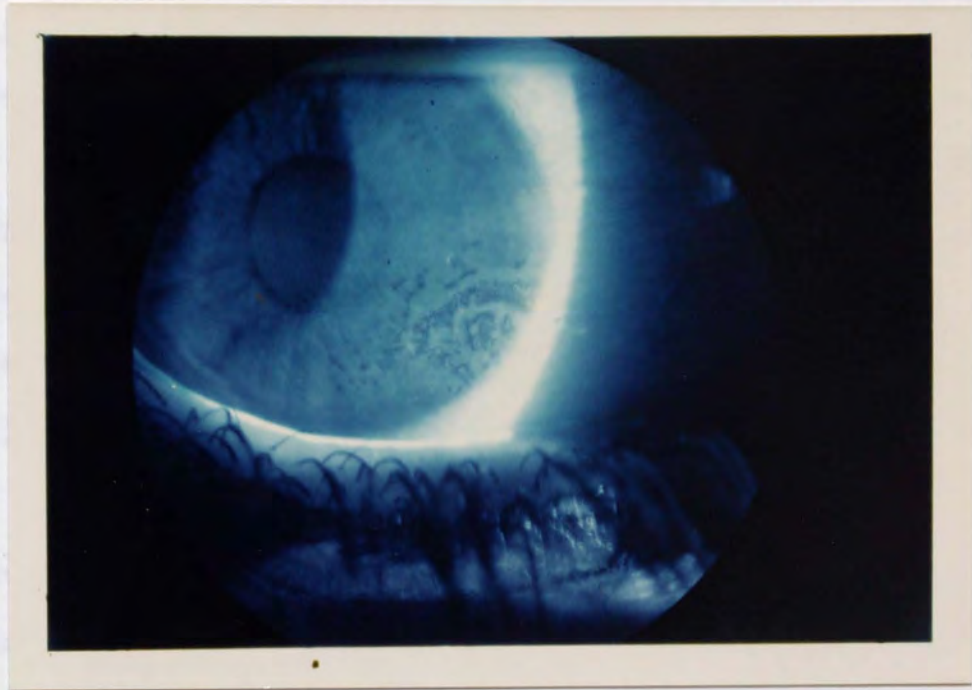
(a) Non-wetting cornea due to a disturbed mucoidal layer.
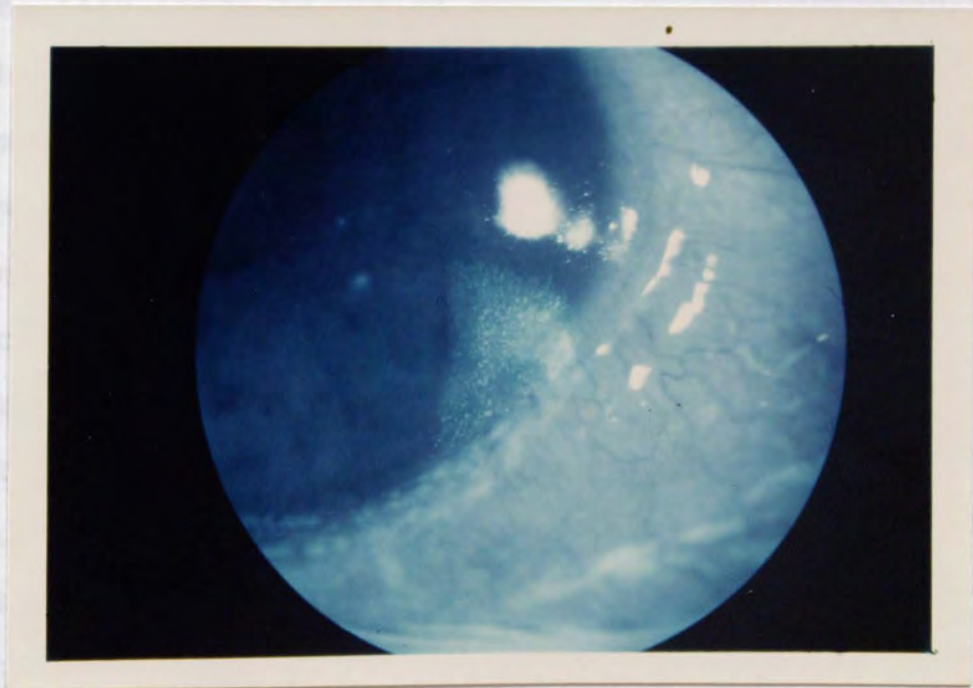
(b) Peripheral punctate stain due to prolonged non-wetting
of the corneal epithelium. Corneal lens wearer.

If the epithelium is not coated with mucus, the
aqueous film cannot wet it, and the tissue begins to dry.
Transitory 'dry spots' are common among corneal lens
wearers. By 'dry spot' one means a corneal area that, on
slit lamp examination immediately after lens removal and
fluorescein instillation, the green stained tear film may
be seen not to cover, giving the cornea a patchy blue
appearance against the overlying green tear film. See
fig.3.

These not-wetting areas are only temporary and a few
minutes normal blinking is sufficient to re-establish the
mucus layer and an even tear film. This type of mild
disturbance occurs randomly over the cornea, but the
central corneal area seems to be protected from any more
severe effects by the presence of the fluid retained beneath
the contact lens, on top of a mucus coating presumably
established to some extent during pre-insertion blinking.
(A haptic lens is, after all, used to conserve moisture
in cases of pathologically impaired tear film). A small
corneal lens however, has an anulus of exposed cornea round
it and, particularly in the case of an imcompletely
blinking patient, or one with a very wide palpebral
aperture (see patient 11CN & 17WP), the portion of this
anulus just below the horizontal uncovered by the lids
may be subject to such prolonged non-wetting that it begins
to dry out.   Instillation of fluorescein then shows a
characteristic 3 o/c - 9 o/c punctate stain as the damaged
epithelial tissue takes up the dye. See fig.3. Longstanding
dessicated areas are prone to infection and ulceration.

Healing of the epithelium after direct mechanical damage - lens pressure areas, foreign body trauma, etc. - is rapid. Complete renewal of epithelial cells occurs anyway every 7 days in a normal undamaged cornea. Small wounds are covered in about 3 hours by neighbouring basal cells which send out pseudopodia to cover the damaged area.[26] In larger areas of damage normal cell mitosis plays a part in the healing process. New epithelium is very prone to damage for some time, although apparently intact,[48] so that a contact lens wearer is advised to discontinue wear for several days after incurring any significant corneal damage.

(viii) <u>Conjunctival Involvement</u>

Conjunctival involvement in corneal lens wear is rather more indirect, although it is of considerable significance in the case of scleral lens wearers.

Complaints of conjunctival infection, particularly towards the end of the day, are not uncommon. There is generally no observable reason for it, and although cosmetically bad is not usually accompanied by any discomfort. It may possibly have some connection with the finding of altered lysozyme-protein fractions in tear fluid collected at the end of the day.[18] See also patient 14JC.

To summarise therefore, it may be seen that the phrase "adaptation to a contact lens" relates to a fairly complex inter-relationship of physiological and possibly psychological factors, many of which are unclear.

The main effects concern the hydration and metabolism
of the corneal structure, its neural sensitivity and its
surface integrity.

Having in this chapter reviewed the direct effect of
a contact lens on a normal eye, it is necessary to go on
to consider what is known about the systemic effects of the
orally administered steroid hormones involved in this study.

It may then be possible to establish, with the aid of
the clinical data accumulated throughout the project, the
points of contact between the steroidal medication and the
lens-eye system, and thus establish an hypothesis regarding
their inter-relationship.

5. A REVIEW OF THE GENERAL PHYSIOLOGICAL EFFECTS
    ASSOCIATED WITH THE USE OF SYNTHETIC OESTROGENIC
    AND PROGESTOGENIC STEROIDS.

(i) Introduction.

The first orally active progestational steroids were
synthesised in 1952, Norethynodrel by Colton[98,99] and
Norethindrone by Djerassi.[12,71] Other compounds, some more
potent than these two original preparations, have since
been developed and utilised.

Chemically, both norethynodrel and norethisterone are
derivatives of the hydrocarbon estrane, but norethisterone
(or alternatively norethindrone) is often termed a 19-nor
derivative to show its close structural and chemical
relationship to testosterone and 19-nortestosterone. There
is confusion in the literature regarding the correct
classification of norethynodrel. Some authors list it
also as a 19-nor derivative, e.g. Diczfalusy[10] and Klopper,[50]
whereas Drill[14,98,99] who was part of the group involved
in the early development and evaluation of the compound
states that the different position of the double carbon-
carbon bond (see fig.4) means that it cannot be classified
as a testosterone derivative.

Drill states "This difference is biologically signifi-
cant. Norethynodrel in addition to being progestational
is oestrogenic and devoid of androgenic effects in both
animals and man. Norethindrone on the other hand, although
progestational, is devoid of oestrogenic properties but is
androgenic in animals and man."[14]

The range of progestogenic preparations now in use in oral contraceptives is quite wide (see Diczfalusy[10] and Haller[34]) so although their primary progestogenic actions are thought to be similar, secondary actions and adverse effects vary considerably.

The position with regard to the oestrogenic component is more clear cut. All oral contraceptives contained ethynylestradiol or its 3-methyl ether, mestranol, although the dosage level varies.

Contraceptive drugs come in two basic types of formulation.

1. Combined pills - containing various combinations of orally active oestrogen and progestogen, administered for 20, 21 or 22 days with 6 or 7 days free of medication.

2. Sequential pill - classified as (i) classical, in which oestrogen only is taken on days 5-20 of the cycle, with an oestrogen - progestogen combination on days 21-25, or (ii) modified sequential: oestrogen only on days 5-16 and a mixed pill on days 17-25.

The bulk of published work available predictably concerns the mode of action of these drugs directly on the reproductive system, although even there much remains to be clarified. Much information is based on animal experiments which must be interpreted with caution since species differences are great. Knowledge of hormonal parameters controlling the normal cycle is itself incomplete, while in experiments these parameters are usually assessed in

Figure 4. Chemical Structure of the most widely used orally active contraceptive steroids.[10]

(a) Progestogenic steroids.

Norethynodrel
eg. Enovid, Conovid
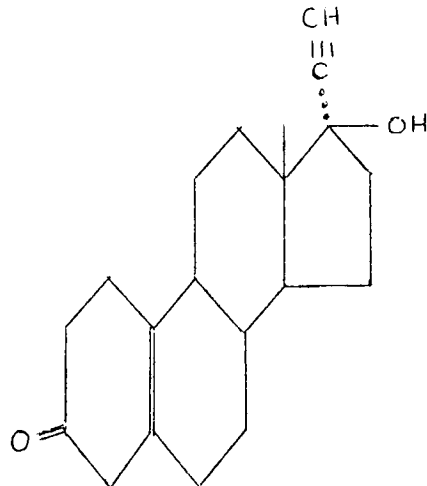
Norethindrone
eg. Ortho-Novin
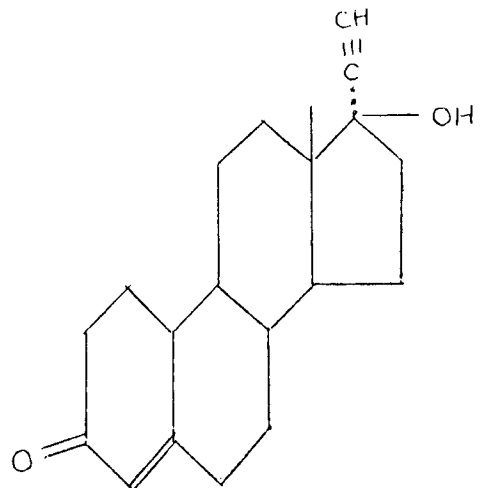
Lynestrol
eg. Minilyn
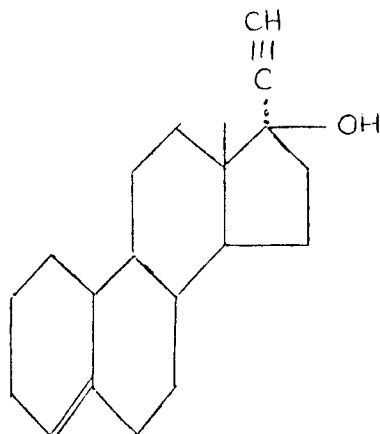
Norethindrone acetate
eg. Gynovlar, Anovlar

(b) Oestrogenic steroids.

17α ethynylestradiol

Mestranol
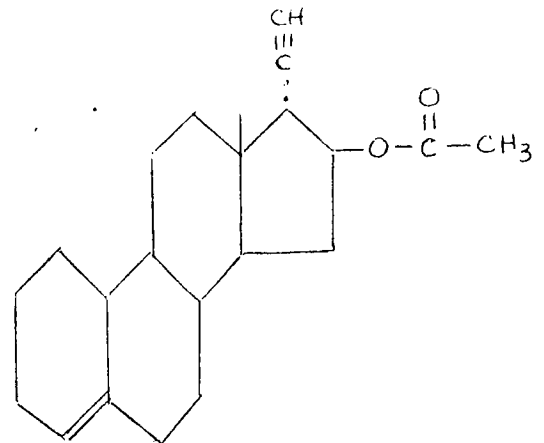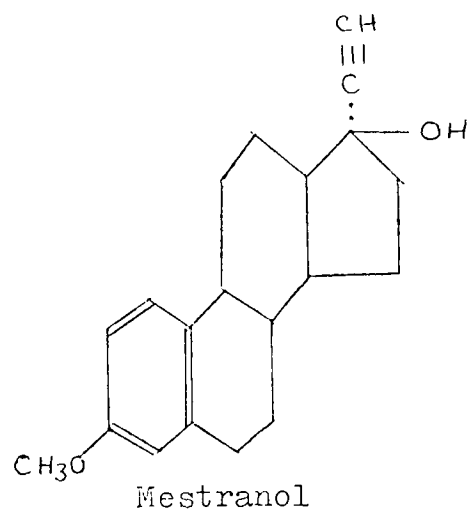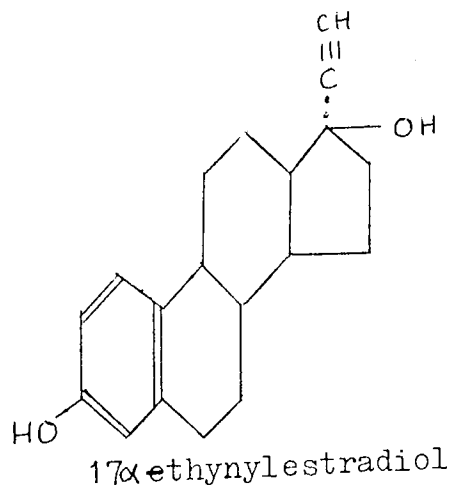
a control cycle followed by a treatment cycle although
there is evidence that the drug effects during the first
treatment cycle may differ considerably from later cycles.

In addition the pharmacologic effects of seemingly
closely related compounds are often different and the
possible points of attack for the various agents numerous.[10]
The mode of action may also change with altered dose levels
or by being combined with other compounds.

It is not relevant to the project to consider the
mechanism of contraception control as such in any great
detail. We are concerned rather with the more general
biochemical and endocrinological adverse effects occurring
throughout the body. These I will sub-divide into basically
oestrogenic, basically progestogenic and undifferentiated
combined effects.

(ii) Oestrogenic Effects

1. The primary contraceptive effect is suppression of
ovulation by inhibition of follicle stimulating hormone
(F.S.H.) secreted by the anterior pituitary gland.
Oestrogen does not appear to affect the secretory pattern
of luteinizing hormone in the early stages of treatment
although suppression has been reported with high dose,
long term treatment. The endometrium and the cervical
mucus are largely unaffected.

Adverse effects:

2. Oestrogen reduces sebum production from sebaceous
glands.[108]

3. Oestrogen stimulates aldosterone release which gives rise to a transitory retention of Na, Cl and water with resultant oedema.[19,101,28]

4. Oestrogens increase protein-bound iodine, but do not seem to influence thyroid function. The use of oestrogenic contraceptives however interferes with tests of thyroid function. (see patient 21DT)

5. Lipid metabolism is affected by oestrogen. There is an increase in low-density lipoprotein and a decrease in high-density lipoprotein. Also increased is the cholesterol level, its turnover and oxidation rate. There are elevated levels of plasma triglyceride and plasma phospholipid. The total lipid in plasma may increase by up to 15%.[57,100]

6. Connective tissue metabolism is affected to give increased collagen synthesis and turnover.

7. Protein metabolism is said to be affected by oestrogen-induced adrenacortical activity.

8. Plasma protein effects vary. Laurell et al[57] consider that oestrogens have the dominating effect for altered protein, the progestogen dose being of little importance. Simpson[101] lists a decrease in the levels of albumin, total serum proteins, haptoglobin and cholinesterase, with an increase in ceruloplasmin, plasminogen testosterone, oestrogen binding proteins remin and fibrinogen. Cerulo-plasmin concentration for example is almost doubled.[57] Olsen and Dich[83] using mestranol on female mice confirm that oestrogen is responsible for the reduced albumin

concentration. Dale and Spivey[8] using an oestrogenic combination pill found a rise in the plasma levels of α_1, α_2 and β-globulin and again a fall in the albumin level, as measured by means of C.A.E. electrophoresis.

(iii) Progestogenic Effects.

1. Primary contraceptive action of progestational agents is again thought to be by interference with pituitary function although by abolition of the mid-cycle L.H. peak. There appears to be no change in the levels of F.S.H. or in the basal secretory levels of L.H.[10] This effects the endometrium and cervical mucus, not the ovaries directly.

The endometrium becomes thinner and basically non-secretory, while the cervical mucus becomes thick and tacky and reduced in volume much earlier in the cycle than normal.

Secondary effects are -

2. Sebum production by the sebacious glands is increased, especially by norethindrone derivatives.[122]

3. Norethindrone derivatives have androgenic properties and can produce signs of fetal masculinization.[14]

4. Norethindrone derivatives also exhibit anabolic effects which may contribute to the increased body weight experienced by some oral contraceptive users (see patient 123L).[14]

5. Progestogens produce excretion of sodium as a result of inhibition of aldosterone or deoxycorticosterone at renal level.[28,126,127]

6. There is no reported effect on lipid metabolism or connective tissue metabolism.

7. Major effects on protein metabolism are said to be due to the catabolic action of progesterone.[101]

8. Recorded progestogenic effects on plasma proteins are contradictory. Laurell et al[57] consider progestin insignificant, but Dale and Spivey[8] find a raised level of total serum protein, albumin and γ-globulin with injectable long acting progestin. Olsen and Dich find megestrol acetate in mice reduces plasma volume and the rate of albumin synthesis, but does not affect its basic concentration.

(iv) Combined Effects

Certain observed effects do not appear to have been evaluated with individual hormones, only with a commercial combined pill. These include:

1. Carbohydrate metabolism which is affected by the secondary pill effect of increased secretion of growth hormone from the pituitary gland. This in turn affects the hypothalmus and causes a rise in blood glucose levels which then stimulates pancreatic production of insulin. Generally, insulin and glucose levels return to normal in six months[30,101] but this mechanism may precipitate trouble in patients with sub-clinical diabetes.

2. Vascular system effects are largely unanswered although there does seem to be an association between blood clotting disorders and oral contraceptive use.[101]

3. Liver function is impaired in most people taking the pill, but in healthy women the effect is clinically insignificant and probably transient.

4. Some evidence of increased histamine in the lungs of experimental animals, and sensitization of the airways following oral contraceptive use might implicate the drug in the production of asthma.[101]

5. There is also the whole question of the rather more subjective patient - complaints which appear to be of psychosomatic origin in many cases judging by the results of double-blind cross over studies. See Goldzieher et al.[30] Subjective complaints are - nausea, vomiting, abdominal discomfort, breast discomfort, headache, depression, leg pain. Goldzieher found a surprisingly high incidence of all these symptoms in control cycles before administering any medication, and only nausea and vomiting actually increased with the pills (especially with high oestrogen preparations). All other symptoms in fact decreased, including those from the placebo groups.

6. Contact lens intolerance.

(v) Summary

It may be seen therefore that the use of oestrogens and progestogens potentially affects many systems within the body. In a large number of cases we are still at the level of recording observed changes, without any understanding of the chain of events underlying that change. As is illustrated in the case of carbohydrate metabolism, the endocrine system is completely inter-related, on a negative feed-back basis in many cases, and steroid induced effects on a pituitary clearly have widespread repercussions throughout the body.

Fortunately it would seem that the various systems have quite large intrinsic safety margins, preventing any major disruption, e.g. liver function, providing no sub-clinical pathology is present.

Prediction of the intensity of a drug effect is particularly difficult in the case of the hormone-endocrine system because of the scarcity of our knowledge about its normal balance and behavior. When one adds to this the complexity of the oestrogen/progestogen compounds themselves, it is hardly surprising that so much information is on an empirical basis only.

For example, Jackson et al[46] in atttempting to establish what dose level of ethynyl estradiol or mestranol inhibited ovulation in normal women found completely erratic results, varying between women and between cycles, with no correlation to weight or height or any other parameter that they could determine.

There is also a tremendous variation in oestrogen-progestogen balance in combination pills. Rooks et al[94] using weight gain of the mouse uterus test, compared relative oestrogenicity of 5 current oral contraceptives. Norinyl-1 as the least oestrogenic of their sample was taken as the reference standard = 1.

They found -

Tablet 1.	Norinyl-1	=	1	
" 2.	Norinyl-2	=	2.1 x	⎫
" 3.	Ovulen	⇌	2.7 x	⎬ more
" 4.	Enovid -E	=	6 x	⎬ estrogenic
" 5.	Enovid -5	=	8 x	⎭

This corresponds to tablet composition as follows:-

Tablet

1	Mestranol	0.05 mg	Norethisterone	1 mg
2	"	0.1 mg	"	2 mg
3	"	0.1 mg	Ethynodiol diacetate	1 mg
4	"	0.1 mg	Norethynodrel	2.5 mg
5	"	0.1 mg	"	5.0 mg

This illustrates clearly that one cannot expect a common effect from all contraceptive preparations. The enhanced estrogenic effect of Enovid is presumed due to the conversion of norethynodrel to oestrogenic compounds within the body. Ethynodiol diacetate is also estrogenic, but on the basis of these findings, appreciably less so than norethynodrel.

Norethisterone on the other hand is non-oestrogenic and will have quite different secondary effects.

There are marketed at present throughout the world some 40 different combinations of hormone and dose level combined pills, making up some 90 trade named preparations. In addition, approximately 20 trade-named sequential preparations in some 12 different formulations are available.[34] Others are being developed.

On the whole, therefore, general guidelines only can be established and wide individual variation must be expected.

6. RESULTS SECTION

Part 1.

(1) Individual Clinical Summary Sheets. Pages 82-106.

(2) Analysis and Discussion of Schirmer test results.
Pages 107-113.

(3) Analysis and Discussion of corneal curvature results.
Pages 114-120

(1) Individual Clinical Summary Sheets

These sheets summarise the data concerning Schirmer test results at various stages of the project. They also indicate basic refractive details of each patient, the contact lens supplied and the prognosis of wear.

Following the start of lens wear, the change in corneal curvature (in mm.) from the mean initial level is recorded at certain specific stages of lens wear and medication use. + indicates a steepening and - a flattening of the corneal curvature relative to the mean pre-fitting level.

The presence of oedema, visible on slit lamp examination (not merely implied by curvature change) is indicated as follows:-

+ = mild; ++ = moderate; +++ = severe.

For the purpose of classification of corneal stain, the epithelial surface has been sub-divided into central and peripheral regions. The central region is considered to be that portion of the cornea, approximately 7 mm diameter, covered by the central optic zone of a centred corneal lens. The peripheral portion is then the 2-3 mm wide anulus surrounding this central region, which is

partially covered by a corneal lens.

Various types of stain occur during corneal lens wear. A classification and coding for use on the summary sheets has been adopted as follows:-

1.   Scattered punctate stain: central: C.S.P.

Peripheral: P.S.P.

2.   Peripheral 3o/c - 9 o/c stain.

Any tendancy for either 1 or 2 category stain to become confluent in nature has been indicated by (c) after the basic classification coding.

3.   Arc stain: which in all cases encountered in this study was within the central corneal region.

4.   Central granular stain: C.G.S. (other than arc stain).

A full discussion of wearing performance related to the curvature, Schirmer and electrophoresis results may be found in Chapter 7.

Name: 1HG   Medication Group: Control

SCHIRMER TEST RESULTS. R.E.only

Initial level: 16   results. Mean 16.1 $\pm$ 3 mm
With medication: 15   results. Mean 14.6 $\pm$ 3 mm
With CLs, 1-4weeks: 5   results. Mean 23.4 mm
With CLs, 5-10weeks   7   results. Mean 18.3 mm
   plus medication.
With CLs, post-medication: 4   results. Mean 17.3 mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-3.00 DS 6/5	-3.00 DS 6/5
Initial Keratometry (mean10 rdgs.)	7.61 x 180 $\pm$ 0.02 / 7.47 x 90 $\pm$ 0.03	7.63 x 180 $\pm$ 0.01 / 7.47 x 90 $\pm$ 0.02
Keratometry with medication.10 rdgs.	7.59 x 180 $\pm$ 0.01 / 7.46 x 90 $\pm$ 0.01	7.60 x 180 $\pm$ 0.01 / 7.46 x 90 $\pm$ 0.01

C.L.supplied: BCOR 7.45 BVP -3.50D   BCOR 7.45 BVP -3.50D
Prognosis of wear: Excellent

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.11x180 / +0.11x90	−	−	+0.15x180 / +0.07x90	−	CSP
After 4wks CL wear 12 hrs/day-all day	+0.19x180 / -0.03x90	−	mild arc	+0.20x180 / +0.11x90	−	−
After 6wks wear, plus medication.	+0.10x180 / -0.03x90	−	PSP	+0.15x180 / +0.09x90	−	−
After 11wks wear, plus medication.	+0.03x180 / -0.07x90	−	PSP	+0.13x180 / +0.03x90	+	PSP
After 14wks wear, no medication.	+0.09x180 / +0.07x90	+	CSP	+0.15x180 / +0.05x90	+	CSP & 3 o/c

Final result after 19 weeks wear:

Keratometry: 7.52 x180 / 7.40 x 90 i.e +0.09 x 180 / +0.07 x 90   7.52 x 180 / 7.42 x 90 i.e +0.11 x 180 / +0.05 x 90

Spectacle Rx: -3.00 DS 6/5   -3.00/-0.50 x 180 6/5

SUMMARY OF WEARING PERFORMANCE:

No problems, straightforward adaptation. Only complaints concerned
a tendency to conjunctival injection in the evening and a sensation
of dryness from time to time.

Name: 2EC   Medication Group: Control

SCHIRMER TEST RESULTS.   R.E.only

Initial level: 12   results. Mean 8.4 $\pm$ 2.5 mm
With medication: 16   results. Mean 6.7 $\pm$ 2.5 mm
With CLs, 1-4weeks: 6   results. Mean 20.5 mm
With CLs, 5-10weeks   8   results. Mean 15.8 mm
  plus medication.
With CLs, post-medication: 3   results. Mean 18 mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-5.75/-075x105 6/18	-2.50/-0.50 x90 6/5
Initial Keratometry (mean 10 rdgs.)	7.49 x 180 $\pm$ 0.02 7.46 x 90 $\pm$ 0.03	7.51 x 180 $\pm$ 0.03 7.48 x 90 $\pm$ 0.04
Keratometry with medication. 9 rdgs.	7.48 x 180 $\pm$ 0.02 7.45 x 90 $\pm$ 0.06	7.52 x 180 $\pm$ 0.02 7.49 x 90 $\pm$ 0.02
C.L.supplied:	BCOR 7.40 BVP -5.75D	BCOR 7.45 BVP -3.75D
Prognosis of wear:	Excellent	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.16x180 +0.14x90	–	–	+0.12x180 +0.04x90	–	–
After 4wks CL wear 12 hrs/day-all day	+0.09x180 +0.06x90	–	–	+0.09x180 +0.08x90	–	–
After 6wks wear, plus medication.	+0.15x180 +0.12x90	+	CSP	+0.07x180 +0.06x90	–	–
After 11wks wear, plus medication.	+0.09x180 +0.0 x90	–	faint arc	+0.09x180 +0.04x90	–	PSP
After 14wks wear, no medication.	+0.08x180 +0.10x90	–	faint arc	+0.06x180 +0.03x90	–	–

Final result after 20 weeks wear:

Keratometry: 7.38 x 180 i.e +0.11 x 180   7.46 x 180 i.e +0.05 x 18
               7.38 x 90   +0.08 x 90   7.50 x 90   -0.02 x 90

Spectacle Rx: -6.50 DS 6/18   -4.00 DS 6/5

SUMMARY OF WEARING PERFORMANCE:

Rapid adaptation, no difficulty at any stage. Intermittent
mild arc stain on R.E. Final spectacle V.A. equal to pre-
fitting levels, R.E. amblyopic.

Name: 3KS   Medication Group: Control

SCHIRMER TEST RESULTS.   R.E.only

Initial level:	16 results. Mean	16.6 ± 3 mm
With medication:	13 results. Mean	18.2 ± 2.5 mm
With CLs, 1-4weeks:	6 results. Mean	23.5 mm
With CLs, 5-10weeks plus medication.	8 results. Mean	22.5 mm
With CLs, post-medication:	4 results. Mean	20.5 mm

CORNEAL CURVATURE RESULTS.

	R.E.		L.E.	
Initial Spec.Rx	-6.00/-1.00+105 6/6		-5.50/-0.50x85 6/6-	
Initial Keratometry (mean10 rdgs.)	7.95 x 10 ± 0.02		8.02 x 180 ± 0.02	
	8.03 x 100 ± 0.04		8.04 x 90 ± 0.05	
Keratometry with medication. 9 rdgs.	7.96 x 10 ± 0.02		8.02 x 180 ± 0.03	
	8.02 x 100 ± 0.03		8.04 x 90 ± 0.03	

C.L.supplied: BCOR 7.95 BVP -6.00/-1.00x90 Initial: 7.85:-6.25/-1.00
Prognosis of wear: Good.Prism Ballast Lenses. Final: 7.75:-7.50 DS x90

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.17x180 +0.15x90	+	mod. P+CSP	+0.18x180 +0.27x 90	+	P+CSP
After 4wks CL wear 12 hrs/day-all day	+0.12x180 +0.13x90	–	PSP	+0.08x180 +0.11x 90	+	Arc & PSP
After 6wks wear, plus medication.	+0.11x180 +0.11x90	+	CSP	+0.12x180 +0.10x 90	–	–
After 11wks wear, plus medication.	+0.02x180 +0.10x90	–	mod. CSP arc	+0.12x180 +0.19x 90	–	–
After 14wks wear, no medication.	+0.02x180 +0.02x90	–	CSP	+0.08x180 +0.19x 90	+	C+ PSP

Final result after 35 weeks wear:

Keratometry: 8.08 x 180 i.e -0.13   8.11 x 180 i.e -0.09
                 8.13 x 90   -0.10   8.07 x 90   -0.03

Spectacle Rx: Not known

SUMMARY OF WEARING PERFORMANCE:

Prism ballast lenses were necessary for residual astigmatism
correction. Both lenses rode low and generalised corneal stain
was present throughout. Subject response was good after replace-
ment of the left lens eliminated some early reading difficulty.

Name: 4AF   Medication Group: Control

<u>SCHIRMER TEST RESULTS.</u>   R.E.only

Initial level:	16	results. Mean	19.6 ± 6 mm
With medication:	16	results. Mean	17.5 ± 5 mm.
With CLs, 1-4weeks:	6	results. Mean	36.2 mm
With CLs, 5-10weeks plus medication.	8	results. Mean	26.5 mm
With CLs, post-medication:	2	results. Mean	22.5 mm

<u>CORNEAL CURVATURE RESULTS.</u>

	R.E.	L.E.
Initial Spec.Rx	+0.50/-1.50x180 6/9+	+0.50/-1.50x180 6/9+
Initial Keratometry (mean 10 rdgs.)	8.06 x 180 0.015 / 7.85 x 90 0.025	8.03 x 180 0.026 / 7.81 x 90 0.038
Keratometry with medication. 9 rdgs.	8.07 x 180 0.013 / 7.84 x 180 0.036	8.03 x 180 0.026 / 7.83 x 90 0.038

C.L.supplied: BCOR 7.85 BVP-1.00D   BCOR 7.80 BVP-1.00D
Prognosis of wear: Reasonable tolerance, high lacrimation

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.21x180 / +0.08x 90	–	CSP	+0.16x180 / +0.05x90	–	CSP
After 4wks CL wear 12 hrs/day-all day	+0.12x180 / -0.12x 90	+	3-9o/c CSP	+0.12x35 / -0.10x145	–	3-9o/c
After 6wks wear, plus medication.	+0.19x180 / -0.09x 90	+	C+PSP	+0.18x40 / -0.09x130	–	PSP
After 11wks wear, plus medication.	+0.09x180 / +0.04x 90	+	3-9o/c	+0.11x30 / -0.09x120	–	3-9o/c
After 14wks wear, no medication.	+0.06x180 / -0.04x 90	–	PSP	+0.11x25 / -0.09x115	–	PSP

Final result after 18 weeks wear:

Keratometry: 8.02 x 165   7.93 x 35
                    7.94 x 75   8.01 x 125

Spectacle Rx: Pℓ/-1.50 x 180 6/7.5   -0.50/-1.50 x 180 6/7.5

<u>SUMMARY OF WEARING PERFORMANCE:</u>

Adapted easily within 4weeks. Mild 3o/c-9o/c punctate stain and
some central mild stain and oedema, reducing over the observation
period. Final spectacle refraction V.A. immediately after removing
lenses slightly better than before lens fitting.

Name: 5HG   Medication Group: Control

<u>SCHIRMER TEST RESULTS.   R.E.only</u>

Initial level:	13	results. Mean	18.9 $\pm$ 5 mm
With medication:	9	results. Mean	20.3 $\pm$ 2.5 mm
With CLs, 1-4weeks:		results. Mean	)
With CLs, 5-10weeks plus medication.		results. Mean	) Not recorded - see below
With CLs, post-medication:		results. Mean	)

<u>CORNEAL CURVATURE RESULTS.</u>

	R.E.	L.E.
Initial Spec.Rx	-1.75 DS 6/6	-3.00 DS 6/6
Initial Keratometry (mean9 rdgs.)	7.70 x 180 $\pm$ 0.02 / 7.73 x 90 $\pm$ 0.03	7.62 x 180 $\pm$ 0.01 / 7.55 x 90 $\pm$ 0.03
Keratometry with medication. 8 rdgs.	7.71 x 180 $\pm$ 0.03 / 7.71 x 90 $\pm$ 0.03	7.61 x 180 $\pm$ 0.01 / 7.55 x 90 $\pm$ 0.03

C.L.supplied: BCOR 7.50   BVP -250D   BCOR 7.50   BVP -3.75D
Prognosis of wear: Reasonable.   Erratic personality

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.13x180 / +0.16x 90	–	–	+0.17x180 / +0.13x 90	–	PSP
After 4wks CL wear 12 hrs/day-all day	–			–		
After 6wks wear, plus medication.	+0.07x180 / +0.12x 90	–	–	+0.09x180 / +0.00x90	–	PSP
After 8wks wear, plus medication.	+0.05x180 / +0.20x 90	–	3o/c	+0.10x180 / +0.07x90	–	3o/c
After 14wks wear, no medication.	+0.12x180 / +o.12x180	–	–	+0.10x180 / +0.13x90	–	PSP

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

<u>SUMMARY OF WEARING PERFORMANCE:</u>

This subject had her lenses inverted after 2 weeks and suffered
a severe overwear attack at $4\frac{1}{2}$ weeks. No lens wear was therefore
attempted for 6 days until the eyes were comfortable again.
Wearing time was built up slowly over 8 weeks to 2 x 6 hours
per day. The patient then lost her spectacles and was forced
to wear the lenses more conscientiously. All day wear quickly
resulted with no further problems.

Name: 6BS   Medication Group: Control/
                                                      Oestrogen

SCHIRMER TEST RESULTS. R.E.only

Initial level:	15 results. Mean	22 ± 5 mm
With medication: Control	13 results. Mean	20 ± 5 mm
With CLs, 1-4weeks:	6 results. Mean	27 mm
with CLs, 5-10weeks plus medication. Oestrogen	7 results. Mean	40 mm
With CLs, post-medication:	6 results. Mean	34.5 mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-3.25 DS 6/5	-4.00 DS 6/5
Initial Keratometry (mean 10 rdgs.)	7.88 x180 $\pm$ 0.02 7.87 x 90 $\pm$ 0.03	7.94 x 180 $\pm$ 0.00 7.81 x 90 $\pm$ 0.05
Keratometry with medication. 9 rdgs.	7.87 x180 $\pm$ 0.02 7.85 x 90 $\pm$ 0.04	7.92 x 180 $\pm$ 0.01 7.82 x 90 $\pm$ 0.03
C.L.supplied:	BCOR 7.75 BVP-4.00D	BCOR 7.75 BVP -5.00D
Prognosis of wear:	Excellent	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.16x180 +0.20x 90	-	-	+0.18x180 +0.18x 90	+	CSP
After 4wks CL wear 12 hrs/day-all day	+0.10x180 +0.12x 90	-	-	+0.16x180 +0.18x 90	-	-
After 6wks wear, plus medication.	+0.21x180 +0.29x 90	-	-	+0.27x180 +0.18x 90	-	-
After 11wks wear, plus medication.	+0.16x180 +0.29x 90	-	-	+0.16x180 +0.20x 90	-	-
After 14wks wear, no medication.	+0.19x180 +0.23x 90	-	-	+0.14x180 +0.12x 90	-	-

Final result after 30 weeks wear:

Keratometry:	7.72 x 180 7.72 x 90	i.e +0.16 x 180 +0.15 x 90	7.69 x 180 i.e +0.24 7.63 x 90 0.18
Spectacle Rx:	-4.75 DS 6/5		-5.00 DS 6/5+

SUMMARY OF WEARING PERFORMANCE:

No subjective problems for the duration of the project, despite
very flat-fitting lenses resulting from the substantial corneal
steepening at the time of oestogen medication. After the end of
the project, however, the subject started taking Minovlar oral
contraceptive and experienced a reduction in comfort necessitating
reduced wearing times. When seen during the second cycle of
Minovlar, K readings were again at the abnormally high levels
of the medication period of the project although the instrument
mires were still quite sharp.

A blending curve was added to the lenses, and when seen again 3 months later, the patient reported that the discomfort had gradually subsided and had not recurred. At this time curvature of the R.eye had reduced although that of the L.eye was still abnormally steep.

Name: 7JR   Medication Group: Control

<u>SCHIRMER TEST RESULTS.</u>   R.E.only

Initial level:	16	results. Mean	24.0 $\pm$ 6	mm
With medication:	14	results. Mean	22.5 $\pm$ 7.5	mm
With CLs, 1-4weeks:	10	results. Mean	27	mm
With CLs, 5-10weeks plus medication.	8	results. Mean	19	mm
With CLs, post-medication:	5	results. Mean	17	mm

<u>CORNEAL CURVATURE RESULTS.</u>

	R.E.	L.E.
Initial Spec.Rx	-0.75 DS 6/6	-0.75 DS 6/6
Initial Keratometry (mean10 rdgs.)	$\dfrac{7.82 \times 180 \pm 0.02}{7.78 \times 90 \pm 0.03}$	$\dfrac{7.73 \times 180 \pm 0.01}{7.72 \times 90 \pm 0.04}$
Keratometry with medication.10 rdgs.	$\dfrac{7.81 \times 180 \pm 0.03}{7.77 \times 90 \pm 0.02}$	$\dfrac{7.72 \times 180 \pm 0.01}{7.72 \times 90 \pm 0.02}$

C.L.supplied: BCOR 7.65   BVP -2.00D   BCOR 7.60   BVP -1.75D
Prognosis of wear: Excellent

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.10x180 +0.04x 90	-	-	+0.02x180 +0.05x90	-	-
After 4wks CL wear 12 hrs/day-all day	+0.17x180 +0.13x 90	+	C+PSP	+0.12x180 +0.11x90	-	-
After 6wks wear, plus medication.	+0.08x180 +0.12x 90	-	PSP	+0.08x180 +0.13x90	-	PSP
After 12wks wear, plus medication.	+0.13x180 +0.08x 90	-	-	+0.09x180 +0.07x90	-	-
After 14wks wear, no medication.	+0.16x180 +0.09x 90	-	-	+0.11x180 +0.08x90	-	-

Final result after 23 weeks wear:

	R.E.	L.E.
Keratometry:	$\dfrac{7.82 \times 180}{7.80 \times 90}$ i.e $\dfrac{0.0}{-0.02}$	$\dfrac{7.75 \times 180}{7.74 \times 90}$ i.e. $\dfrac{-0.02}{-0.05}$
Spectacle Rx:	-1.00 DS	-1.00 DS

<u>SUMMARY OF WEARING PERFORMANCE:</u>

No problems at any stage. Final result at 23 weeks was after
6 weeks on oral contraceptive orthonovum.

Name: 8JH   Medication Group: Progestogen

SCHIRMER TEST RESULTS. R.E.only

Initial level:	16 results.	Mean 41.8 $\pm$ 10 mm
With medication:	11 results.	Mean 34.3 $\pm$ 11 mm
With CLs, 1-4weeks:	6 results.	Mean 50 mm
With CLs, 5-10weeks plus medication.	8 results.	Mean 50 mm
With CLs, post-medication:	5 results.	Mean 50 mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-2.75 DS 6/6+	-2.50 DS 6/6+
Initial Keratometry (mean10 rdgs.)	7.87 x 180 $\pm$ 0.03 / 7.85 x 90 $\pm$ 0.03	7.88 x 180 $\pm$ 0.02 / 7.88 x 90 $\pm$ 0.02
Keratometry with medication. 8 rdgs.	7.86 x 180 $\pm$ 0.01 / 7.85 x 90 $\pm$ 0.02	7.88 x 180 $\pm$ 0.02 / 7.87 x 90 $\pm$ 0.02

C.L.supplied: BCOR 7.70 BVP -3.50D   BCOR 7.75 BVP -2.50D
Prognosis of wear: Excellent   Later7.60 BVP

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.21x180 / +0.22x 90	–	PSP	+0.13x180 / +0.19x 90	–	PSP
After 4wks CL wear 12 hrs/day-all day	+0.12x180 / +0.13x 90	–	CSP'	+0.16x180 / +0.19x 90	–	–
After 6wks wear, plus medication.	+0.29x180 / +0.21x 90	+	–	+0.23x180 / +0.23x 90	–	–
After 11wks wear, plus medication.	–			–		
After 14wks wear, no medication.	+0.26x180 / +0.18x 90	Readaptation period		+0.24x180 / +0.21x 90	Readaptation period	

Final result after20 weeks wear: After lens modification

Keratometry:	7.65 x 180 i.e +0.22 / 7.78 x 90 +0.07	7.69 x 180 i.e+0.19 / 7.69 x 90 +0.19
Spectacle Rx:	-3.75 DS 6/6+	-3.75 DS 6/6

SUMMARY OF WEARING PERFORMANCE:

Initial 4 weeks adaptation proceeded normally. A steep rise in
curvature was manifest after 2 weeks of progestogen medication,
after 4 weeks medication during the Christmas vacation the lenses
became very uncomfortable for the first few hours of wear, and
were discontinued altogether during examination revision in early
January. Within 2 weeks of stopping tablet usage, wear was again
up to 12 weeks. A suggestion of the same problem did recur however
after a further 2 weeks with a period of approximately $\frac{1}{2}$ hour of
discomfort shortly after insertion. The lenses were therefore
modified by the addition of a 4th,blending,curve which re-established
all day wear.

Name: 9PK   Medication Group: Progestogen

SCHIRMER TEST RESULTS. R.E.only

Initial level: 16 results. Mean 18.3 $\pm$ 6 mm

With medication: 14 results. Mean 18.5 $\pm$ 3 mm

With CLs, 1-4weeks: 6 results. Mean 25 mm

With CLs, 5-10weeks   results. Mean   –
  plus medication.

With CLs, post-medication: results. Mean   –

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-4.50 DS 6/7.5	$-1.00/-0.75 \times 10$ 6/6
Initial Keratometry (mean 10 rdgs.)	7.90 x 180 $\pm$ 0.02 / 7.54 x 90 $\pm$ 0.05	7.80 x 180 $\pm$ 0.02 / 7.50 x 90 $\pm$ 0.02
Keratometry with medication. 7 rdgs.	7.91 x 180 $\pm$ 0.01 / 7.51 x 90 $\pm$ 0.03	7.80 x 180 $\pm$ 0.02 / 7.44 x 90 $\pm$ 0.01
C.L.supplied:	BCOR 7.70 BVP -5.50D	BCOR 7.60 BVP -2.25D
Prognosis of wear:	Good	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.29x180 / +0.16x 90	+	Arc ++	+0.15x180 / +0.28x 90	–	–
After 4wks CL wear 12 hrs/day-all day	+0.09x180 / +0.04x 90	–	Arc ++	+0.11x180 / +0.16x 90	–	Mild arc
Refit R.steeper After 6wks wear, plus medication.	+0.16x180 / +0.04x 90	–	Faint arc	+0.17x180 / +0.08x 90	–	–
After 11wks wear, no medication.	+0.14x180 / +0.0 x 90	+++	CGS	+0.11x180 / +0.20x 90	+++	CGS
fenestrate After 14wks wear, no medication.	+0.10x180 / −0.04x 90	+	CSP	+0.11x180 / +0.11x 90	+	CSP

Final result after 20 weeks wear:

Keratometry: 7.83 x 180 i.e. +0.07 / 7.54 x 90 +0.0   7.63 x 180 i.e. +0.17 / 7.32 x 90 +0.18

Spectacle Rx: $-5.50/-1.00 \times 22\frac{1}{2}$ 6/9– $-3.00/-2.00 \times 155$ 6/9

SUMMARY OF WEARING PERFORMANCE:

Good early adaptation until a pronounced arc stain necessitated
a steeper lens refit. The resulting severe central oedema was
finally eliminated by a fenestration hole in each lens. Full
all day wear could then be maintained.

Name: 10RA   Medication Group: Progestogen

SCHIRMER TEST RESULTS. R.E.only

Initial level:	16 results. Mean 19.0 $\pm$ 6 mm	
With medication:	13 results. Mean 20.5 $\pm$ 8 mm	
With CLs, 1-4weeks:	6 results. Mean 40 mm	
With CLs, 5-10weeks plus medication.	7 results. Mean 29 mm	Discomfort
With CLs, post-medication:	2 results. Mean 27 mm	

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-3.75 DS 6/5	-4.25/-0.50 x 90 6/5
Initial Keratometry (mean 10 rdgs.)	7.58 x 180 $\pm$ 0.02 / 7.52 x 90 $\pm$ 0.03	7.59 x 180 $\pm$ 0.02 / 7.50 x 90 $\pm$ 0.03
Keratometry with medication. 8 rdgs.	7.58 x 180 $\pm$ 0.01 / 7.50 x 90 $\pm$ 0.02	7.55 x 180 $\pm$ 0.01 / 7.50 x 90 $\pm$ 0.02

C.L.supplied: BCOR 7.55 BVP -425D   BCOR 7.50 BVP -475D
Prognosis of wear: Reasonable. High lacrimation.

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.21x180 / +0.24x 90	–	–	+0.25x180 / +0.26x 90	–	–
After 4wks CL wear 12 hrs/day-all day	+0.08x180 / +0.18x 90	–	–	+0.17x180 / +0.20x 90	–	–
After 6wks wear, plus medication.	+0.12x180 / +0.14x 90	–	–	+0.15x180 / +0.14x 90		
After 11wks wear, plus medication.	+0.11x180 / +0.24x 90	–	–	+0.13x180 / +0.22x 90	+	CSP
After 14wks wear, no medication.	+0.08x180 / +0.10x 90	–	–	+0.09x180 / +0.16x 90	–	–

Final result after 20 weeks wear:

Keratometry: 7.52 x 180 i.e. +0.06   7.50 x 180 i.e. +0.09
                  7.41 x 90   +0.11   7.38 x 90   +0.12

Spectacle Rx:

SUMMARY OF WEARING PERFORMANCE:

Slow adaptation with persistent reading difficulty. Tolerance
lost during illness after 2 months wear, while taking progestogen
medication. Tolerance improved after ending medication, but was
never fully satisfactory.

Name: 11CN   Medication Group: Progestogen

SCHIRMER TEST RESULTS.   R.E.only

Initial level:	16	results. Mean	16.5 ± 8 mm	
With medication:	15	results. Mean	12.5 ± 4.5 mm	
With CLs, 1-4weeks:	8	results. Mean	23 mm	
With CLs, 5-10weeks plus medication.	7	results. Mean	28 mm	
With CLs, post-medication:	3	results. Mean	23 mm	

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-5.50/-100 x 80 6/12	-5.00/-0.75 x 30 6/6
Initial Keratometry (mean10 rdgs.)	$\dfrac{7.74 \times 180 \pm 0.02}{7.47 \times 90 \pm 0.03}$	$\dfrac{7.68 \times 180 \pm 0.02}{7.49 \times 90 \pm 0.02}$
Keratometry with medication. 9 rdgs.	$\dfrac{7.72 \times 180 \pm 0.02}{7.46 \times 90 \pm 0.02}$	$\dfrac{7.66 \times 180 \pm 0.03}{7.47 \times 90 \pm 0.02}$

C.L.supplied: BCOR 7.45   BVP -7.00D   BCOR 7.50   BVP -5.75D
Prognosis of wear: Excellent

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.18x180 -0.07x 90	–	3-9o/c (c)	+0.16x180 +0.05x 90	–	3-9o/c (c)
After 4wks CL wear 12 hrs/day-all day	+0.16x180 +0.01x 90	–	3-90/c	+0.14x180 -0.01x 90	–	3-9o/c
After 6wks wear, plus medication.	+0.12x180 +0.07x 90	–	3-9o/c	+0.13x180 +0.01x 90	–	–
After 11wks wear, plus medication.	+0.07x180 -0.07x 90	–	3-9o/c (c)	+0.07x180 -0.01x 90	(c)	3-9o/c (c)
After 14wks wear, no medication.	+0.13x180 -0.07x 90	–	3-9o/c	+0.07x180 -0.05x 90	–	3-9o/c

Final result after20 weeks wear:

Keratometry:	$\dfrac{7.57 \times 180}{7.40 \times 90}$ i.e $\dfrac{+0.17}{0.07}$	$\dfrac{7.55 \times 180}{7.40 \times 90}$ i.e $\dfrac{+0.13}{+0.01}$
Spectacle Rx:	-6.50/-1.00x90 6/18+ normal	-6.00/-0.75 x 180 6/6-

SUMMARY OF WEARING PERFORMANCE:

No trouble at any stage. Peripheral dryness due to wide palpebral
aperture and small diameter lenses.

Name: 123L   Medication Group: Progestogen

SCHIRMER TEST RESULTS.   R.E.only

Initial level:	16	results. Mean	14.0 ± 5 mm
With medication:	11	results. Mean	9.5 ± 3 mm
With CLs, 1-4weeks:	6	results. Mean	20.5 mm
With CLs, 5-10weeks plus medication.	6	results. Mean	17.5 mm
With CLs, post-medication:	4	results. Mean	14 mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-4.25 DS 6/5	-4.25 DS 6/5
Initial Keratometry (mean 10 rdgs.)	$\frac{7.56 \times 180 \pm 0.01}{7.43 \times 90 \pm 0.03}$	$\frac{7.53 \times 180 \pm 0.02}{7.36 \times 90 \pm 0.04}$
Keratometry with medication. 8 rdgs.	$\frac{7.57 \times 180 \pm 0.01}{7.43 \times 90 \pm 0.02}$	$\frac{7.53 \times 180 \pm 0.01}{7.35 \times 90 \pm 0.04}$

C.L.supplied: BCOR 7.40   BVP -5.00D   BCOR 7.40   BVP -5.25D
Prognosis of wear: Excellent

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	$\frac{+0.21 \times 180}{+0.14 \times 90}$	−	−	$\frac{+0.21 \times 180}{+0.14 \times 90}$	−	−
After 4wks CL wear 12 hrs/day-all day	$\frac{+0.14 \times 180}{+0.09 \times 90}$	−	−	$\frac{+0.19 \times 180}{+0.14 \times 90}$	−	−
After 6wks wear, plus medication.	$\frac{+0.20 \times 180}{+0.13 \times 90}$	+	−	$\frac{+0.15 \times 180}{+0.11 \times 90}$	−	−
After 11wks wear, plus medication.	$\frac{+0.12 \times 180}{+0.07 \times 90}$	−	−	$\frac{+0.17 \times 180}{+0.14 \times 90}$	−	PSP
After 14wks wear, no medication.	$\frac{+0.16 \times 180}{+0.11 \times 90}$	−	−	$\frac{+0.17 \times 180}{+0.14 \times 90}$	−	−

Final result after 18 weeks wear:

Keratometry: $\frac{7.33 \times 180}{7.30 \times 90}$ i.e $\frac{+0.18}{0.13}$ $\frac{7.42 \times 180}{7.30 \times 90}$ i.e $\frac{+0.11}{+0.06}$

Spectacle Rx: -5.50D 6/5-   -4.75 DS 6/4-

SUMMARY OF WEARING PERFORMANCE:

No problems with lenses at any stage. This subject reacted
very badly to the progestogen tablets however, putting on a
lot of weight and becoming very depressed and subdued.

Name: 13WD   Medication Group: Progestogen

SCHIRMER TEST RESULTS.   R.E.only

Initial level:	16	results. Mean	17.0 $\pm$ 3 mm
With medication:	11	results. Mean	12.9 $\pm$ 2.5 mm
With CLs, 1-4weeks:	6	results. Mean	26 mm
With CLs, 5-10weeks plus medication.		results. Mean	—
With CLs, post-medication:		results. Mean	

CORNEAL CURVATURE RESULTS.

	R.E.		L.E.	
Initial Spec.Rx	-5.00/-0.50 x 85	6/6+	-7.25/-0.75 x 85	6/6+
Initial Keratometry (mean 10rdgs.)	7.55 x 180 $\pm$ 0.02 7.43 x 90 $\pm$ 0.02		7.61 x 180 $\pm$ 0.01 7.44 x 90 $\pm$ 0.02	
Keratometry with medication. 8 rdgs.	7.56 x 180 $\pm$ 0.02 7.41 x 90 $\pm$ 0.01		7.61 x 180 $\pm$ 0.02 7.42 x 90 $\pm$ 0.02	

C.L.supplied: BCOR 7.35 BVP -7.50/-100x90   BCOR 740 BVP -7.75/-150x9(

Prognosis of wear: Reasonable

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.26x180 +0.21x 90	—	—	+0.23x180 +0.14x 90	—	—
After 4wks CL wear 12 hrs/day-all day	+0.15x180 +0.13x 90	—	—	+0.13x180 +0.14x 90	—	—
After 6wks wear, plus medication.	Overwear					
After 11wks wear, plus medication.	Erratic wear					
After 14wks wear, no medication.						

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

SUMMARY OF WEARING PERFORMANCE:

Normal good adaptation to all day wear in 4 weeks, followed by
a bad overwear attack shortly after starting progestogen tablets,
although this is felt probably to be coincidental. Wear there-
after was erratic and no useful results may be concluded from it.

Name: 14JC   Medication Group: Progestogen

SCHIRMER TEST RESULTS. R.E.only

Initial level:	16	results. Mean	7.7 ± 3	mm
With medication:	13	results. Mean	6.5 ± 3	mm
With CLs, 1-4weeks:	6	results. Mean	7.5	mm
With CLs, 5-10weeks plus medication.	6	results. Mean	5.8	mm
With CLs, post-medication:	1	results. Mean	9	mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-5.50 DS 6/6+	-3.50/-0.75 x 90 6/5
Initial Keratometry (mean 10rdgs.)	7.85 x 180 ± 0.02 / 7.84 x 90 ± 0.04	7.85 x 180 ± 0.03 / 7.74 x 90 ± 0.04
Keratometry with medication. 8 rdgs.	7.87 x 180 ± 0.02 / 7.86 x 90 ± 0.02	7.84 x 180 ± 0.01 / 7.78 x 90 ± 0.03
C.L.supplied:	BCOR 7.70 BVP -6.25D	BCOR 7.70 BVP -5.25D
Prognosis of wear:	Excellent	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.21x180 / +0.08x 90	–	PSP	+0.18x180 / +0.05x 90	–	PSP
After 4wks CL wear 12 hrs/day-all day	+0.13x180 / +0.10x 90	+	–	+0.11x180 / +0.05x 90	+	–
After 6wks wear, plus medication.	+0.21x180 / +0.12x 90	+	–	+0.22x180 / +0.07x 90	+	PSP
After 11wks wear, plus medication.	+0.09x180 / +0.12x 90	–	Arc PSP(c)	+0.09x180 / -0.04x 90	–	PSP (c)
After 14wks wear, no medication.	+0.18x180 / -0.04x 90	+	–	+0.07x180 / -0.01x 90	+	–

Final result after 20 weeks wear:

Keratometry:	7.69 x 180 i.e. +0.16 / 7.74 x 90 +0.10	7.69 x 180 i.e. +0.16 / 7.74 x 90 +0.00
Spectacle Rx:	-6.00 DS 6/5	-4.00/-0.75 x 90 6/4

SUMMARY OF WEARING PERFORMANCE:

Basically low tear flow. No difficulty with lenses until 4 weeks after starting progestogen, when lenses became rather greasy and very, very marked conjunctival injection developed. This injection remained unsightly until medication ceased, then subsided within 2 weeks to give a good cosmetic appearance. Corneal disturbance was very much more marked also during the medication period.

Name: 15AMcN   Medication Group: Progestogen

SCHIRMER TEST RESULTS. R.E.only

Initial level:	16 results. Mean	26.3 ± 11 mm
With medication:	12 results. Mean	22.2 ± 8 mm
With CLs, 1-4weeks:	5 results. Mean	34.5 mm
With CLs, 5-10weeks plus medication.	5 results. Mean	30 mm
With CLs, post-medication:	1 results. Mean	23 mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	$-2.50/-0.50$ x 130 6/6	-1.50 DS 6/6+
Initial Keratometry (mean10 rdgs.)	$\dfrac{7.12 \times 180 \pm 0.01}{0.96 \times 90 \pm 0.02}$	$\dfrac{7.12 \times 180 \pm 0.01}{6.96 \times 90 \pm 0.03}$
Keratometry with medication.6 rdgs.	$\dfrac{7.09 \times 130 \pm 0.04}{6.97 \times 90 \pm 0.01}$	$\dfrac{7.12 \times 180 \pm 0.01}{6.96 \times 90 \pm 0.03}$
C.L.supplied:	BCOR 690 BVP -3.75D	BCOR 6.90 BVP -1.75D
Prognosis of wear:	Moderate	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.03x180 / +0.00x 90	–	–	+0.03x180 / +0.00x 90	–	–
After 4wks CL wear 12x hrs/day-allxday	+0.08x180 / +0.01x 90	+	CSP	+0.08x180 / +0.04x 90	–	–
After 6wks wear, plus medication.	+0.06x180 / – 90	–	3o/c	+0.07x180 / +0.01x 90	–	CSP
After 11wks wear, plus medication.	+0.03x180 / –	–	–	+0.03x180 / +0.01x 90	–	–
After 14wks wear, no medication.						

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

SUMMARY OF WEARING PERFORMANCE:

Build up of tolerance very slow, 12 hours after 3 months wear, not apparently affected in any way by progestogen tablets. Troubled throughout by high lacrimation.

Name: 16MH   Medication Group: Oestrogen

SCHIRMER TEST RESULTS. R.E. only

Initial level:	15 results. Mean	22.3 ± 5 mm
With medication:	12 results. Mean	14.6 ± 4 mm
With CLs, 1-4weeks:	3 results. Mean	23.0 mm
With CLs, 5-10weeks plus medication.	results. Mean	–
With CLs, post-medication:	results. Mean	–

CORNEAL CURVATURE RESULTS.

	R.E.		L.E.	
Initial Spec.Rx	+0.50/-3.00 x 10	6/5	+0.50/-2.50 x 160	6/5
Initial Keratometry (mean 9 rdgs.)	7.78 x 180 $\pm$ 0.03 7.37 x 110 $\pm$ 0.04		7.77 x 150 $\pm$ 0.01 7.49 x 60 $\pm$ 0.04	
Keratometry with medication. 7rdgs.	7.75 x 10 $\pm$ 0.03 7.36 x 110 $\pm$ 0.02		7.34 x 150 $\pm$ 0.02 7.51 x 60 $\pm$ 0.02	

C.L.supplied: BCOR 7.55 BVP -2.00D BCOR 7.60 BVP -2.00
Prognosis of wear: Excellent patient response

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.30x180 +0.21x 90	+	CSP	+0.25x180 +0.13x 90	–	–
After 4wks CL wear 12 hrs/day-all day	+0.26x180 +0.13x 90	+	Mild CSP	+0.09x180 +0.00x 90	–	Mild CSP
After 6wks wear, plus medication.	+0.33x130 +0.14x 90	+	–	+0.10x180 -0.01x 90	+	CSP
After 11wks wear, plus medication.	–			–		
After 14wks wear, no medication.	–			–		

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

SUMMARY OF WEARING PERFORMANCE:

Compromise lens fit owing to high corneal astigmatism. Adaptation
good up to start of oestrogen medication, when subjective comfort
reduced although retaining all day wear. During Christmas vacation,
after approximately 6 weeks medication, she was advised by
ophthalmologist to discontinue lens wear due to extensive central
abrasions B.E. On returning to Birmingham, lens adaptation was
restarted but satisfactory wear never achieved. At the end of the
project fenestrated conoid lenses were fitted. The results of this
are not known.

Name: 17WP   Medication Group: Oestrogen

<u>SCHIRMER TEST RESULTS. R.E.only</u>

Initial level:	16	results.	Mean	22.0 $\pm$ 9 mm
With medication:	10	results.	Mean	18.8 $\pm$ 5 mm
With CLs, 1-4weeks:	5	results.	Mean	27 mm
With CLs, 5-10weeks plus medication.	5	results.	Mean	25 mm
With CLs, post-medication:	1	results.	Mean	32 mm

<u>CORNEAL CURVATURE RESULTS.</u>

	R.E.	L.E.
Initial Spec.Rx	-3.00 DS 6/6	-2.50/-0.50 x 170 6/6
Initial Keratometry (mean 10 rdgs.)	7.85 x 180 $\pm$ 0.01 / 7.67 x 90 $\pm$ 0.02	7.83 x 180 $\pm$ 0.02 / 7.68 x 90 $\pm$ 0.03
Keratometry with medication. 6 rdgs.	7.86 x 180 $\pm$ 0.02 / 7.65 x 90 $\pm$ 0.02	7.83 x 180 $\pm$ 0.02 / 7.69 x 90 $\pm$ 0.02

C.L.supplied: BCOR 7.65   BVP -4.00D   BCOR 7.65   BVP 2.75DS
Prognosis of wear: Excellent

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.11x180 / -0.05x 90	–	3-9o/c	+0.07x180 / -0.05x 90	–	3-9o/c
After 4wks CL wear 12 hrs/day-all day	+0.08x180 / -0.01x 90	–	–	+0.05x180 / -0.01x 90	–	–
After 6wks wear, plus medication.	+0.18x180 / -0.02x 90	–	9o/c PSP	+0.09x180 / -0.00x 90	–	–
After 11wks wear, plus medication.	+0.10x180 / -0.08x 90	+	3-9o/c	+0.00x180 / -1.12x 90	–	3-9o/c
After 14wks wear, no medication.	+0.09x180 / -0.09x 90	+	3-9o/c (c)	+0.02x180 / -0.01x 90	+	3-9o/c

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

<u>SUMMARY OF WEARING PERFORMANCE:</u>

No real subjective problems, lenses tended to dislodge. Objectively,
considerable 3o/c-9o/c stain will require refitting with larger
lenses. Keratometry readings show an atypical flattening of the
vertical meridian, producing an almost spherical cornea in the R.E.

Name: 18GS

Medication Group: Oestrogen

SCHIRMER TEST RESULTS. R.E.only

Initial level:	16	results. Mean 28.0 ± 6 mm
With medication:	9	results. Mean 23.1 ± 2 mm
With CLs, 1-4weeks:	6	results. Mean 32 mm
with CLs, 5-10weeks plus medication.	6	results. Mean 37 mm
With CLs, post-medication:	4	results. Mean 34.6

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-2.50/0.75 x 160 6/5	-0.75 DS 6/5
Initial Keratometry (mean 10 rdgs.)	7.92 x 180 ± 0.03 7.64 x 90 ± 0.04	7.97 x 180 ± 0.02 7.60 x 90 ± 0.04
Keratometry with medication. 9 rdgs.	7.92 x 180 ± 0.01 7.63 x 90 ± 0.04	7.97 x 180 ± 0.02 7.58 x 90 ± 0.03
C.L.supplied:	BCOR 7.70 BVP -3.25D	BCOR 7.70 BVP -1.50 DS
Prognosis of wear:	Excellent	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.23x180 +0.22x 90	–	–	+0.19x180 +0.23x 90	–	–
After 4wks CL wear 12 hrs/day-all day	+0.18x180 +0.11x 90	–	–	+0.14x180 +0.16x 90	–	–
After 6wks wear, plus medication.	+0.20x180 +0.10x 90	–	–	+0.21x180 +0.22x 90	–	–
After 11wks wear, plus medication.	+0.17x180 +0.06x 90	–	CSP	+0.14x180 +0.08x 90	–	CSP
After 14wks wear, no medication.	+0.16x180 +0.06x 90	–	–	+0.14x180 +0.14x 90	–	PSP

Final result after 20 weeks wear:

Keratometry:	7.76 x 180 i.e +0.16 7.58 x 90 +0.06		7.80 x 180 i.e +0.17 7.42 x 90 +0.18
Spectacle Rx:	-3.00 DS 6/5		-1.50 DS 6/5+

SUMMARY OF WEARING PERFORMANCE:

No problems throughout. Subject observed that the lenses were always uncomfortable for 2 days before her menstrual period, but then became quite comfortable again. Corneal steepening L.E. during early stage of medication.

Name: 19VH   Medication Group: Oestrogen

SCHIRMER TEST RESULTS.   R.E.only

Initial level:	15 results. Mean 7.94 $\pm$ 3 mm	
With medication:	13 results. Mean 10.6 $\pm$ 4 mm	
With CLs, 1-4weeks:	6 results. Mean 28 mm	
With CLs, 5-8weeks plus medication.	4 results. Mean 11 mm	
With CLs, post-medication:	results. Mean -	

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-5.25/-075 x 75 6/6+	-7.50/-1.25 x 90 6/6+
Initial Keratometry (mean 10 rdgs.)	7.94 x 180 $\pm$ 0.03 / 7.75 x 90 $\pm$ 0.02	7.94 x 180 $\pm$ 0.03 / 7.75 x 90 $\pm$ 0.04
Keratometry with medication. 9 rdgs.	7.93 x 180 $\pm$ 0.01 / 7.74 x 90 $\pm$ 0.03	7.92 x 180 $\pm$ 0.02 / 7.73 x 90 $\pm$ 0.03
C.L.supplied:	BCOR 7.80 BVP -6.75 D	BCOR 7.80 BVP -8.50 D
Prognosis of wear:	Reasonable	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.16x180 / +0.10x 90	-	PSP (c)	+0.13x180 / +0.16x 90	+	C+PSP (c)
After 4wks CL wear 12 hrs/day-all day	+0.06x180 / -0.05x 90	+	CSP	+0.14x180 / -0.04x 90	+	P+CSP
After 6wks wear, plus medication.	+0.07x180 / -0.08x 90	+	Mild CSP	+0.11x180 / -0.01x 90	+	Mild CSP
After 11wks wear, plus medication.	-			-		
After 14wks wear, no medication.	-			-		

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

SUMMARY OF WEARING PERFORMANCE:

Adaptation to all-day wear very satisfactory within the allotted
4 weeks. 10 days after starting oestrogen medication lenses began
to grease over. This got progressively worse until after a further
week they became thickly coated in grease immediately on insertion,
reducing vision to 6/36. During the Christmas vacation both medi-
cation and lens wear were discontinued. Adaptation was then restarted
in January, but greasing continued to be a problem and was not helped
by Zincfrin eye drops.
A refit with 9.70 mm C4 lenses was therefore carried out in February,
and all-day wear achieved.

Name: 20AMcL   Medication Group: Oestrogen

SCHIRMER TEST RESULTS.   R.E.only

Initial level: 15 results. Mean 17.0 $\pm$ 5 mm
With medication: 12 results. Mean 12.0 $\pm$ 4 mm
With CLs, 1-4weeks: 8 results. Mean 17.0 mm
With CLs, 5x10weeks   8 results. Mean 20 mm Discomfort
  7-13
  plus medication.
With CLs, post-medication: 4 results. Mean 17 mm

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	-0.75/-0.50 x 90 6/5	-0.75/-0.50 x 80 6/5
Initial Keratometry (mean 10 rdgs.)	7.78 x 180 $\pm$ 0.02 7.86 x 90 $\pm$ 0.05	7.75 x 180 $\pm$ 0.03 7.88 x 90 $\pm$ 0.05
Keratometry with medication. 8 rdgs.	7.78 x 180 $\pm$ 0.01 7.86 x 90 $\pm$ 0.02	7.77 x 180 $\pm$ 0.02 7.88 x 90 $\pm$ 0.03
C.L.supplied:	BCOR 7.65 BVP -1.75DS	BCOR 7.65 BVP -1.75DS
Prognosis of wear:	Excellent	

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	+0.11x180 +0.12x90	–	–	+0.09x180 +0.15x90	–	–
After 4wks CL wear 12 hrs/day-all day	+0.11x180 +0.11x90	–	–	+0.08x180 +0.15x90	–	–
After 6wks wear, plus medication.	+0.04x180 +0.07x90	–	PSP	+0.03x180 +0.12x90	–	PSP
After no 13 wks wear, plus medication.	+0.15x180 +0.26x90	+	–	+0.12x180 +0.14x90	+	–
After 19 wks wear, no medication.	+0.02x180 +0.04x90	–	–	+0.02x120 +0.08x90	–	–

Final result after   weeks wear:

Keratometry: –   –

Spectacle Rx: –   –

SUMMARY OF WEARING PERFORMANCE:

Difficulty with very close range precision vision required in job
(dental student) produced a slightly erratic wearing pattern through-
out the project. 14 hours per day wear was achieved however by the
time oestrogen therapy was started after 6weeks. After a further
week she contracted bronchitis and discontinued lens wear for 2 weeks,
although continuing to take the project tablets. When seen 4 weeks
after restarting lens wear, the wearing time was still only 3-4 hours,
owing to considerable discomfort described as a hot, dry gritty
sensation quite dis-similar to that experienced when first adapting
to lenses. At this point the cornea was very much steeper than at
any previous time during the adaptation process.

4 weeks after stopping oestrogen therapy the lenses
were subjectively much better, being described as 'excellent'
outside; reading vision was improved and wearing time back
to all day if desired. The corneal curvature had reverted
almost to pre-fitting levels.

Name: 21DT   Medication Group: Oestrogen/Control

SCHIRMER TEST RESULTS. R.E. only

Initial level:	15 results. Mean 13.7 ± 5 mm
With medication:	13 results. Mean 14.5 ± 6.5 mm
With CLs, 1-4weeks:	results. Mean
With CLs, 5-10weeks plus medication.	results. Mean
With CLs, post-medication:	results. Mean

CORNEAL CURVATURE RESULTS.

	R.E.	L.E.
Initial Spec.Rx	+2.50/-1.00 x 180 6/6	+2.75/-1.00 x 180 6/6
Initial Keratometry (mean 10 rdgs.)	$\dfrac{7.56 \text{ x } 180 \pm 0.02}{7.42 \text{ x } 90 \pm 0.05}$	$\dfrac{7.59 \text{ x } 180 \pm 0.03}{7.47 \text{ x } 90 \pm 0.05}$
Keratometry with medication. 8 rdgs.	$\dfrac{7.55 \text{ x } 180 \pm 0.03}{7.43 \text{ x } 90 \pm 0.02}$	$\dfrac{7.58 \text{ x } 180 \pm 0.04}{7.46 \text{ x } 90 \pm 0.02}$

C.L.supplied: BCOR 7.45 BVP +0.75 D   BCOR 7.45 BVP +1.25 D
Prognosis of wear: Excellent

	K Rdg	Oedema	Stain	K Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day	$\dfrac{+0.09x180}{+0.08x\ 90}$	−	PSP	$\dfrac{+0.14x180}{+0.17x\ 90}$	−	PSP
After 4wks CL wear 12 hrs/day-all day	$\dfrac{+0.10x180}{+0.11x\ 90}$	+	CSP	$\dfrac{+0.12x180}{+0.27x\ 90}$	+	CSP
After 6wks wear, plus medication.	$\dfrac{+0.02x180}{+0.06x\ 90}$	−	CSP	$\dfrac{+0.09x180}{+0.16x\ 90}$	−	CSP
After 13wks wear, plus medication.	$\dfrac{+0.06x180}{+0.02x\ 90}$	−	−	$\dfrac{+0.09x180}{+0.05x\ 90}$	−	−
After 14wks wear, no medication.	−			−		

Final result after 22 weeks wear:

Keratometry:	$\dfrac{7.50 \text{ x } 180}{7.34 \text{ x } 90}$	i.e $\dfrac{+0.06}{+0.08}$	$\dfrac{7.50 \text{ x } 180}{7.42 \text{ x } 90}$	i.e. $\dfrac{+0.09}{+0.05}$
Spectacle Rx:	+0.75/-1.00 x 180 6/4		+1.50/-1.00 x 180 6/4	

SUMMARY OF WEARING PERFORMANCE:

This subject had a tumour of the thyroid gland diagnosed during the second half of the project and was therefore unable to take the final quantity of oestrogen tablets (see p.74). She also experienced considerable eyelid swelling during the early stages of lens wear, which appeared to be an adverse reaction to Transol wetting agent, since it disappeared on discontinuing use of this solution. Adaptation and wear were then normal. Spectacle refraction immediately after lens removal at 22 weeks shows a myopic shift, but with excellent corrected acuities.

Name: 22YF   Medication Group: Oestrogen

<u>SCHIRMER TEST RESULTS. R.E.only</u>

Initial level:	16 results. Mean	17.3 ± 3.0 mm
With medication:	13 results. Mean	18.0 ± 4.0 mm
With CLs, 1-4weeks:	results. Mean	–
With CLs, 5-10weeks plus medication.	results. Mean	–
With CLs, post-medication:	results. Mean	–

<u>CORNEAL CURVATURE RESULTS.</u>

	R.E.	L.E.
Initial Spec.Rx	$-2.50/-0.50 \times 90$ 6/5	$-2.00/0.75 \times 80$ 6/5
Initial Keratometry (mean10 rdgs.)	$8.03 \times 180 \pm 0.02$ $8.01 \times 90 \pm 0.04$	$8.11 \times 180 \pm 0.02$ $8.05 \times 90 \pm 0.02$
Keratometry with medication. 9 rdgs.	$8.07 \times 180 \pm 0.02$ $8.06 \times 90 \pm 0.03$	$8.12 \times 180 \pm 0.02$ $8.07 \times 90 \pm 0.03$

C.L.supplied: BOOR 7.95 BVP -3.25 D   BOOR 7.95 BVP -3.25 D

Prognosis of wear: Poor

	X Rdg	Oedema	Stain	X Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day						
After 4wks CL wear 12 hrs/day-all day						
After 6wks wear, plus medication.						
After 11wks wear, plus medication.						
After 14wks wear, no medication.						

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

<u>SUMMARY OF WEARING PERFORMANCE:</u>

Failed to adapt to lenses.

Name: 23CM   Medication Group: Oestrogen

<u>SCHIRMER TEST RESULTS. R.E. only</u>

Initial level: 16 results. Mean 22.1 mm. S.D.4.0 mm
With medication: 15 results. Mean 17.9 ± 4.0 mm
With CLs, 1-4weeks: results. Mean   —
With CLs, 5-10weeks   results. Mean   —
   plus medication.
With CLs, post-medication: results. Mean   —

<u>CORNEAL CURVATURE RESULTS.</u>

	R.E.	L.E.
Initial Spec.Rx	-4.00/-0.75 x 150 6/5	-4.00/-0.75 x 150 6/5
Initial Keratometry (mean 10 rdgs.)	7.40 x 180 ± 0.02 7.23 x 90 ± 0.02	7.48 x 180 ± 0.02 7.27 x 90 ± 0.02
Keratometry with medication. 9 rdgs.	7.41 x 180 ± 0.02 7.26 x 90 ± 0.04	7.47 x 180 ± 0.02 7.27 x 90 ± 0.03

C.L.supplied: BCOR 7.2 BVP -5.25D   BCOR 7.25 BVP -5.25D
Prognosis of wear: Poor. Excessive lacrimation.

	R Rdg	Oedema	Stain	R Rdg	Oedema	Stain
After 2wks CL wear 2 x 4hrs/day						
After 4wks CL wear 12 hrs/day-all day						
After 6wks wear, plus medication.						
After 11wks wear, plus medication.						
After 14wks wear, no medication.						

Final result after   weeks wear:

Keratometry:

Spectacle Rx:

<u>SUMMARY OF WEARING PERFORMANCE:</u>

   Failed to adapt to lenses due to persistent excessive
   lacrimation.

(2) Schirmer Tear Secretion Results.

Schirmer's test provides an estimate of the amount of moisture present in the precorneal film and some guide to the rate of secretion. In a Schirmer test as performed in this study, the filter paper strip remains in position behind the lower lid for 3 minutes.

The data collected during the M.Sc. project[128] prior to this study, gave an average Schirmer reading in normal eyes of 16.7 mm. This corresponds well with the figure of 18 mm. obtained in this study.

In addition, experiments were carried out during the M.Sc. project with a constant flow injection apparatus. A syringe was connected to the apparatus which could be set to deliver microlitre amounts of fluid at various rates onto a Schirmer filter paper strip. Normal saline was used, and the effect of an average Schirmer test in the eye was found to be most closely paralleled by an initial application of 5 μl N. saline to the end of the test strip, followed by 22 μl min.$^{-1}$ during the next 3 minutes.

This suggests that approximately 5 μl is absorbed initially from the conjunctival sac, with a secretion rate of approximately 2 μl min^{-1} thereafter. This will clearly be an overestimate in the case of low readings and an underestimate in moist eyes. The filter paper is unlikely to absorb all fluid present, so an estimate of 5-10 μl fluid in the conjunctiva sac would seem reasonable. This agrees well with the findings of Mishima et al[77] of a tear secretion rate of 1.2 μl min.$^{-1}$ and a tear volume of 6.2 $\pm$ 2 μl which were obtained by a fluorimetric technique.

A tear secretion rate of 1-2 ul min^{-1} corresponds to a production of 1.4-1.8 ml/day.

Individual results for each person are tabulated on the clinical summary sheets. The findings have been divided into 5 major groups; initial level, tear volume with medication, with contact lenses during adaptation, with contact lenses plus medication and, where available, the post medication period.

The two major results series, before and during medication, have been compared by a Student t-test on the means of the paired observations, i.e. for each medication group the mean level of tear flow under both experimental conditions has been calculated for each subject. The difference between the means has been used to measure the direction and magnitude of any induced medication effect. The results are shown in table 1.

This experimental design is a two-tailed test with df = 7 in each medication group. The results are as follows.

Control group   - t = 1.414 df = 7 Non significant

Progestogen group - t = 2.982 df = 7 Significant at p = 0.05

Oestrogen group   - t = 1.982 df = 7 Marginally non-significant p = 0.10

The direction of the change may be seen by inspection of table 1 to be a decrease in the rate of secretion during the medication period.

The direction and magnitude of the induced change is not constant however, even within each medication group, ranging from a decrease of 7.6 mm to an increase of 2.3 mm

Table 1. Schirmer Results: t-test on the difference between the means of Paired Observation

	Control Group				Progestogen Group				Oestrogen Group		
Subject No.	Mean Basic Level (mm)	Mean Medic Level (mm)	M_1-M_2 (mm)	Subject No.	Mean Basic Level (mm)	Mean Medic Level (mm)	M_1-M_2 (mm)	Subject No.	Mean Basic Level (mm)	Mean Medic Level (mm)	M_1-M_2 (mm)
1HG	16.09	14.56	-1.53	8JH	41.76	34.31	-7.45	16HH	22.25	14.59	-7.66
2EC	8.41	6.71	-1.70	9PK	18.31	13.33	+0.02	17AP	22.00	18.38	-3.12
3KS	16.59	18.18	+1.59	10RA	19.12	20.40	+1.34	18GS	28.00	23.11	-4.89
4AF	19.60	17.50	-1.10	11CH	16.59	12.70	-3.39	19VH	7.94	10.60	+2.64
5JG	18.88	20.28	+1.40	12SL	14.13	9.54	-4.59	20AMcL	17.20	11.83	-5.37
6BS	22.00	20.00	-2.00	13MD	17.00	12.90	-4.10	21DT	13.73	14.46	+0.73
7JR	24.15	22.40	-1.75	14JC	7.71	6.50	-1.21	22YF	17.26	18.11	+0.86
				15AMCN	26.30	22.15	-4.15	23CM	22.11	17.93	-4.17
	17.97 +5.09	17.10 +5.20	-0.37 +1.63		20.12 +10.26	17.12 +3.80	-3.00 +2.35		18.80 +6.17	16.16 +4.10	-2.58 +3.67
Non-significant result				Significant p 0.05				Non-significant p 0.10			

in the two affected groups. The control group also showed
a slight decrease overall during the medication period.
This is likely to be due to improved weather conditions
during May-July compared to the January-March period of
the initial sample taking.

These results confirm the preliminary findings of
radically reduced tear flow associated with oral contraceptive
use which were noted during the preceding M.Sc. project study.

The actual size of the tear reduction encountered in
this study is not very large. This may well be a reflection
of the uncontrolled environmental factors prevailing before
sample taking. After any length of time indoors, it would
seem likely that evaporation would reduce the fluid volume
to a level considerably below that recorded in this study
where the subjects have just walked some distance outside.

An average tear reduction of approximately 3 mm.
which may appear insignificant when viewed in terms of the
elevated levels recorded during the project, may in fact
represent quite a definite effect under indoor wearing
conditions.

It is a general experience of contact lens wearers
that lenses are most comfortable out of doors, and that hot
indoor atmospheres, especially when combined with close
work are detrimental to lens comfort.

A reduction in the amount of tear fluid available in
these latter conditions may therefore have wide repercussions
in terms of corneal integrity and subjective response.

It should be noted that both oestrogen and progestogen
produced a tear reduction. The effect of combined contra-
ceptive medication is therefore likely to produce an
additive effect and greater tear reduction than that
encountered with either hormone alone. This may be the
explanation of the much larger reduction found with full
contraceptive preparations during the M.Sc. project.

During the early stages of lens wear, the tear level
rises considerably (mean 28 mm) and then declines again
as adaptation proceeds to a final level which is usually
slightly above the pre-fitting level.

The medication response during lens wear was not as
noticeable as it had been prior to lens use. This is pre-
dictable in view of the fact that a reduction in secretion
during this stage of contact lens wear is the normal
response to increased adaptation. A medication-induced
reduction is therefore likely to be masked by the differing
rates of subsidence of the normal reflex lacrimation.

The fact that the experimental conditions involved
were not conducive to precision monitoring of tear volume
changes is illustrated by some results obtained from an
adapted lens wearer not directly concerned in this project.

This patient, who had worn corneal lenses for 2 years,
consistently experienced discomfort in the 2 or 3 days
immediately prior to the onset of her menstrual period.
She was asked to carry out a Schirmer test on herself each
morning during the affected period. This she did very
conscientiously for 9 months. The results are shown in

full in table 2, and show a quite remarkable degree of consistency. This highlights the difference in the precision of the test when applied under the controlled conditions which were possible with the understanding and co-operation of this lens wearer, compared with the very variable conditions which relate to the project as a whole.

The fact that a medication-induced difference could be detected despite the adverse conditions, indicate the positive nature of the induced effect. It is possible also that the reduction in secretion would have been found to be larger under controlled conditions.

The other significant point raised by the data in table 2, is that normal hormone variation by itself is capable of inducing ocular change leading to lens discomfort. Geographical factors made it impossible to measure corneal curvature and lacrimal protein during this portion of each month, so it is not known whether the Schirmer change is the only occurring effect, although it seems unlikely that it should be.

The size of the Schirmer volume reduction (6-10 mm) during this period is greater than the mean reduction obtained experimentally during this project, but similar to some individual results, thus confirming that drug induced changes in the lacrimal gland may contribute to, or possibly cause, corneal lens discomfort.

Table 2. Results of Schirmer tests carried out by an adapted lens wearer on her right eye for the 4 days prior to and 5 days of menstruation.

Days relative to Menstruation	Aug.	Sept.	Oct.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
-4	22	22	23	22	24	23	22	24	22
-3	18	21	22	23	21	23	21	23	21
-2	17	19	20	19	20	21	19	21	21
-1	16	17	17	18	17	18	19	18	19
M	14	15	15	16	14	16	15	14	16
+1	17	16	16	17	16	16	16	15	15
+2	20	18	17	17	17	17	16	17	17
+3	21	21	18	19	19	19	19	19	20
+4	21	21	20	20	21	21	21	21	22

All results in mm. of filter paper moistened in 3 mins.

(3) <u>Corneal Curvature Results</u>.

A Bausch and Lomb Keratometer was used to measure the central corneal curvature. A measurement was taken weekly on both eyes during the initial control and medication periods. 9 or 10 results were therefore obtained per person in each results series and the means of these figures are listed on the individual clinical summary sheets (p.82-106).

In the normal cornea, prior to the use of corneal lenses, in no subject did the medication make any difference to the central curvature, which remained constant (within $\pm$ 0.03 mm in the majority of patients). The horizontal meridian was found to show slightly more consistent results than the vertical meridian. This seems likely to be due to minor corneal deformation during blinking or to a deeper pre-corneal film along the lid margins which might interfere with the reflection of the vertical keratometer mires.

When contact lenses had been supplied, keratometry readings were taken after 2, 4, 6, 11 and 14 weeks wear. Individual results on pages 82-106 are expressed in terms of the difference in mm between the prefitting level and the new lens wearing level. A plus sign indicates a steepening relative to the prefitting level and a negative sign a relative flattening. Horizontal results are always listed first.

The amount of corneal change occurring at each stage has been compared between the three medication groups by means of an analysis of variance technique including an allowance for unequal sized sample groups.[82] The summary

tables for this A.N.O.V.A are shown on page 116.

This shows that the amount of corneal change is similar in the three groups at all stages of lens wear except after 6 weeks, i.e. after 2 weeks medication.

After this 2 week medication period the F-ratio, indicating the existence of a difference between the groups, is significant at the 5% probability level. Inspection of the original data indicates that the difference arises primarily within the oestrogen group, where the corneal radii may be seen to steepen again during this early stage of steroid use. There was a suggestion of a similar effect in the progestogen group, but it was of a smaller, less consistent nature. The effect was absent in the control group.

After a further 5 weeks medication use, the group difference was no longer present, and mathematically it did not recur when medication ceased, i.e. the F-ratio remained non-significant, although some corneas did show a steepening for approximately 3 weeks after medication was stopped. That a renewed corneal steepening after 6 weeks wear is not explicable on the basis of normal corneal response to lenses as is confirmed by the absence of any such effect in the control group. In this group corneas continued to flatten as adaptation to the lenses improved.

It can only be assumed therefore to be a specific medication effect, induced by the oestrogen. This effect was not evident in the normal stable corneas during the

Table 3.

Corneal Curvature Results.

Analysis of Variance: Unequal sized sets. Olivetti programma
No.ST.1001

Week 2

Source of variation	Sum of Squares	df	Est. of variance	F ratio
Between groups	0.0189	2	0.0094	1.492
Within groups	0.5110	81	0.0063	
Total	0.5299	83		

Non significant

Week 4

Source of variation	Sum of Squares	df	Est. of Variance	F ratio
Between groups	0.0019	2	0.0009	0.2727
Within groups	0.2155	65	0.0033	
Total	0.2174	67		

Non significant

Week 6

Source of variation	Sum of Squares	df	Est. of variance	F ratio
Between groups	0.0319	2	0.0159	3.6136
Within groups	0.2597	59	0.0044	
Total	0.2916	61		

Significant at 5% level

Week 11

Source of variation	Sum of Squares	df	Est. of variance	F ratio
Between groups	0.0210	2	0.0105	1.4788
Within groups	0.4368	61	0.0071	
Total	0.4578	63		

Non significant

Week 13-14

Source of variation	Sum of Squares	df	Est. of variance	F ratio
Between groups	0.0070	2	0.0035	0.573
Within groups	0.3762	61	0.0061	
Total	0.3832	63		

Non significant

Figure 5. Graph showing the curvature change (Right eye, horizontal meridian) for a sample patient from each medication group.
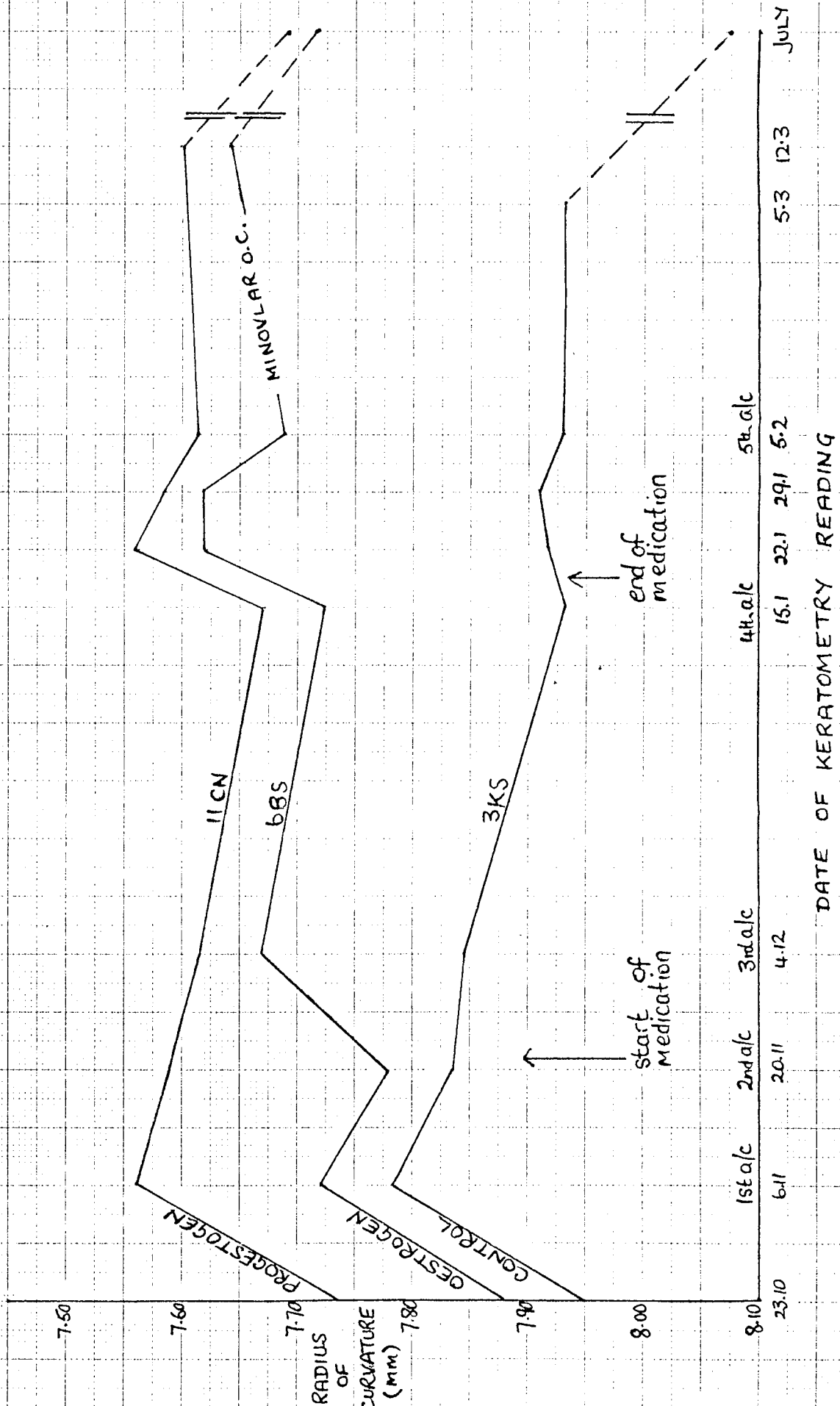
early part of the project. It therefore only becomes
apparent when the underlying corneal metabolism is disturbed,
as is the case certainly during the early stages of contact
lens wear.

The amount of renewed steepening in some cases pro-
duced corneas which were steeper than at any previous stage
in the initial adaptation period. The effect is to produce
a flat-fitting contact lens with a central bearing area
and excessive edge lift. Clinically this is undesirable
because of the likelihood of a resultant central erosion.
Subjectively it is likely to be uncomfortable with the edge
stand-off causing appreciable lid irritation. The flat lens
would also tend to be excessively mobile. This too causes
discomfort as the lens collides with the corneal limbal
regions. The lacrimation induced by the discomfort would
tend to confirm the central oedema and keep the problem in
being.

During the very early stages of lens adaptation, the
oxygen deprivation and reflex lacrimation which precipitate
the normal adaptive corneal steepening are associated with
much shorter wearing times than is the case with drug-
induced steepening in an all day wearer. In the former
case the cause of oedema is removed when the lens is removed
such that the cornea has a much greater opportunity to
recover. In addition the other neural and physiological
adaptive changes previously described are occurring. The
corneal steepening and oedema is therefore unlikely to
become established and, as is found in reality, quickly

subsides from its early high levels to less extreme values.

By comparison, the cornea of the established wearer with drug-induced oedema has little opportunity of re-establishing a normal physiological state before lens discomfort and reflex lacrimation re-inforce the situation. Since the neural sensibility of an adapted wearer may be low, it is possible for a central erosion to develop without the subject being aware of more than general slight discomfort (see patient 16MH).

Induced steepening is thus of much greater significance once all day wear has become established than it is during early adaptation.

Individual sensitivity to oestrogen and progestogen varies.[46,10] The ocular response must therefore be expected to vary. The length of time which elapses before the body re-establishes its normal physiological fluid balance is also variable, symptoms are generally most severe during the first month of oral contraceptive use but then subside to a great extent in the majority of women.[30]

This is in line with the finding in this study that there was little difference in corneal steepening between the 3 groups after 6-7 weeks hormone use.

The 'overshoot' steepening of the corneas after the termination of lens use may be explained in the case of progestogen by its aldosterone link (see p.204). The occurrence of the effect after oestrogen use is not readily explicable. It is a short term effect however and as such unlikely to be of great clinical significance.

Summarising therefore, both oestrogenic and progesto-
genic hormones appear capable of inducing corneal steepening
in all day corneal lens wearers. The effect is most pro-
nounced with oestrogen. 3 subjects from the sample 14 taking
a hormone preparation suffered a disruption of lens wear
which it seems reasonable to attribute directly to this
corneal steepening (see p.188). It is possible however
for a cornea to be shown to have steepened (e.g. patient 6BS)
without precipitating any adverse reaction. Tear volume and
composition are also involved and much depends on the severity
and duration of the individual drug response and the basic
lens fit. All are contributory factors to the potential
development of a drug-induced lens intolerance or difficulty.

Part 2.

1.   Quantitation of Electrophoresis Results.

(i)   Introduction

Quantitation of the amount of protein present in the
stained bands of a support medium is done photometrically
in one of two ways. The support medium is either scanned
with a recording densitometer, yielding a graph of absorbance
along the length of the support, or cut into individual bands,
the dye eluted and its optical density measured in a
spectrophotometer.

The elution technique, although sometimes considered
to be more exact, is very much more time consuming and was
not considered feasible for this reason in view of the large
volume of material to be processed.

A design for a recording densitometer was therefore
devised with the aid of the Physics Department workshop.
Difficulty with the electronic part of the instrument
however eventually necessitated the purchase of a Chromoscan
recording densitometer (manufactured by Joyce Loebl & Co.Ltd).
Owing to delivery delay this did not become available until
some 4 months after the start of the clinical section of the
project. Electrophoresis results are not available therefore
for the early part of the project and data concerning the
normal tear protein pattern and the effect on this of the
selected hormones, is incomplete in a number of cases.

The Chromoscan is an optical system producing a slit
beam through which the sample is moved at a fixed rate.
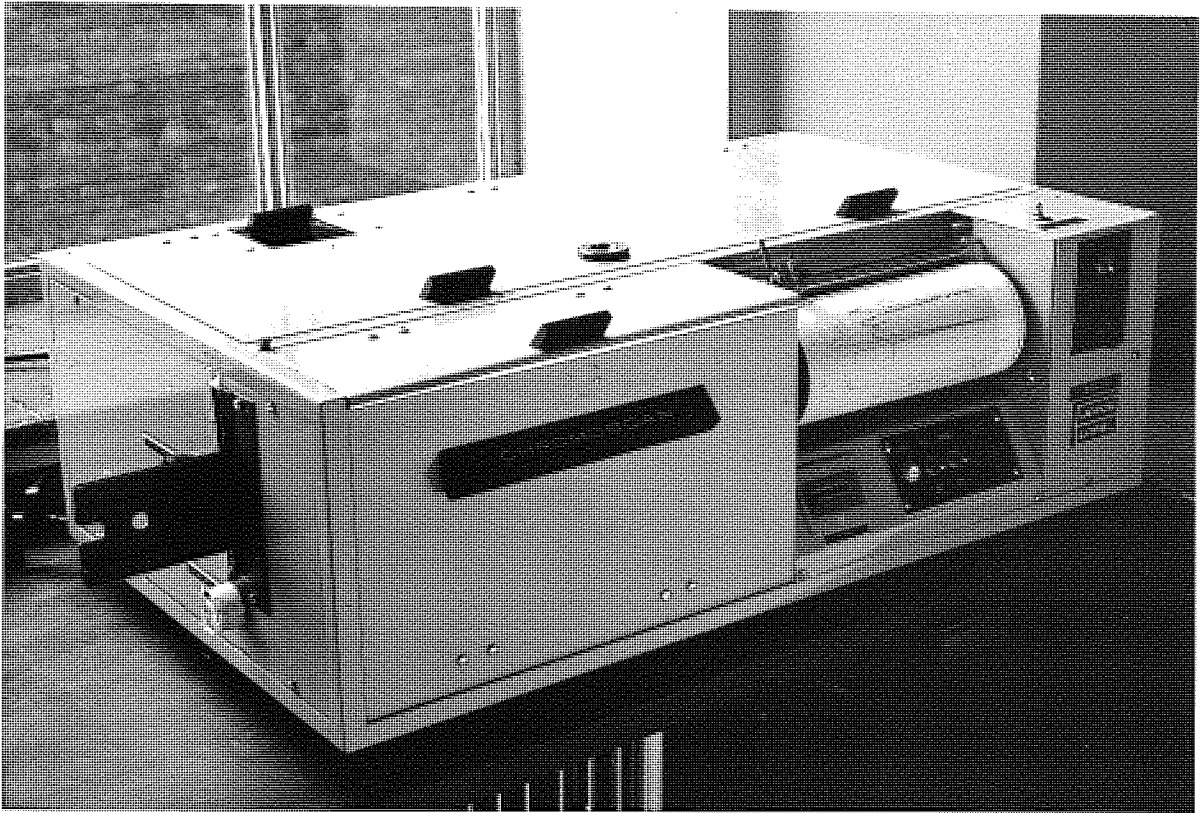
Plate 2. Chromoscan Recording Densitometer

A photocell monitors the light as it is transmitted or reflected by the sample. Variation in the photocell output is finally converted to a drive mechanism for a pen recorder.

(ii) Ultra violet densitometry.

References are available[15,114] indicating that it is possible, by means of ultra-violet densitometry, to quantitate protein in acrylamide gel without the need for time consuming staining procedures. Acrylamide itself is transparent to ultra-violet light at λ 280 nm, a wavelength which corresponds to a high absorbance peak for protein. Scanning an unstained gel with ultra-violet light at this wavelength should produce an absorbance graph of similar form to that which would result from scanning the same, stained, gel with visible light.

On receiving the Chromoscan, this technique was tried out in the hope of being able to eliminate the staining procedure. For use at 280 nm. all optical parts must be of quartz. A low pressure mercury lamp and interference filter provide the required wavelength, which is picked up in an ultra-violet sensitive photomultiplier.

Quartz sample tubes were obtained, since direct scanning of the gel within the tube would further reduce the time factor and eliminate the possibility of damage to the gel during removal from the tubes. Unfortunately, the tubes were found to be too irregular in their transmission properties to permit this. The gels were therefore removed and placed in a dilute acetic acid filled, parallel-sided quartz cuvette designed for use with the Chromoscan.

It was found however that at the formulation used the gels were insufficiently transparent to the ultra-violet light, such that all but the very largest protein bands were masked by the gel background. This was later found to be due to the 5% proportion of the cross-linking monomer 'BIS' present in Cyanogum-41. Some 'BIS' is essential for gelation, but its concentration must be radically reduced to 0.2%, to produce ultra-violet transparent gels. By the time this fact was appreciated however, the project was too far advanced to alter the gel composition, so visual densitometry was used at $\lambda 620$ nm, a region of high absorbance for amido black stain.[30]

(iii) <u>Use of the Chromoscan Densitometer.</u>

Detailed instructions for the use of the Chromoscan may be obtained from the manufacturers handbook. Certain controls are variable however, and the settings used on all current work are as follows:

(a) Slit: 10 mm x 0.5 mm

(b) ND. filter 2.5

(c) Interchangeable cam D.

(d) Sample speed: drum speed 1:3

(e) Sample height control set to give maximum pen deflection. This control determines the position of the sample in relation to the slit beam and the photocell. Once set it should be taped down to avoid accidental movement.

(f) Baseline control is reset for each sample to correct for minor variation in the background clarity of the gel, photocell variations, etc.

The region behind the lysozyme band was selected as the reference portion of the gel. This resetting is necessary to ensure that integer counts on successive graphs are comparable.

The Chromoscan trace of a typical gel is shown in fig.7. Figures on the graph refer to the integer count for that section of the graph and are a measure of the area under the curve.

(iv) Interpretation of Chromoscan Traces.

The tear fluid sample used throughout the project consisted of a 5 mm. diameter disc cut from the end of a Schirmer filter paper strip. This is therefore the portion of the strip which has been held against the eyeball by the lower lid.

It may be argued that this is not the most representative portion of the strip and that it would be preferable to use as a tear fluid sample some portion of the strip which had been moistened purely by absorption of tear fluid without having been in any direct contact with the conjunctiva or lids. However, since the cornea is in reality in close contact with both the inner surface of the lids and the lacrimal fluid, I consider the end portion of the strip to be more representative of the conditions directly affecting the cornea and therefore also the contact lens.

From a practical point of view also it should be realised that the lid portion of the strip is the only part common to all patients. A subject with a very low tear flow may not produce sufficient length of moistened

paper beyond the point of contact with the lid for this
to be used as a sample.

The end section of the Schirmer strip is in contact
with the lacrimal fluid for 3 min. It is thus fully
saturated before being removed and allowed to air dry.
It was found by experiment that a 5 mm. disc of this filter
paper is saturated by 4 μl of aqueous solution. Calculations
of the total protein concentration of the tear samples were
made therefore on the basis of a sample volume of 4 μl.

The problem of translating the graphical Chromoscan
trace into terms of "quantity of protein" either in total
or in individual peaks is complicated by the fact that
different proteins have different affinities for the dye
used. Albumin, for example, has a relatively greater
affinity for amido black than have the globulins.[80]

It is necessary therefore to establish standardisation
curves for each major protein group under the experimental
conditions involved. Five reference substances were obtained:
Koch-Light Laboratories Ltd.

1.   Albumin. Human Cohn fraction V. Batch No.57442
2.   α-globulins. Human Cohn fraction IV. Batch No.48159
3.   β-globulins. Human Cohn fraction III. "   " 59590
4.   γ-globulins. Human Cohn fraction II.   "   " 57712
      Sigma Chemical Company.
5.   Lysozyme. Egg white, grade 1. 3x crystallized.

Lysozyme, α and γ-globulin were found to be readily soluble in distilled water. Albumin formed a fine suspension, especially at the higher concentrations used, while β-globulin was quite insoluble in distilled water and could only be used in solid form in the reference experiments.

With the exception of β-globulin, all the reference substances were made up in distilled water into stock solutions to cover the concentration range expected,

```
i.e.    3%  solution ≈ 0.12 mg.    )
        2%     "      ≈ 0.08 mg.    )
       1.5%    "      ≈ 0.06 mg.    )
        1%     "      ≈ 0.04 mg.    )  applied in 4 ul sample
       0.5%    "      ≈ 0.02 mg.    )         volume
      0.25%    "      ≈ 0.01 mg.    )
      0.12%    "      ≈ 0.005mg     )
```

With the aid of disposable microcapillary tubes 4 ul units of test solution were applied to 5 mm filter paper discs and allowed to air-dry to simulate the conditions applying to the routine tear samples. After electrophoresis the test sample gels were stained, destained and scanned in the normal way.

A minimum of six electrophoretic runs were performed on each protein at each concentration. Standardisation curves were then constructed for each protein, relating the Chromoscan integer total to the quantity of protein in the sample. These standard curves are shown, with the 1st stand. deviation, in fig.6. The standard deviation may be observed to increase with increasing sample concentration, e.g. Albumin.

| 0.1% solution | 54 ± 12 integer counts |
| 0.5% solution | 194 ± 20 integer counts |
| 1% solution | 300 ± 30 integer counts |

This high variation on concentrated samples is felt to be a feature of the photocell response to extreme light reduction. In the great majority of cases tear fluid results are found to involve only the early portion of each graph where the standard deviation is small. Albumin exhibits the greatest variation, a fact which may be expected in view of its form as a suspension not a solution.

(v) Identification of tear fluid protein bands.

The peaks of the Chromoscan trace have been numbered for ease of reference and a composite of several traces to show all the potential peaks separable by this electrophoretic technique is shown in fig.8. Of these 10 peaks, IV, VII, VIII and X are invariably present and peaks V and IX occasionally seen. I, II, III and VI are normally present, although they are more prominent in subjects with lower levels of tear flow.

In one volunteer, not actually part of the project but used frequently during the early development part of the electrophoretic work, peaks I and II were invariably absent, see fig.11, although the electrophoretic pattern corresponded well with other subjects in all other respects. The significance of this difference is unknown.

During the electrophoretic separation of the reference proteins described above, a tear sample was always included among the reference samples for comparison. Chromoscan
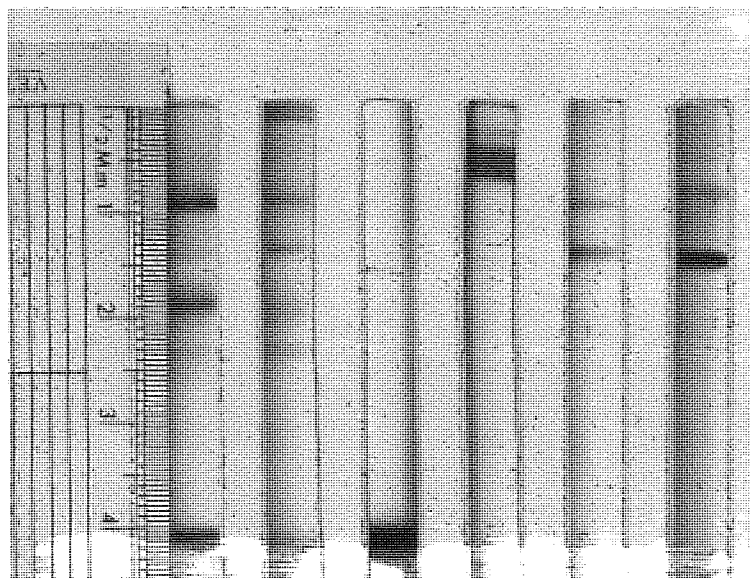
a   b   c   d   e   f

Gel a: Normal Lacrimal Fluid without bands I and II.

Gel b: Normal Lacrimal Fluid showing bands I and II.

Gel c: Reference protein Egg-white Lysozyme, 1% strength.

Gel d: Reference protein ɣ – Globulins, 2% strength.

Gel e: Reference protein ∝ – Globulins, 2% strength.

Gel f: Reference protein Serum Albumin, 2% strength.

Figure 6. Standardization curves of protein concentration related to the Chromoscan integer total. (λ 620nm.) 4ul sample volume; 7.5% gels; stained with amido black.
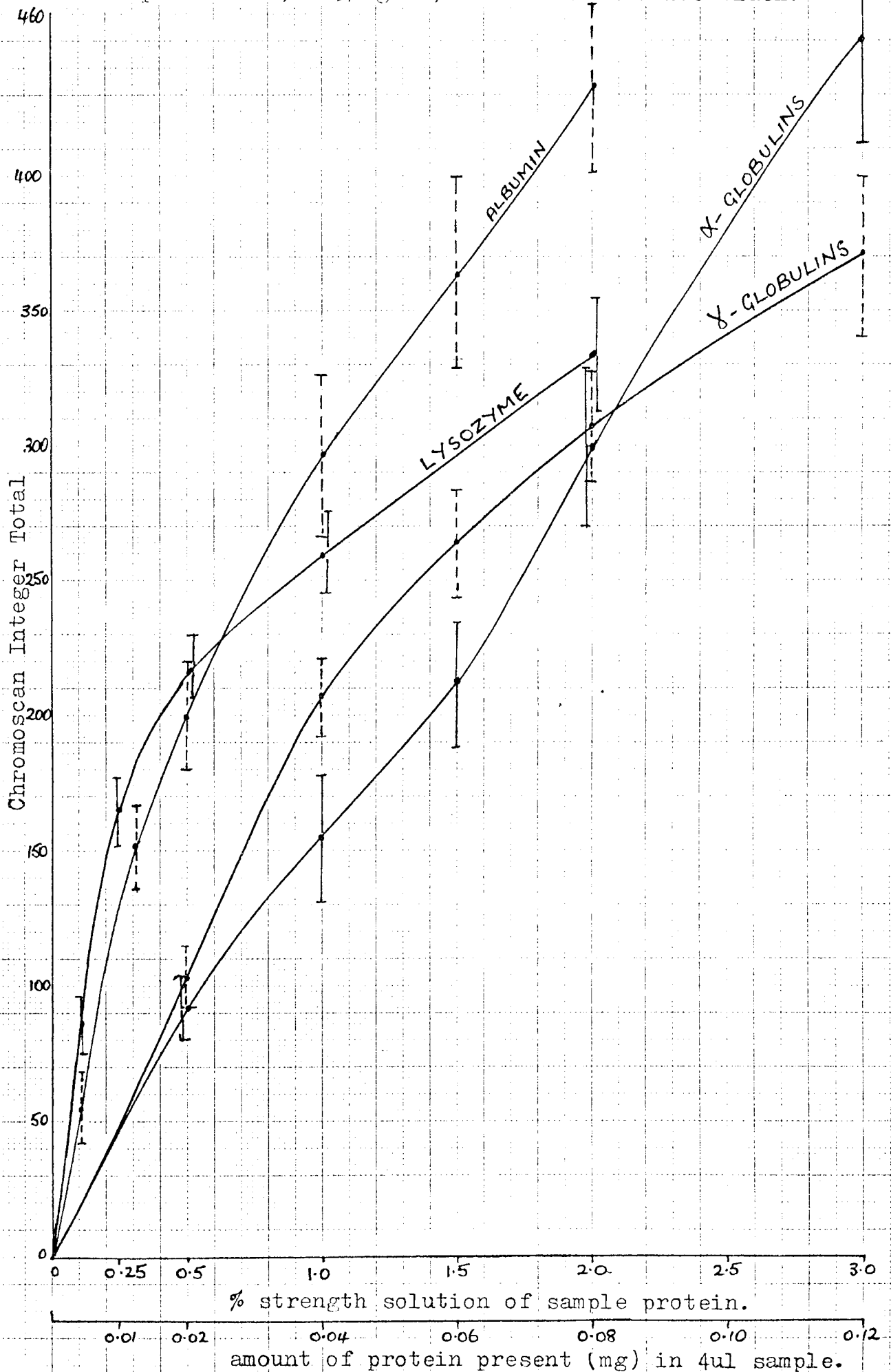
Figure 7. Typical Tear Fluid Protein Trace as obtained from the Chromoscan Recording Densitometer.
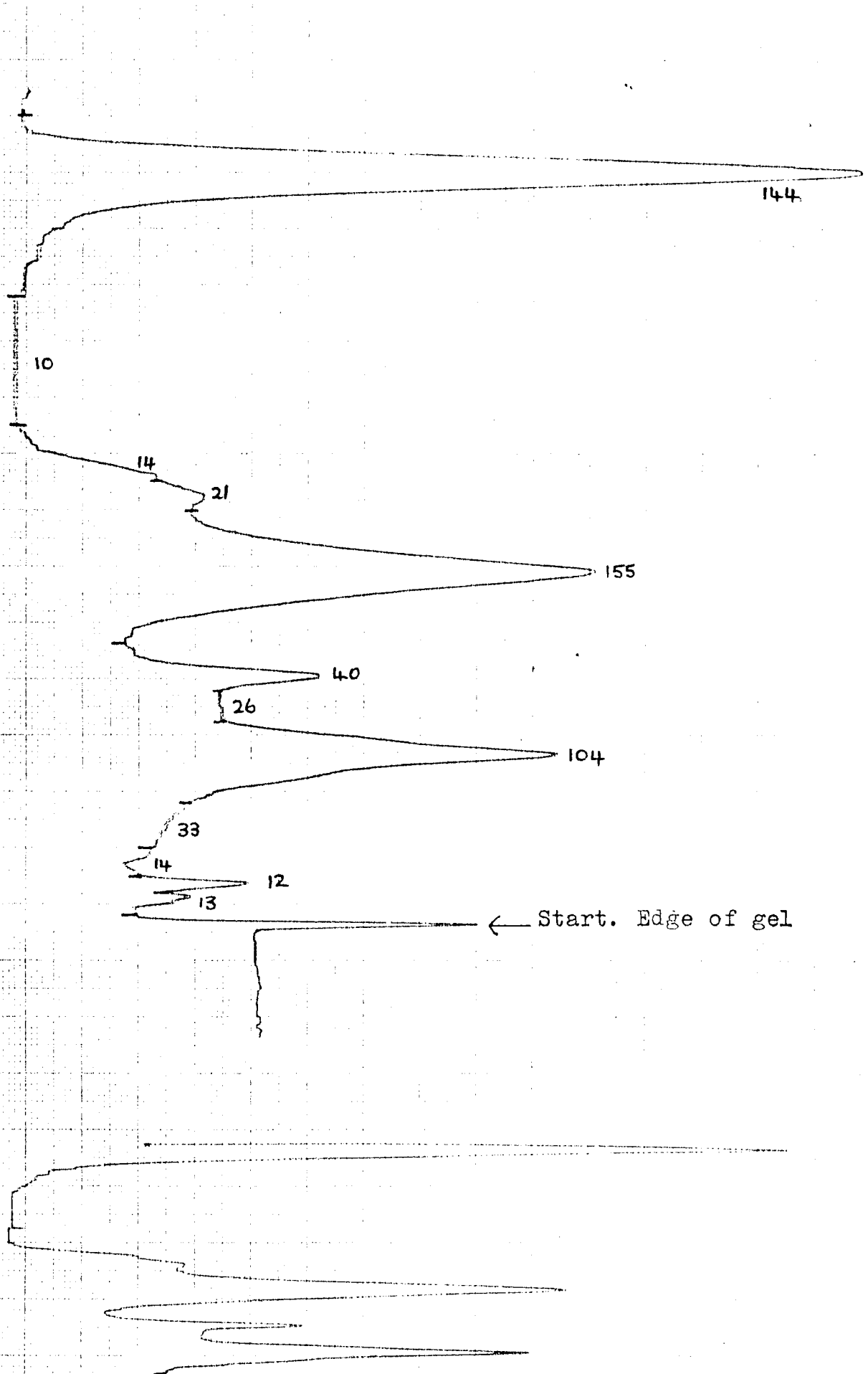
Figure 8. Composite Chromoscan trace to illustrate all possible protein fractions separable from normal unconcentrated lacrimal fluid by this form of acrylamide gel electrophoresis.
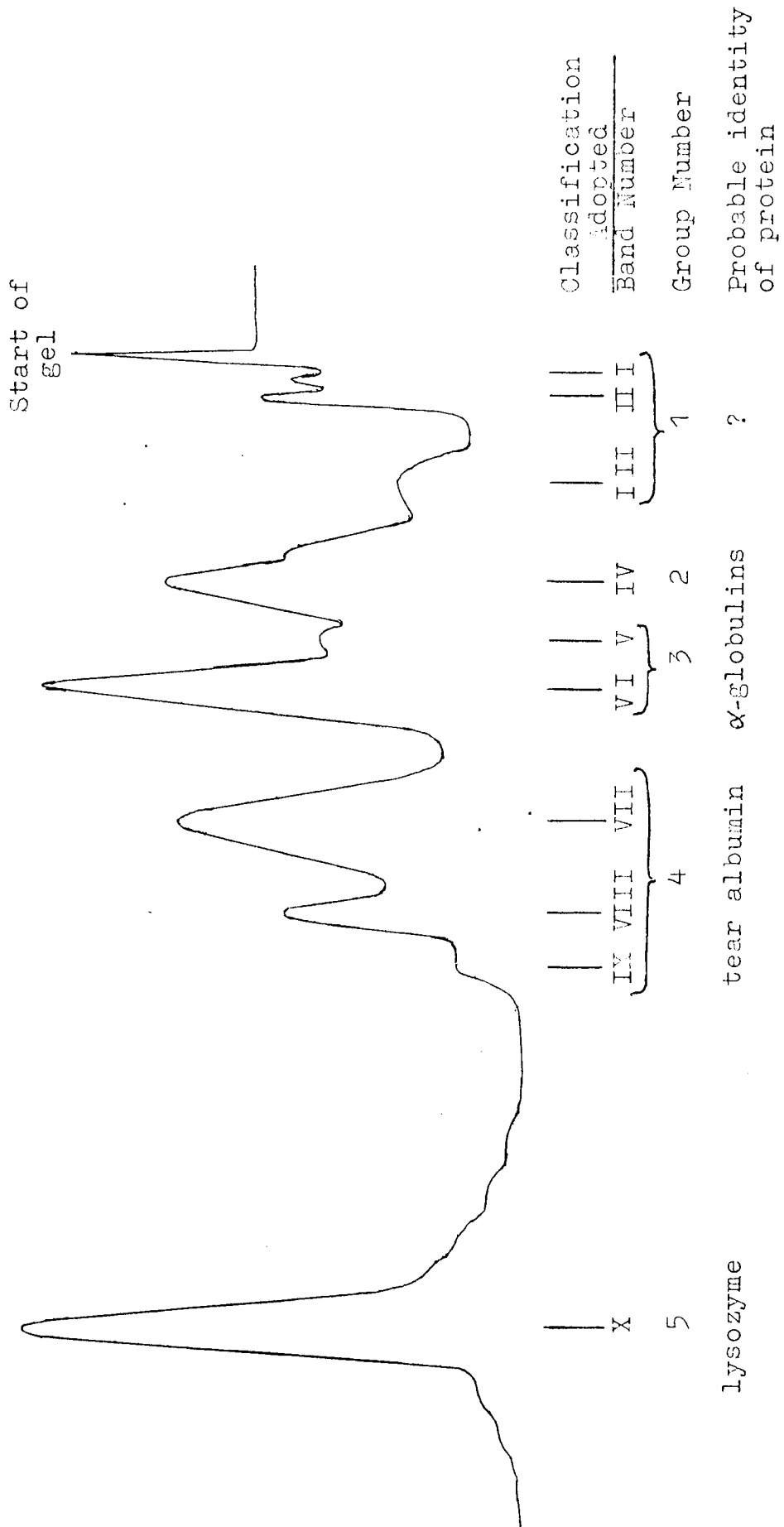
Figure 9.   Chromoscan traces of serum Albumin and serum
α-globulins.
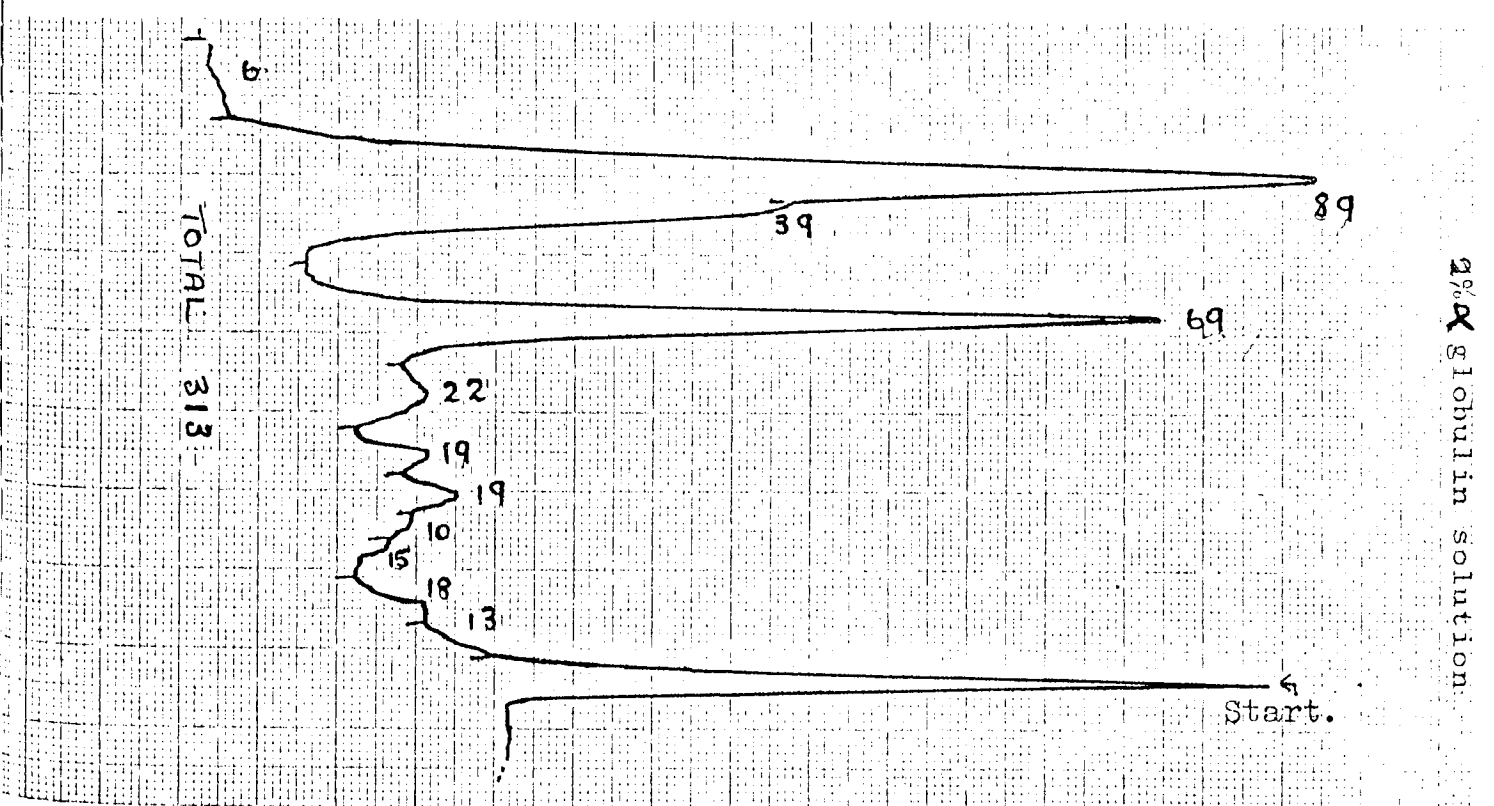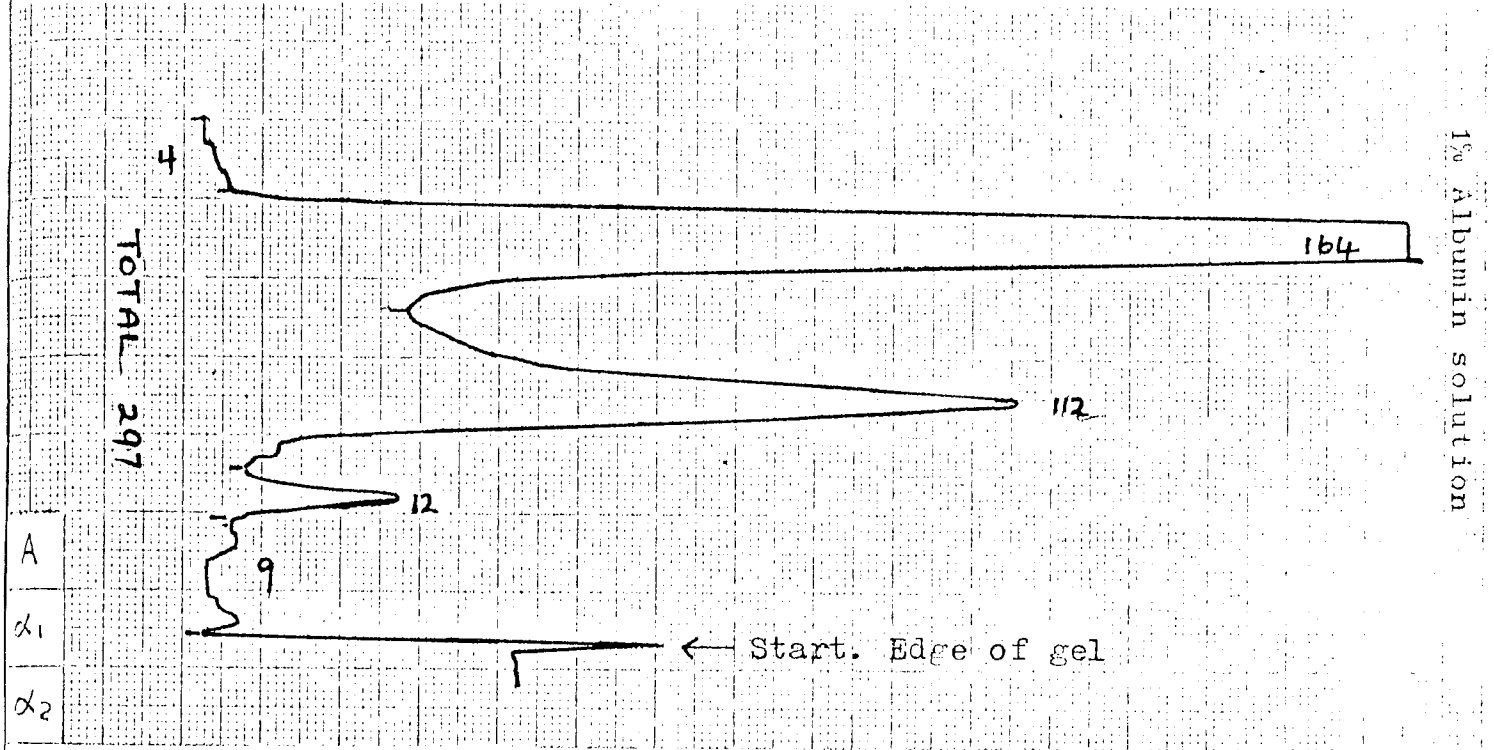
Figure 10. Chromoscan traces of β and γ serum globulins.
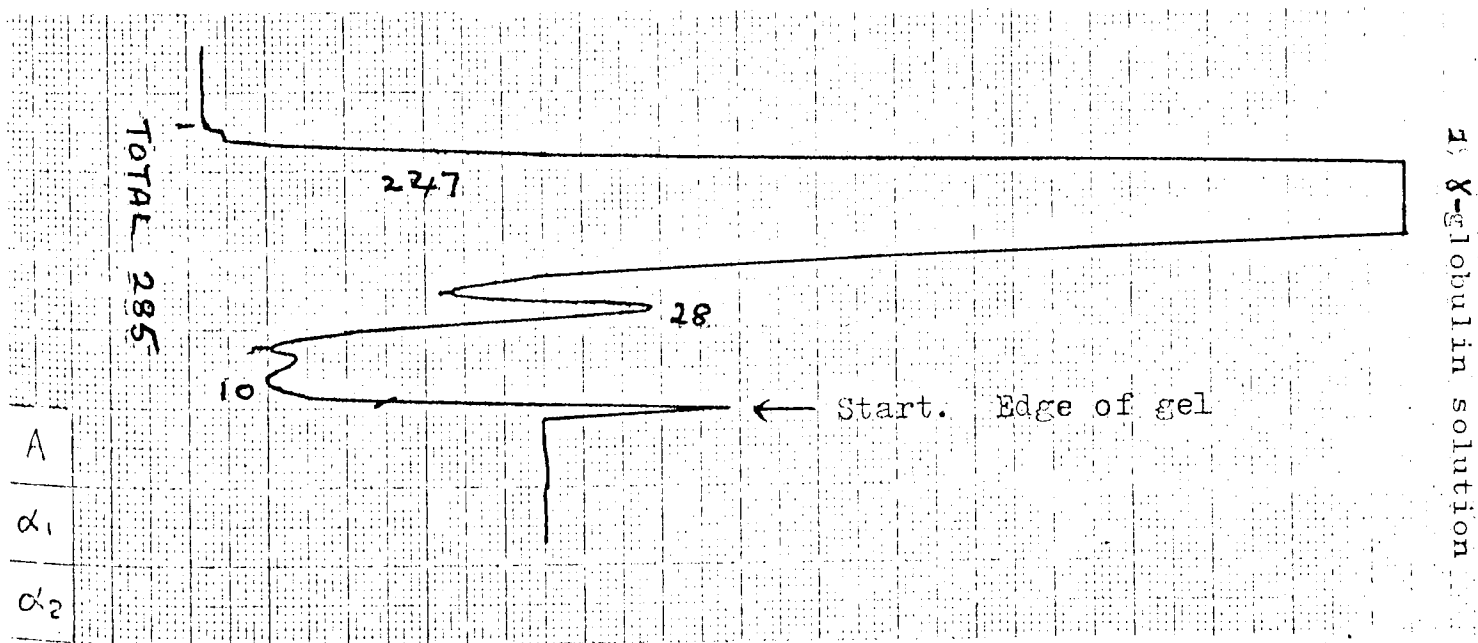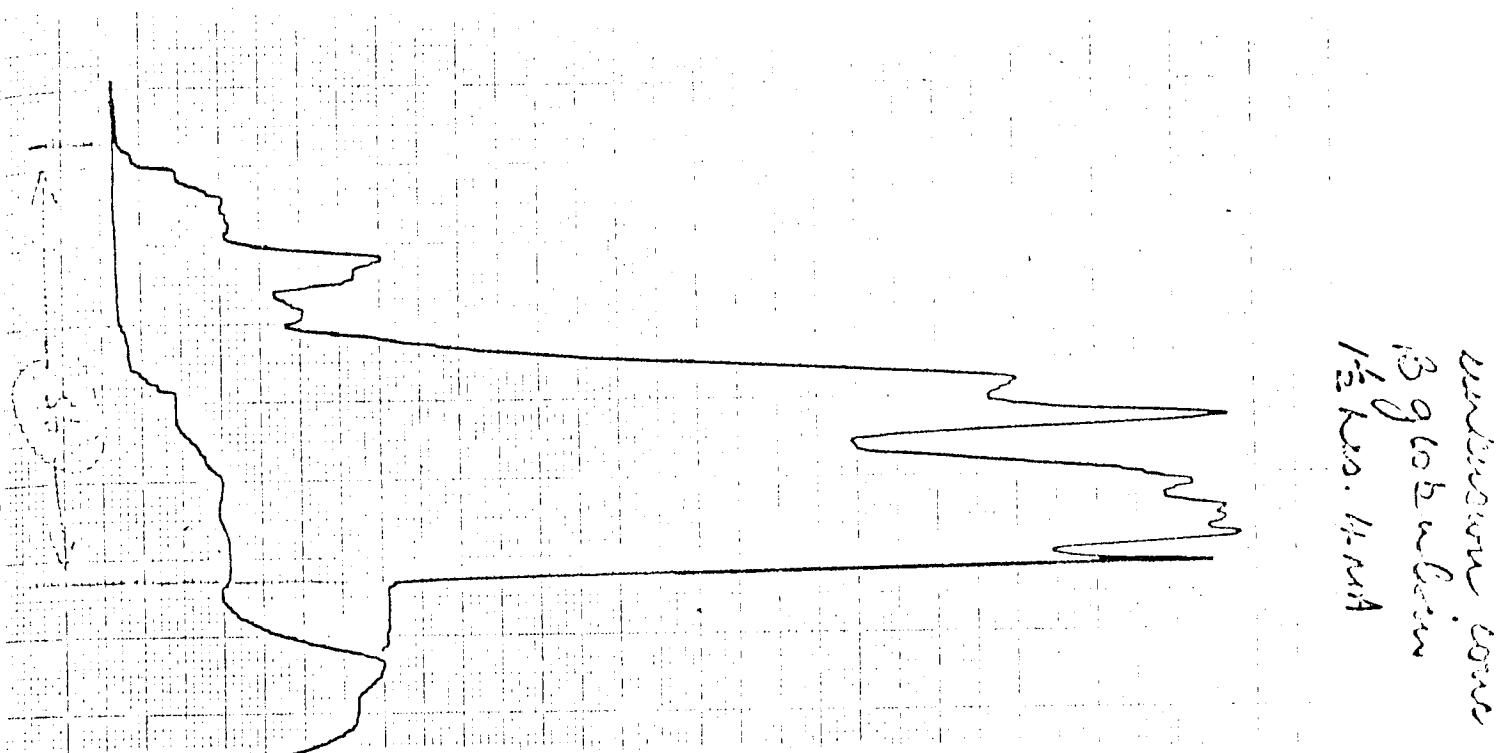
Figure 11.   Superimposed Chromoscan traces of tear fluid,
serum albumin and serum α-globulin samples.

Electrophoresis carried out in 7.5% acrylamide gels,
current 4mA/gel for 90min.   Stain: Amido black.

_____ Tear fluid sample.

_ _ _   Serum albumin, 1% solution, 4ul sample volume.

......   Serum α-globulins,   "   "

All samples processed simultaneously.

Lysozyme

Start.
Top edge of gel
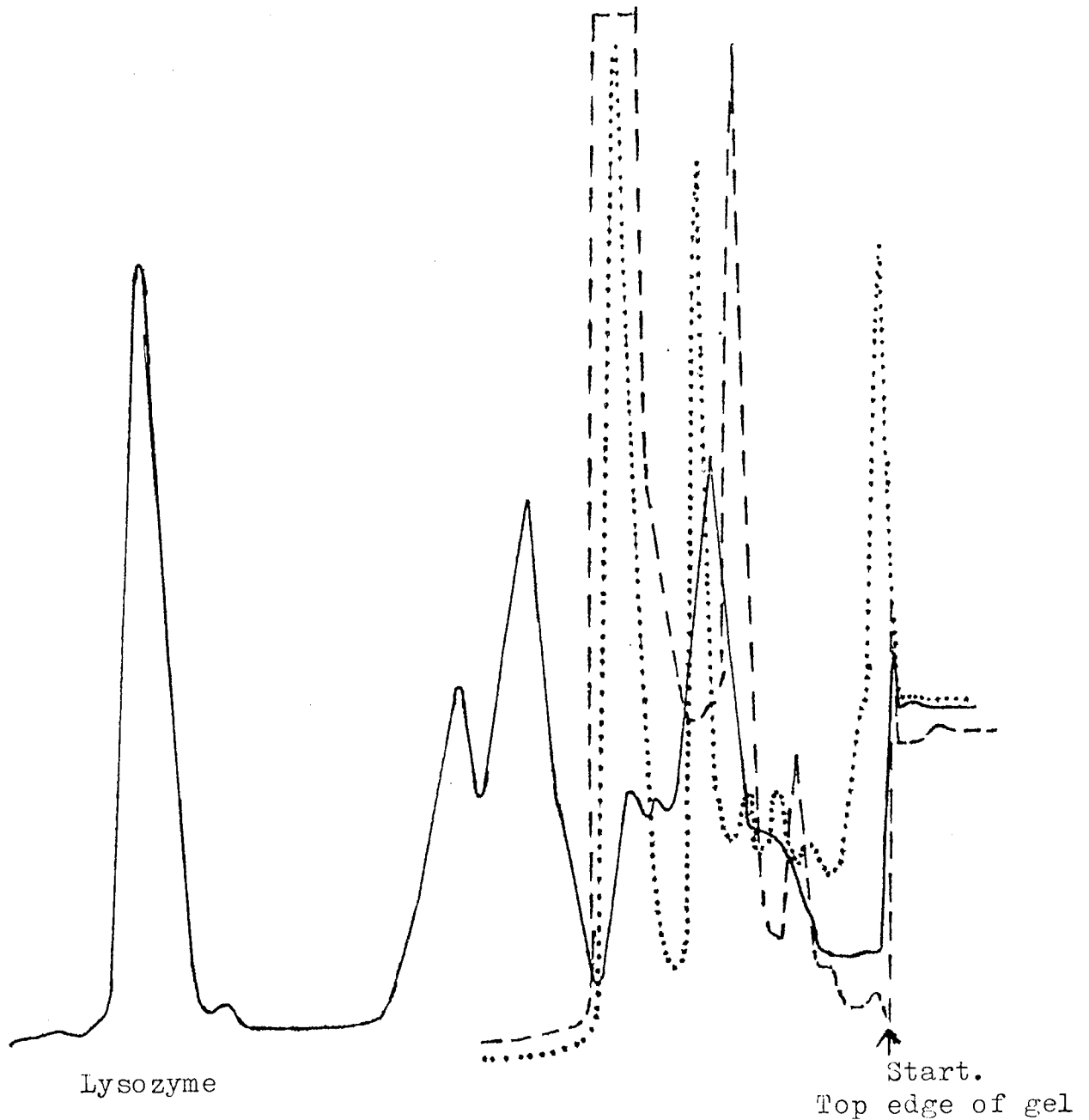
traces of typical 1% strength reference solutions are shown
separately in fig. 9 and 10, and superimposed on a tear
fluid trace run in parallel with them in fig.11.

Lysozyme.

Excellent correspondence was always demonstrable between
peak X and the hen egg-white lysozyme samples.

The presence of lysozyme may be confirmed by its lytic
activity against suspensions of Micrococcus Lysodeicticus
cells. Following the method of Harrison et al[120] a suspen-
sion of Micrococcus Lysodeicticus cells, concentration
25 mg/100 ml, was prepared in 0.067 M. phosphate buffer
pH 6.2 An SP500 Unicam spectrophotometer was used to measure
the change in absorbance (at 450 nm) of the bacterial
suspension induced by the action of lysozyme.

For the purposes of determining which portions of the
processed gel possessed lytic activity, groups of 3 gels,
2 containing tear fluid samples and the third blank, under-
went electrophoresis. The blank gel and one sample gel
were then sliced into 3 mm portions. Each piece was placed
in 1 ml phosphate buffer and left overnight to allow the
unfixed protein to diffuse out of the gel. The third gel
was stained and destained in the usual way. None of the
buffer samples from the blank gel produced any effect on
the Micrococcus Lysodeicticus suspension.

Nor was there any activity from buffer samples corres-
ponding to protein bands I-IX. Buffer solution in which the
gel slice containing band X had been soaked however produced
a marked change in absorbance of the bacterial substrate.

A similar, but smaller, change was evident also from the sample corresponding to the 5 mm of clear gel immediately behind band X, through which the enzyme had just migrated.

Consideration of particle size and isoelectric point also make it likely that this fastest moving protein is lysozyme. Lysozyme is a small molecule, molecular size less than 50 $\overset{\circ}{A}$, which will suffer little frictional resistance from the gel matrix. In addition the isoelectric point $\simeq$ 10.8 in a running system pH 3.9, will induce a high electric charge on the molecule.

It may be firmly concluded that band X is tear lysozyme. It will therefore be designated by the letter L.

Albumin.

The identification of other bands on the gel is far less precise. By reference to fig.11 it may be seen that the major serum albumin and α-globulin peaks coincide, but correspond only to the minor tear fluid peak VI and that the second albumin peak does not match any particular portion of the tear fluid graph.

This apparent virtual absence of albumin is quite illogical. All existing work indicates that albumin contributes approximately 40% of the total tear fluid protein. No electrophoretic problems had been experienced with the serum albumin reference protein and its staining properties had been shown to accord well with established results. There was no reason therefore to suppose that the protein was being adversely affected in any way by the electrophoretic technique.

It had to be concluded either that the properties of the reference albumin had been altered during its previous manufacturing treatment, or that tear albumin and serum albumin are not identical proteins and react differently during the gel separation.

In view of the singular absence of any reference protein peak corresponding to the major tear fluid fractions VII and VIII, it was felt that these were the missing albumin fractions, that tear albumin and serum albumin were not identical, and that under these experimental conditions tear albumin had a greater mobility.

There is some supporting evidence for this assumption of variation between the two proteins. McEwan[69] states that on paper electrophoresis a difference in mobilities can be demonstrated. Albumin is also a protein which is prone to structural alteration, especially at an acid pH.[124]

If the hypothesis relating bands VII and VIII with tear albumin is correct, certain assumptions may be made about the molecule. If it is of the same shape and size as serum albumin, it must possess a less acid, i.e. higher, isoelectric point. This however does not agree with the paper electrophoresis findings which indicate a more acid isoelectric point.

Alternatively, it must be a smaller, more regularly shaped molecule than serum albumin, allowing an easier passage with less frictional resistance through the gel matrix.

Band IX is uncommon but VII and VIII are two of the four invariably occurring proteins. This further supports

their identity as albumin, but indicates the existence of two distinct, although closely related, albumin-type proteins. The slightly slower moving faction is usually present in a greater concentration approximately 2:1, although equal amounts do occur and the ratio is occasionally reversed.

In all other types of electrophoresis, tear albumin is present as a single unit. This finding of two and occasionally three clearly distinct subfactions of the protein is quite new. The source of the difference is most likely to be in a shape or structural variation rather than a change effect and it is the "filtering" effect of the gel matrix which has made this greater resolution possible.

Although it is not completely certain that this protein group is albumin, it appears so likely that the bands may be designated A_1, A_2 and A_3 in increasing order of mobility.

Globulins.

Early paper electrophoresis differentiated tear fluid only into lysozyme, albumin and globulin type proteins. More sophisticated paper or C.A.M. techniques may differentiate the globulin-type group into 3 subfactions. McEwan[69] identifies them as similar to the α_2, β and γ-serum globulins and states that the α_2 and possibly the β-factions are mucoproteins.

As may be seen from fig.11, there is excellent correlation between peaks IV and VI and the 2 major factions of the α - reference protein. Confirmation of the identity of these two tear fluid components is found in the results of the Periodic-Acid Schiff reagent process for the identification

of protein-bound carbohydrate.[4]

Glycoprotein and mucoprotein are found only in
conjunction with the globulins, predominantly the α-globulins,
and are never linked to albumins. When a tear-fluid sample
is stained with the P.A.S. reagent, band IV stains clearly
pink and band VI a faint pink. The rest of the gel remains
completely clear. This confirms the globulin-type nature
of bands IV and VI and further rules out the likelihood of
band VI being a small albumin peak.

It is not possible on the basis of this study to
identify band III, other than to say that it is likely to
be either β or γ-globulin. This is due to the very large
overlap between these two reference substances.

Bands I and II which are often very prominent in the
tear fluid separation (see plate 3) have no obvious parallel
among the reference proteins used. They most nearly match
the initial peak of the β-globulins, but the insolubility of
this protein limited the amount of comparative work that
could be done with it. It is possible that I and II are
another substance altogether. Their limited penetration
into the gel suggests a very large molecule.

2. Tear Fluid Electrophoresis Results.

The electrophoresis results may be conveniently considered in four sections.

(i) Basic Protein levels and site of origin of proteins.

(ii) Protein levels under the influence of medication.

(iii)Protein levels during initial contact lens adaptation.

(iv) Protein levels of contact lens wearer during medication.

Each Chromoscan trace was sub-divided into 5 major proteins, or groups of protein, in line with the provisional identification of the stained bands previously described. These 5 groups were:

Group 1. Unknown protein bands I-III

"   2. Major α -globulin band IV.

"   3. Minor α -globulin bands V and VI.

"   4. Presumed Albumin bands VII-IX.

"   5. Lysozyme.

Groups 2-5 were referred to the appropriate protein standardisation curve (fig.6) while group 1 was referred to a combined $\alpha + \gamma$ globulin curve.

All Chromoscan results for each person were tabulated (see Appendix 2). They were listed in terms of the % strength reference solution which, in 4 ul quantities, gives rise to the same Chromoscan integer count. These % strength values may be readily translated into absolute amounts of protein, assuming 4 ul sample volume, by reference to the alternative scale below the reference graph (Fig.6). Total protein concentration of each sample is arrived at by addition of all 5 subgroups.

(i) Basic Protein Levels.

It had been intended to utilise the initial 10 week period of the clinical project for the establishment of the basic levels of all the variables. The absence of a densitometer for the whole of this period and for two-thirds of the following, medication period prevented this and has considerably reduced the amount of electrophoretic information available.

The tear samples from which the following basic protein information is derived were collected during the two weeks immediately preceding the supply of contact lenses. This has limited the number of samples per person to a maximum of 5. This is quite sufficient, when results from all subjects are grouped together, to illustrate an overall pattern. It is not, however, adequate to confirm a definite quantitative normal level for each individual subject. This is due to the random results which periodically occur, as they do with Schirmer volume readings, and which are thought to be due to the uncontrolled atmospheric conditions prevailing before samples were taken. In a large series of readings these uncharacteristic results are clearly obvious as such, and may be discarded accordingly. In a group of 3 or 4 readings however, there is not sufficient information available to reasonably do this, so there often appears to be little consistency between successive results from the same subject, particularly in subjects prone to large variation in tear volume.

Taking all results from this two week period as a whole, scattergraphs were plotted relating each major protein

group to the corresponding Schirmer reading (p. 148-159).

A Bravais Pearson Correlation test (Olivetti Programma card 132)[82c] was performed on each set of data, and the results of this and the mean level of each variable are indicated on the graphs. Visual examination indicates that only lysozyme exhibits an overall correlation with the Schirmer value. This is confirmed by the correlation coefficient of +0.67, which indicates that the concentration of the enzyme increases with increasing tear secretion and conversely, decreases with decreasing rate of tear production.

As the scattergraph and the correlation coefficient show however, the relationship is not perfect and a fair amount of variation does occur. It is also only valid when dealing with unstimulated tears. An electrophoretic separation of stimulated tears shows only minimal quantities of the three major proteins and is quite dissimilar to the type of pattern resulting even from a very moist Schirmer strip.

That this method of sample taking does not cause significant stimulation of the reflex lacrimal system is further shown by the occurrence of very low Schirmer values of 4 mm and 5 mm. Results at this level could not occur if the reflex system were materially involved.

The higher concentration of lysozyme in lacrimal fluid compared with blood serum has always been considered as evidence for an active secretory mechanism for lysozyme within the lacrimal gland. The positive correlation between lysozyme and tear volume further confirms this. It implies

that the more actively the gland is secreting as a whole
then the more actively it is producing lysozyme. The
finding of an absence of lysozyme in the tear fluid of
patients suffering from keratoconjunctivitis sicca is also
understandable on this basis.[68,69]

Reflex irritant induced lacrimation is by implication
produced by a different mechanism within the gland. It
may be visualised as a rapid, short-term alteration in the
permeability of the gland, allowing passage of serum, freed
from its large molecular components by a filtration effect
of the glandular tissue itself.

This theory would seem to explain both the phenomenon
of reflex lacrimation and the very dilute nature of the
resulting fluid. The means by which the neural stimulus
triggers the hypothetical barrier permeability change is
unknown. The gland receives a parasympathetic secretomotor
supply from the facial nerve and has in addition a sympathetic
and a trigeminal nerve supply. Experimental results are
conflicting, but suggest that stimulation of any of these
nerve paths may cause reflex secretion.[125]

Other protein groups present in the normal tear fluid
do not show any such definite relationship with tear volume.
Nevertheless, inspection particularly of the scattergram
of groups 2 and 3, show a tendency for the high protein
values to occur in conjunction with low Schirmer readings
and for the high Schirmer readings to correlate to low
protein values. This tendency is most marked in the case
of the minor α-globulin peak VI.

When the individual results of subjects with widely varying tear volume levels are considered this tendency may be seen more clearly, e.g.

Subject 8JH

Date	1	2	3	4	L	Total	Schirmer
11.10	0.22%	.30	.17	.13	.165	1.035	50 mm
19.10	0.22%	.27	.54	.16	.057	1.247	36 mm
23.10	0.10	.32	.07	.20	.19	0.875	50 mm

Subject 15AHcH

10.10	0.08%	.34	.09	.18	.30	0.99	50 mm
11.10	0.22%	.34	.34	.125	.06	1.065	12 mm
23.10	0.20	.30	.65	.16	.09	1.40	9 mm

Subject 21DT

17.10	0.27%	.54	.27	.51	.115	1.705	23 mm
19.10	0.50%	.96	.32	.42	.07	2.77	6 mm
23.10	0.17%	.34	.12	.44	.11	1.18	20 mm

Although there is no relationship within a large group between protein groups 1-3 and tear volume, it is possible that treating the results in this way obscures the existence of such a relationship within the tear fluid of an individual person. The three sets of results shown indicate that this might well be the case although it cannot be definitely confirmed or disproved on the limited number of samples available for each person. The absence of a correlation on the scattergraph would then simply be a reflection of the wide individual variation in basic protein concentrations existing within a population.

The albumin (group 4) and the slow moving fractions (group 1) demonstrate no obvious relationship at all with tear volume. The group 1 proteins are the most erratic in their occurrence, while albumin tends to show a greater measure of consistency than any other protein within the results series of each individual.

The results of subject 21DT consistently shows a very elevated albumin concentration, quite outside the range of other subjects. α-globulin is also high. This patient had a thyroid gland tumor diagnosed during the project. An increased B.M.R. is one result of a hyper-secretion of this gland and this is in turn thought to produce elevated levels of blood constituents. Thyroxin is transported round the body by albumin, pre-albumin and α-globulin,[124] and although it cannot be proved, it is possible that the elevated tear protein levels were a reflection of the overall metabolic disturbance of this patient.

Site of origin of lacrimal protein fractions.

Lysozyme is derived from the lacrimal gland by active secretion.

Albumin: Fractions VII, VIII and IX.

It is not possible on the basis of this study alone to conclusively identify the origin of this protein group. It has been established that tear albumin is not identical with serum albumin, that it has a greater mobility under the gel filtration conditions applied, suggestive of a smaller more regular shaped molecule. Its concentration is found to remain relatively constant for a given patient regardless of large changes in tear volume. Its concentration is considerably less in tear fluid than in blood serum.

These 3 factors suggest the possibility that tear albumin is derived from serum albumin following some form of filtration through the lacrimal gland. Serum albumin is not a homogeneous protein and it is reasonable to visualise

only the smaller molecules being passed through the glandular meshwork and possibly undergoing a further minor modification in the process. This hypothesis explains both the structural and concentration differences between the two fluids and further, the concentration gradient across the barrier would permit a continuous passive activity, independent of the active secretory activity of the gland. This diffusion would explain the fairly constant tear albumin concentration since the osmotic pressure would have a self-regulating effect.

In addition histological study of the rabbit eye shows no evidence of albumin within the conjunctiva, the goblet cells or the cyptoplasm of any cells.[130]

This fact, in conjunction with the electrophoretic results would seem to confirm the plasma origin of the tear albumin.

Globulin-type fractions.

Since serum contains all globulin fractions in considerably greater concentration than occur in tear fluid, it is possible that they also are derived by passage through the lacrimal gland. The globulins are very much larger molecules than albumin and would therefore require either an active secretory mechanism across the glandular meshwork, or a large pore-size "sieve" in the case of passive diffusion. A large pore size would be expected to freely permit the passage of the smaller albumin molecules, which would produce a much greater concentration than is actually found. An active secretion would suggest that a correlation with tear volume should exist as it does for lysozyme. For both these reasons

therefore, it is considered highly unlikely that the tear globulins are derived from serum via the lacrimal gland.

Fraction VI.

This is the only fraction which is completely absent from either tear fluid samples collected with a micropipette or from portions of the Schirmer paper strip which has not been in contact with the lid or conjunctiva. This protein fraction is therefore being collected directly from the palpebral or bulbar conjunctiva or from the lid margin and Meibomian glands. This latter possibility seems to be the most likely since a filter paper disc applied directly to the bulbar conjunctiva shows very little trace of this band.

Band VI stains positive for protein bound carbohydrate and matches well with a major peak in the α-globulin reference protein. Meibomian gland secretion is predominantly a lipid substance. An attempt was therefore made to use the standard range of Sudan reagents to identify any lipid material in the gel. The alcohol-based stains however dehydrated and warped the gels, so the attempt had to be abandoned.

The fairly strong inverse relationship of this protein with tear volume would also be understandable on the basis of an origin along the lid margin, i.e. in a very moist eye the total amount of material would be low compared with the fluid volume, but if secreted at the same rate, its relative concentration would be high when the fluid volume were low.

Fraction IV.

This major protein band stains positive for glyco-
protein. It is present in microcapillary liquid samples
and in samples taken from all portions of a Schirmer strip.
When a filter paper disc is applied to the bulbar conjunctiva,
an electrophoretic pattern is produced containing relatively
greater amounts of this protein.

The goblet cells secrete a mucus substance made up
largely of glycoprotein.[60] It is probable therefore that
this protein band is derived from the goblet cells of the
bulbar conjunctiva and fornix. As is the case with fraction V,
this view is supported by the relationship of high protein
concentration and low tear flow.

Fraction I-III.

The electrophoretic mobility of these proteins indicates
the probability of their being large molecules which would be
unlikely to diffuse through the lacrimal gland.

It must be assumed that they originate within the
accessory gland system of the anterior segment, but no more
definite identification can be attempted.
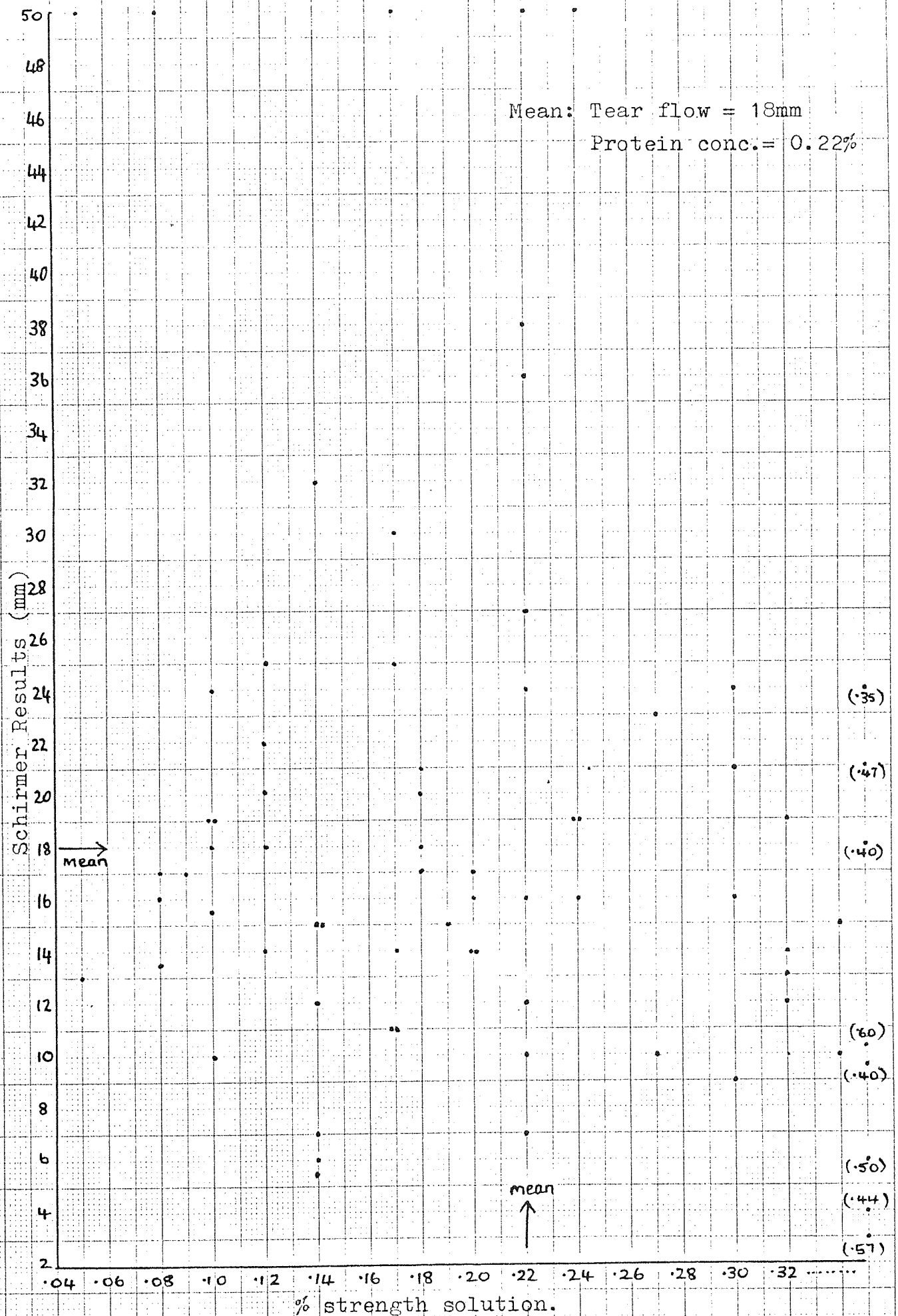
Figure 12. Group 1 protein (Fraction I-III) Control Period.
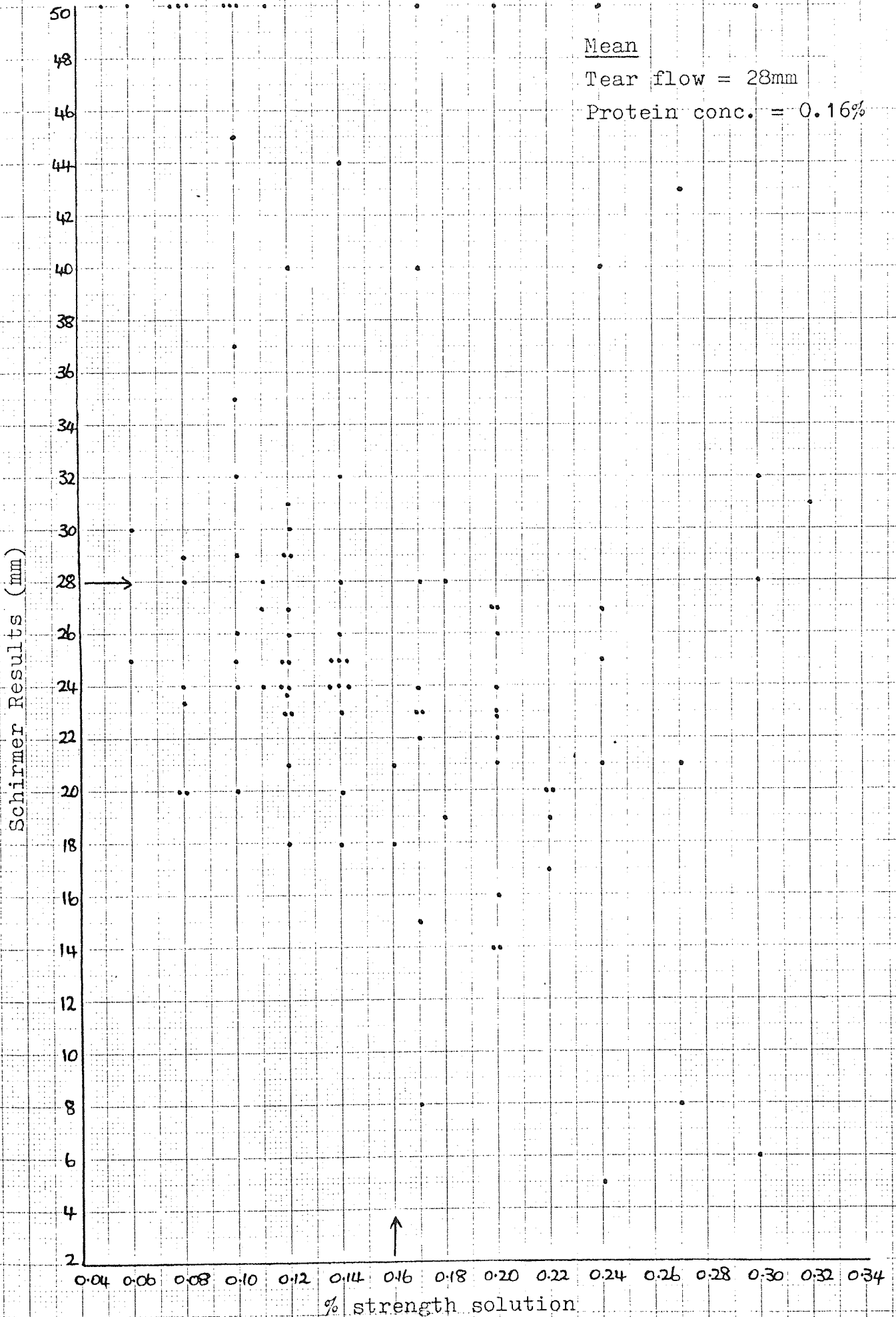
Figure 13. Group 1 protein (Fraction I-III). Lens adaptation period.
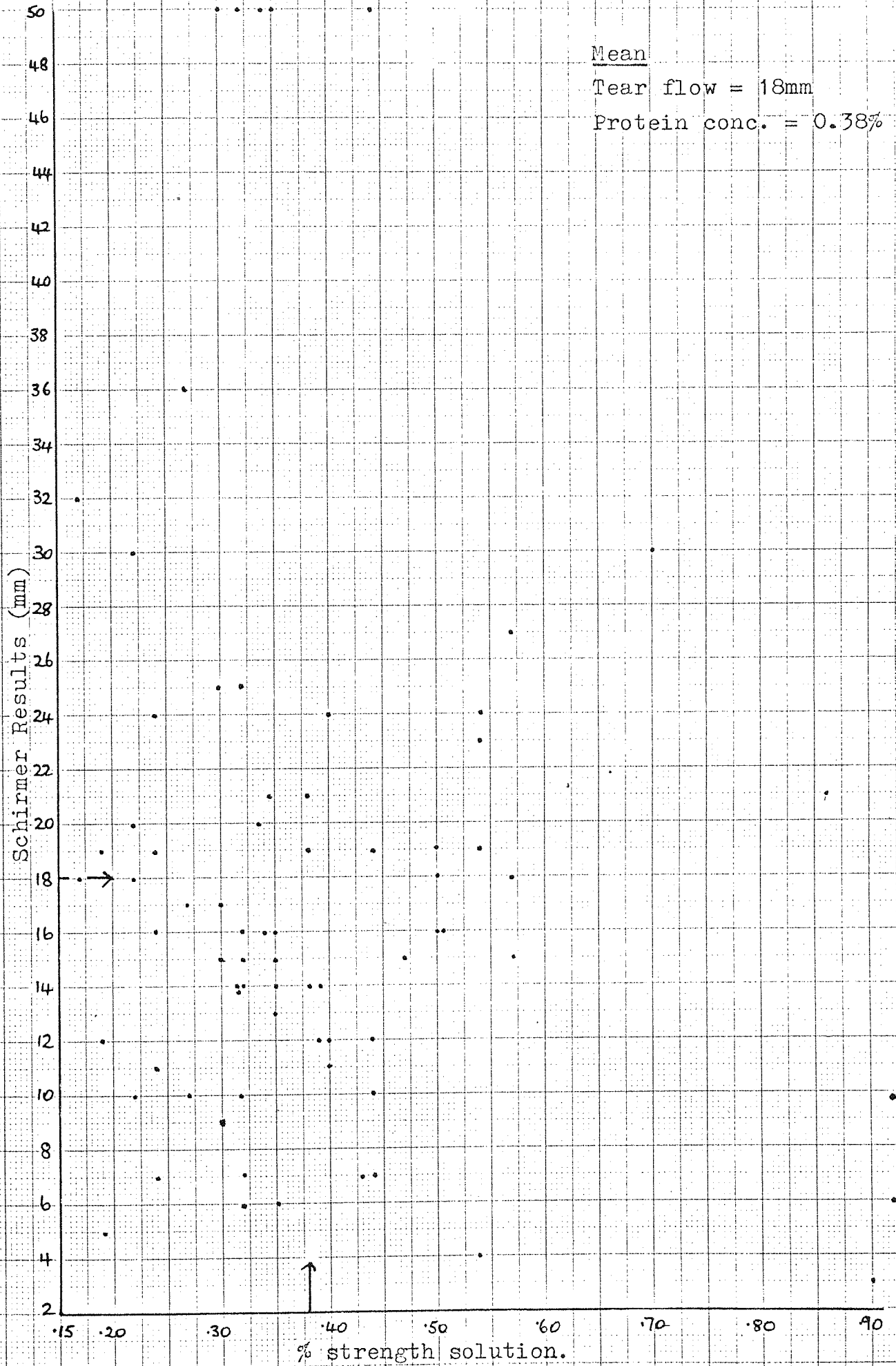
Figure 14. Group 2 protein (Fraction IV). Control period.
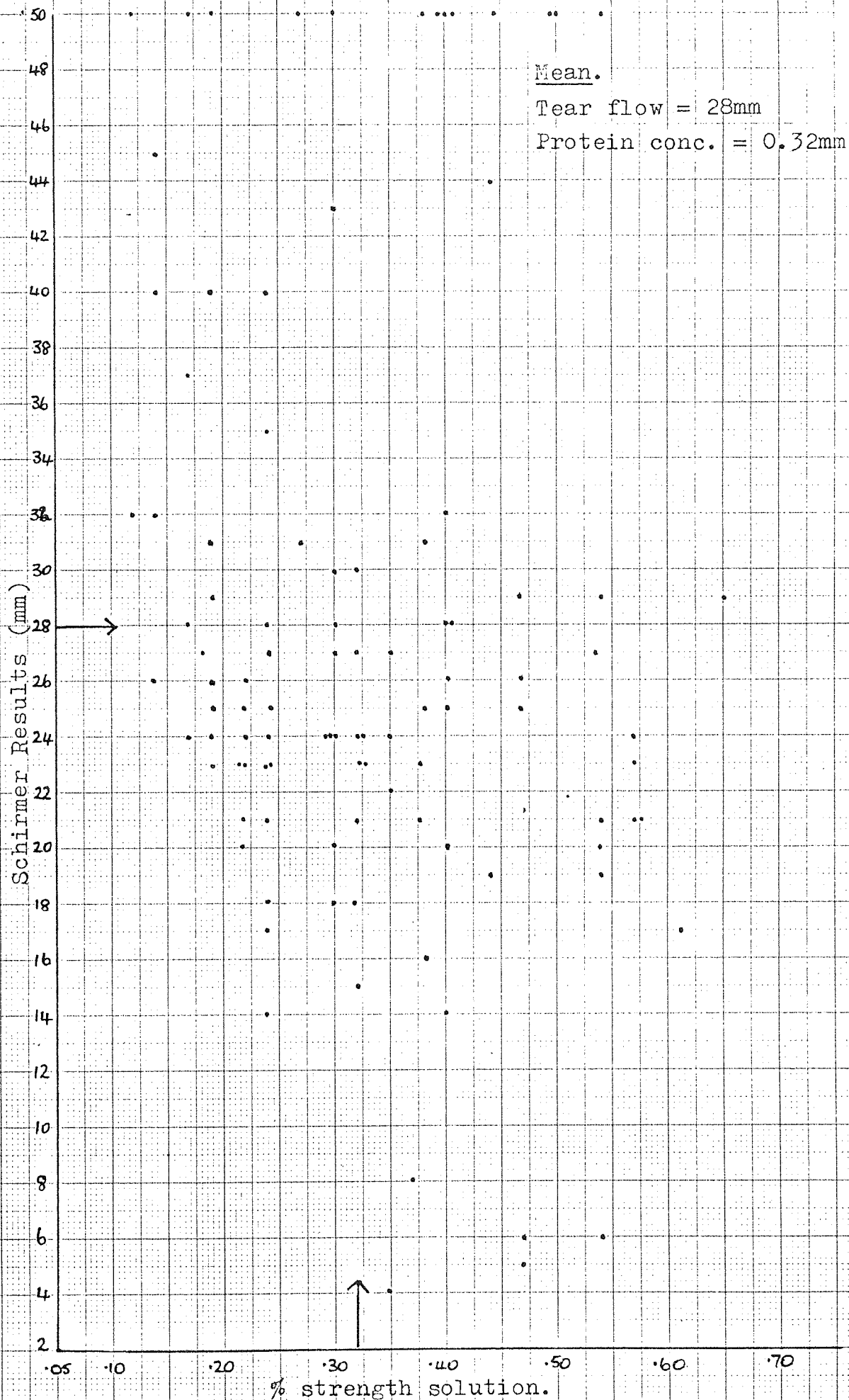
Figure 15. Group 2 protein (Fraction IV). Lens adaptation period.

Mean.
Tear flow = 28mm
Protein conc. = 0.32mm

Mean.
Tear flow = 18mm
Protein conc. = 0.33%
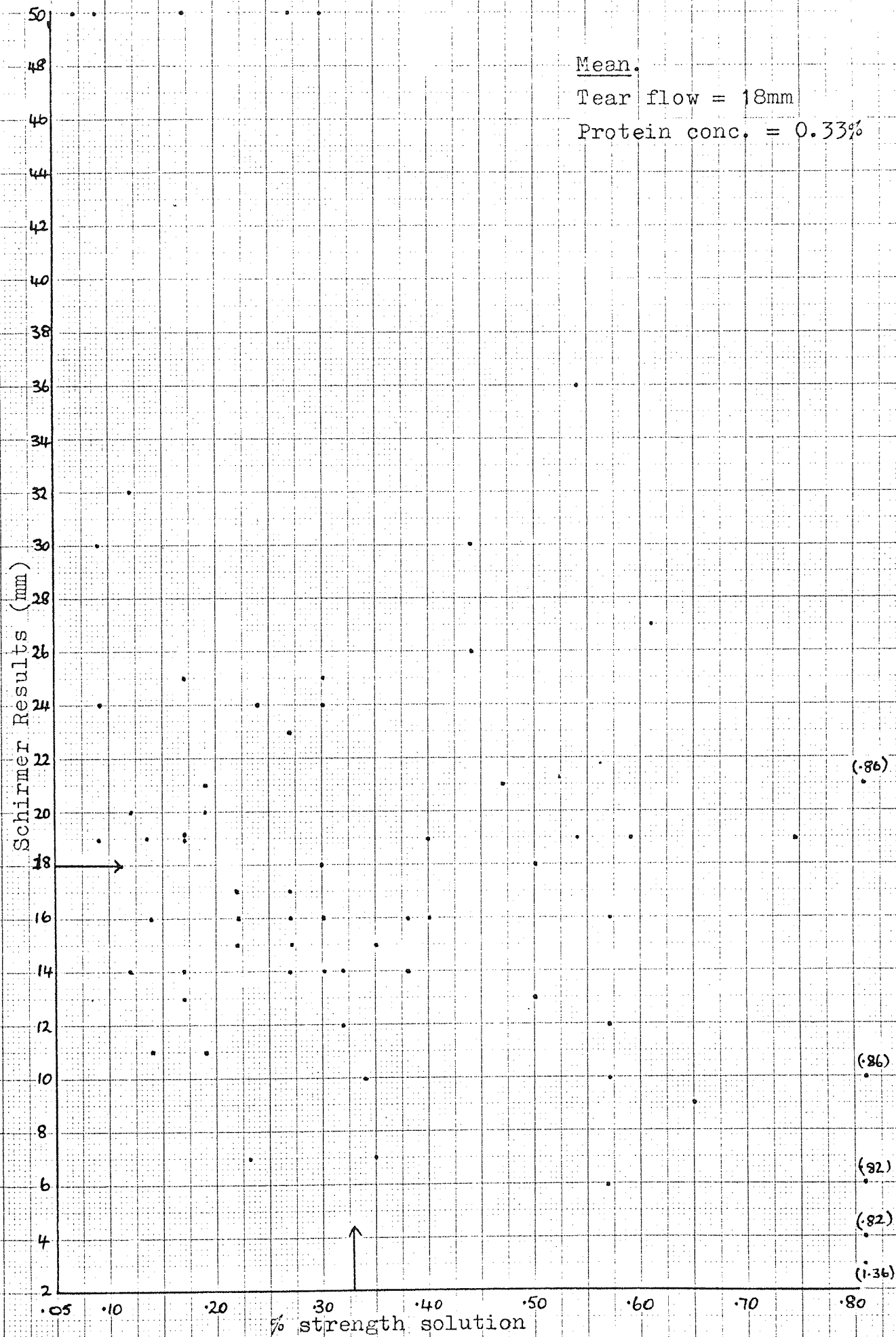
Figure 16. Group 3 protein (Fraction V-VI). Control period.

Mean.

Tear flow = 28mm

Protein conc. = 0.17%
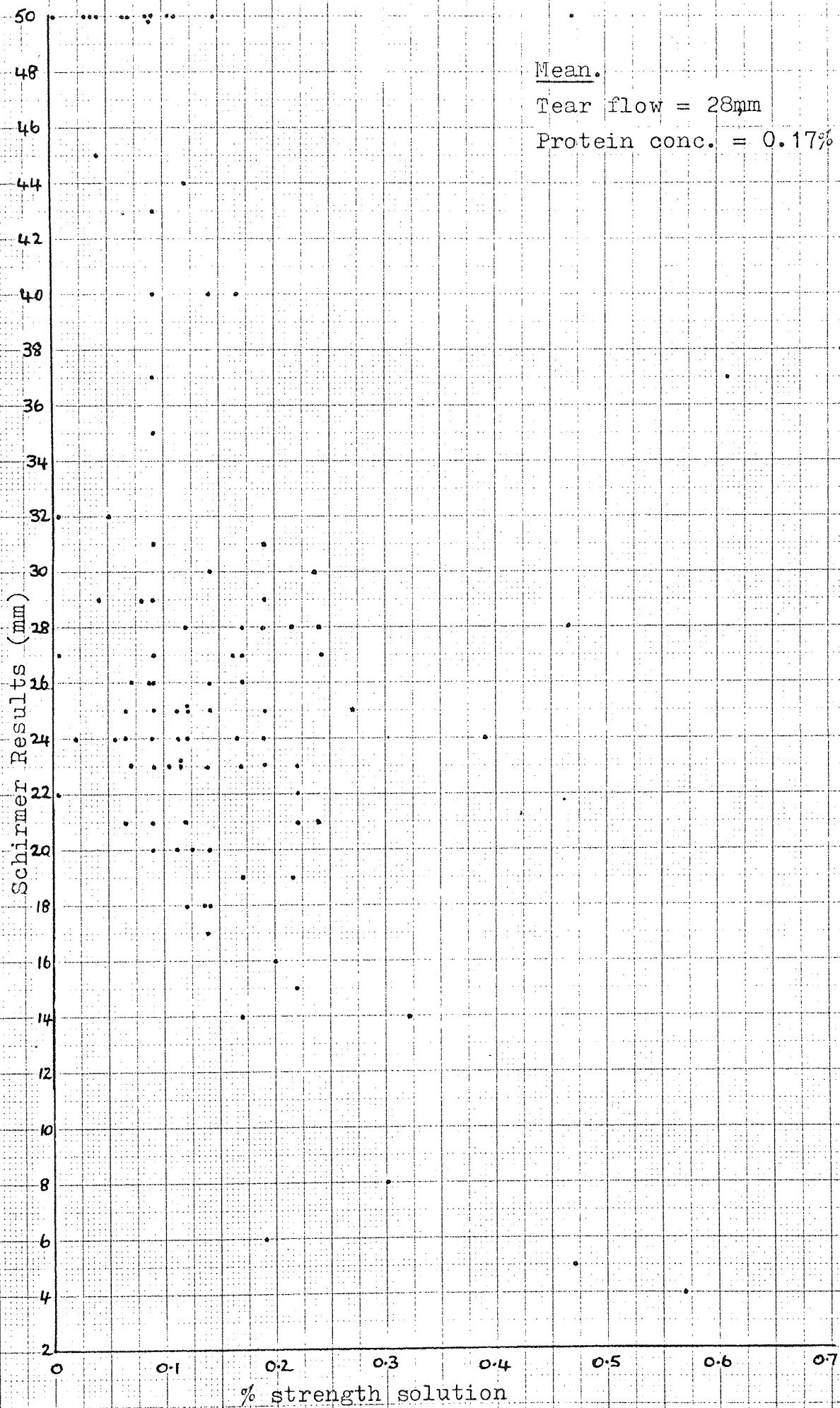
Figure 17. Group 3 protein (Fraction V-VI). Lens adaptation period.
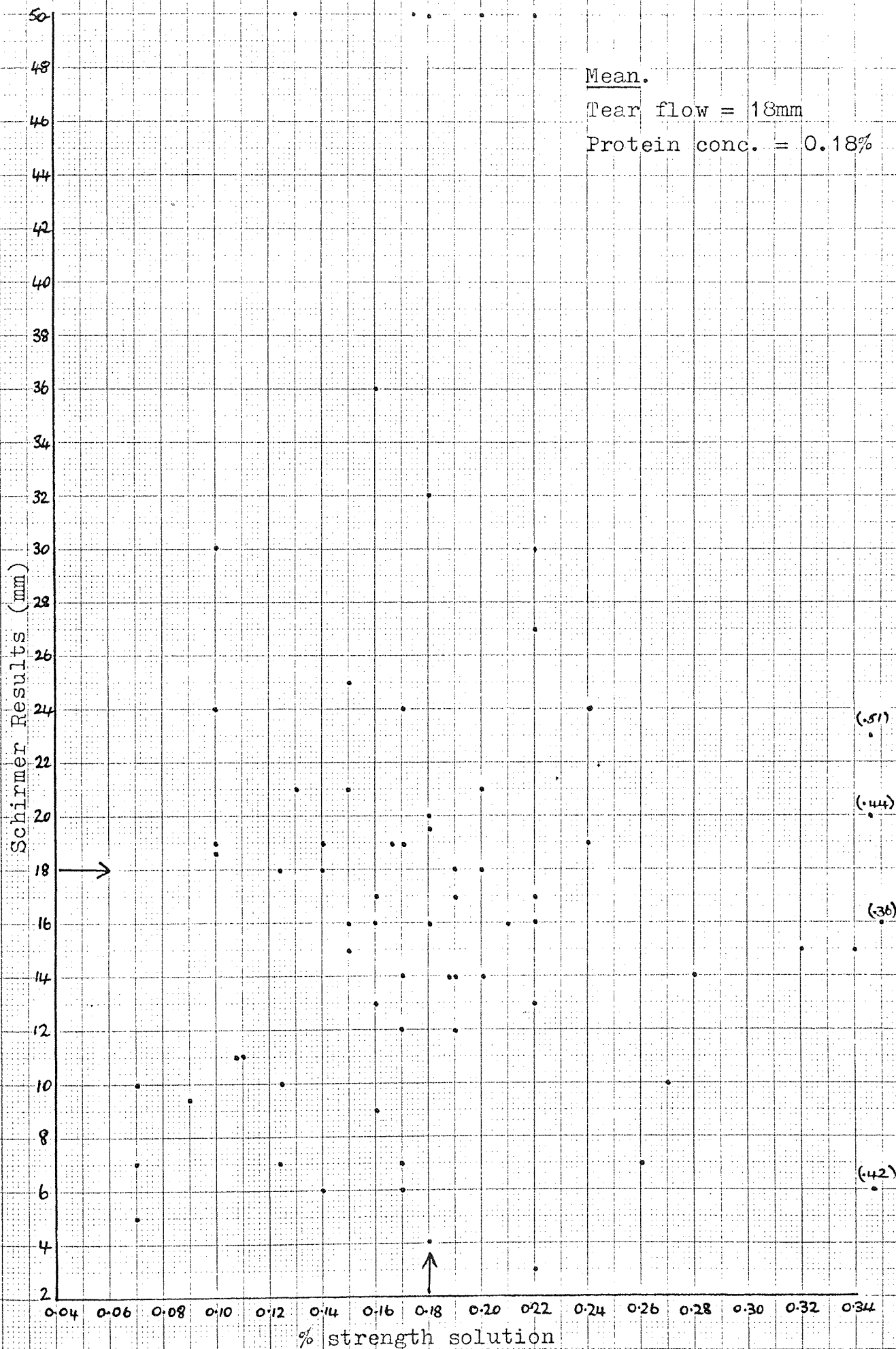
Figure 18. Group 4, Albumin (FractionVII-IX). Control period.

Mean.
Tear flow = 28mm
Protein conc. = 0.29%
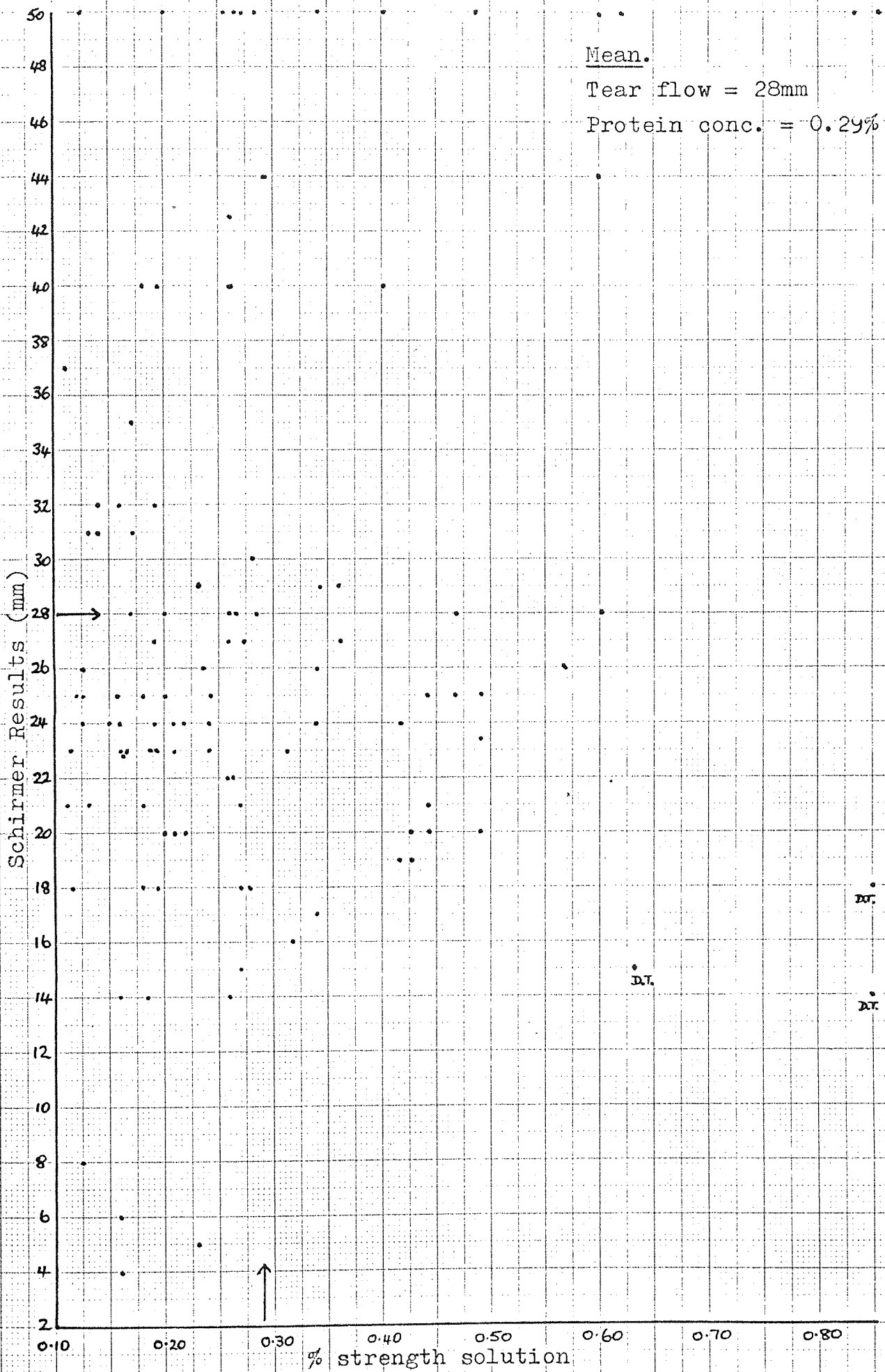
Figure 19. Group 4, Albumin (Fraction VII-IX). Lens adaptation period.
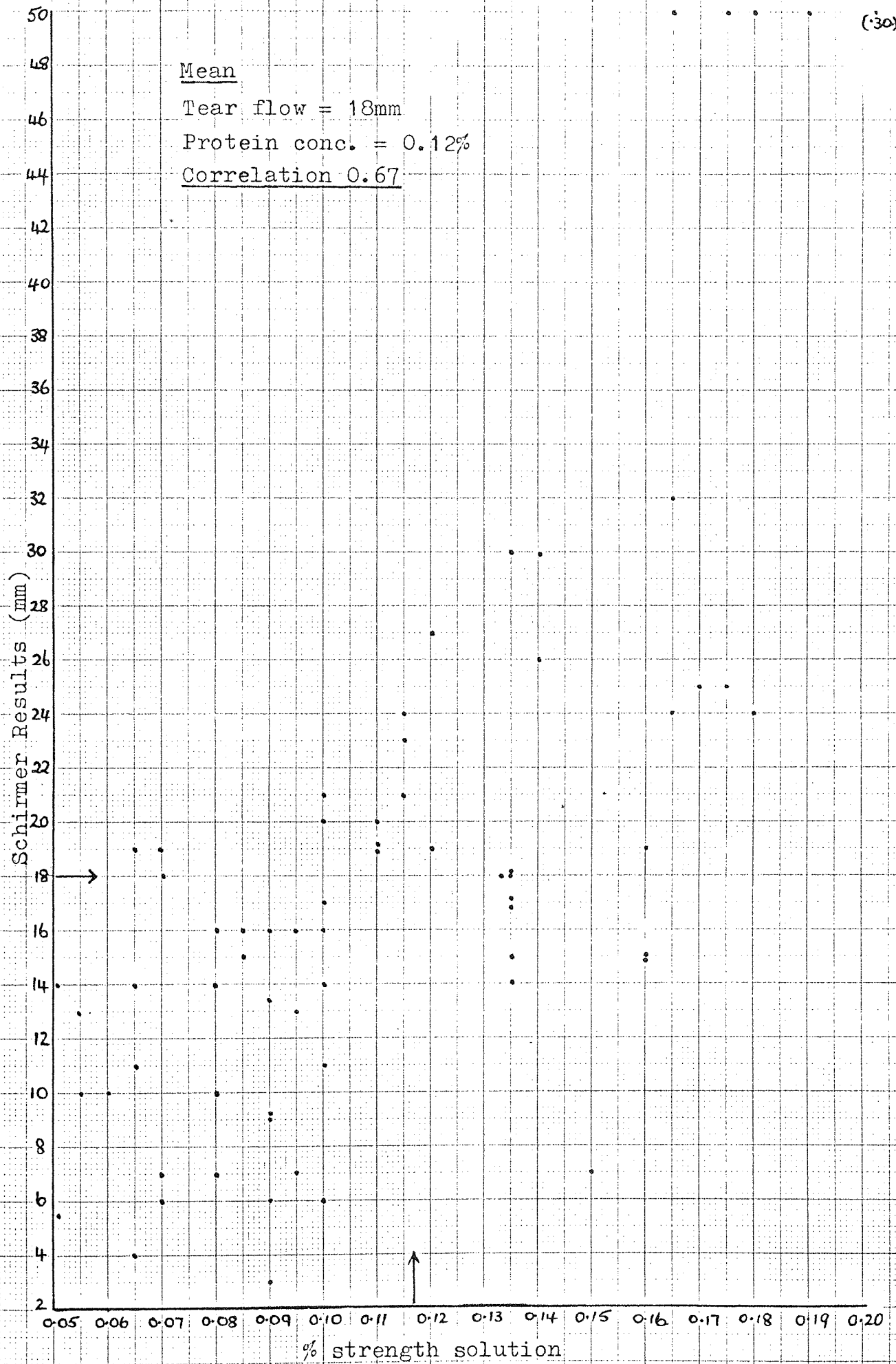
Figure 20. Lysozyme. Control period.
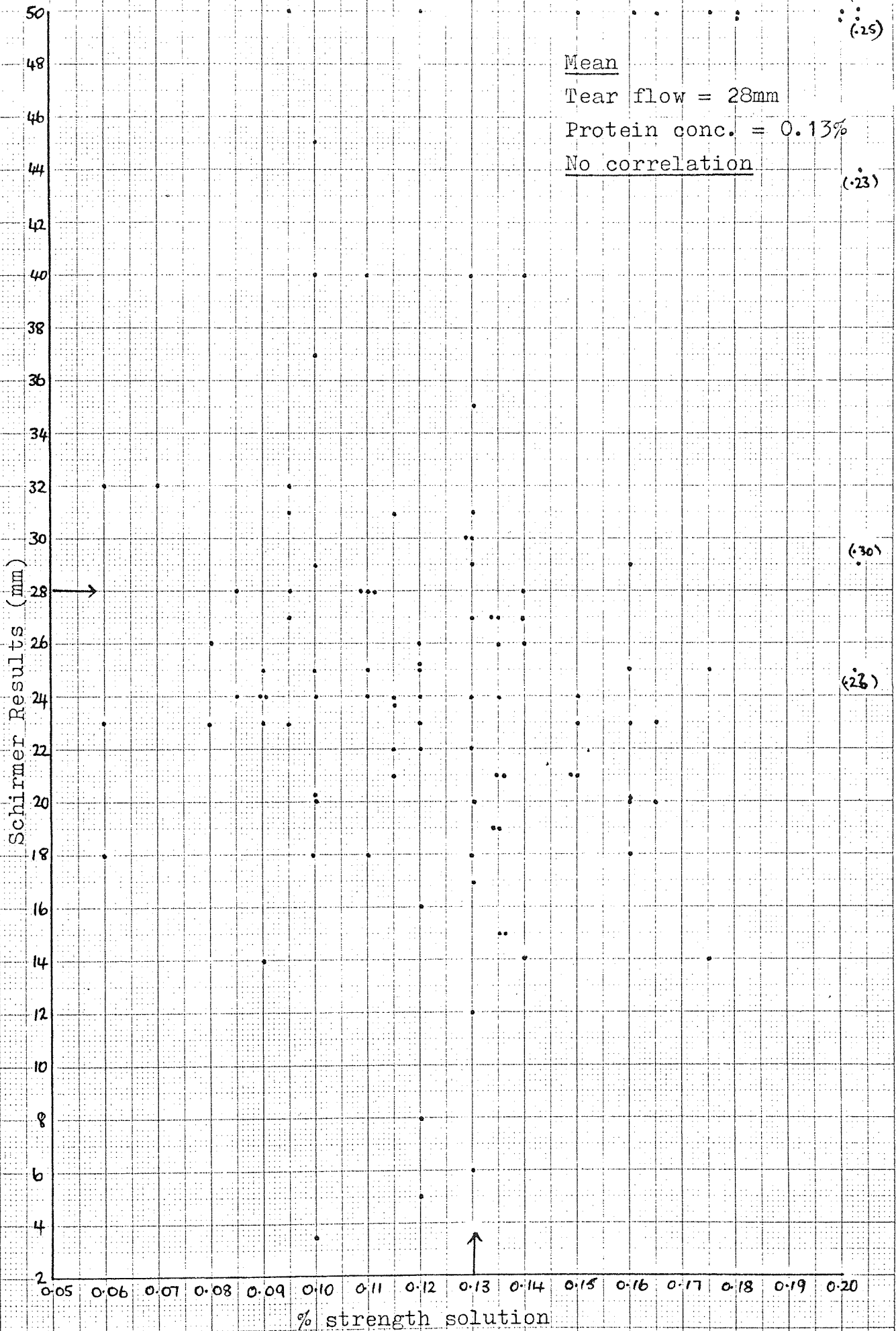
Figure 21.   Lysozyme.   Lens adaptation period.
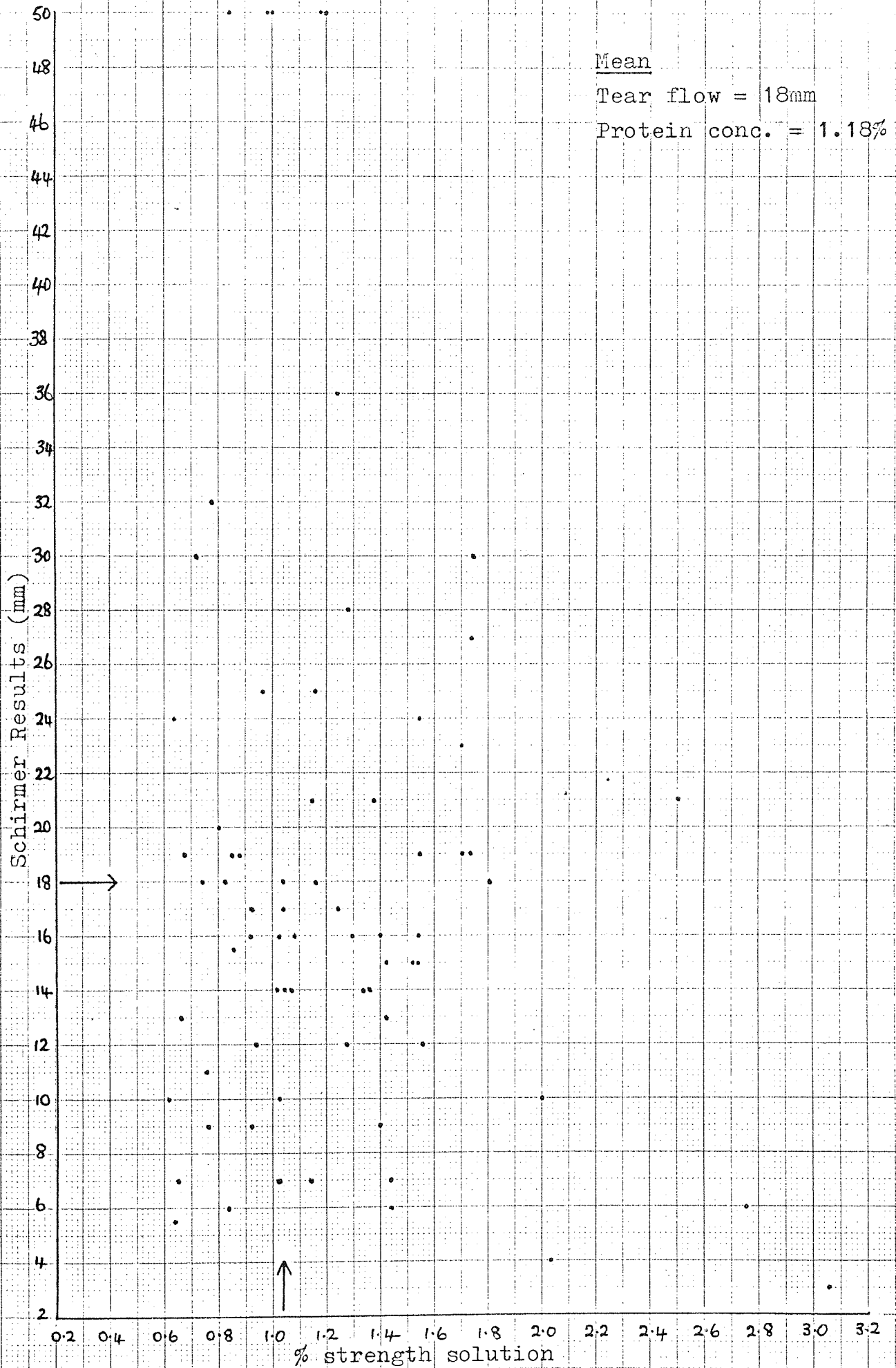
Figure 22. Total protein concentration. Control period.
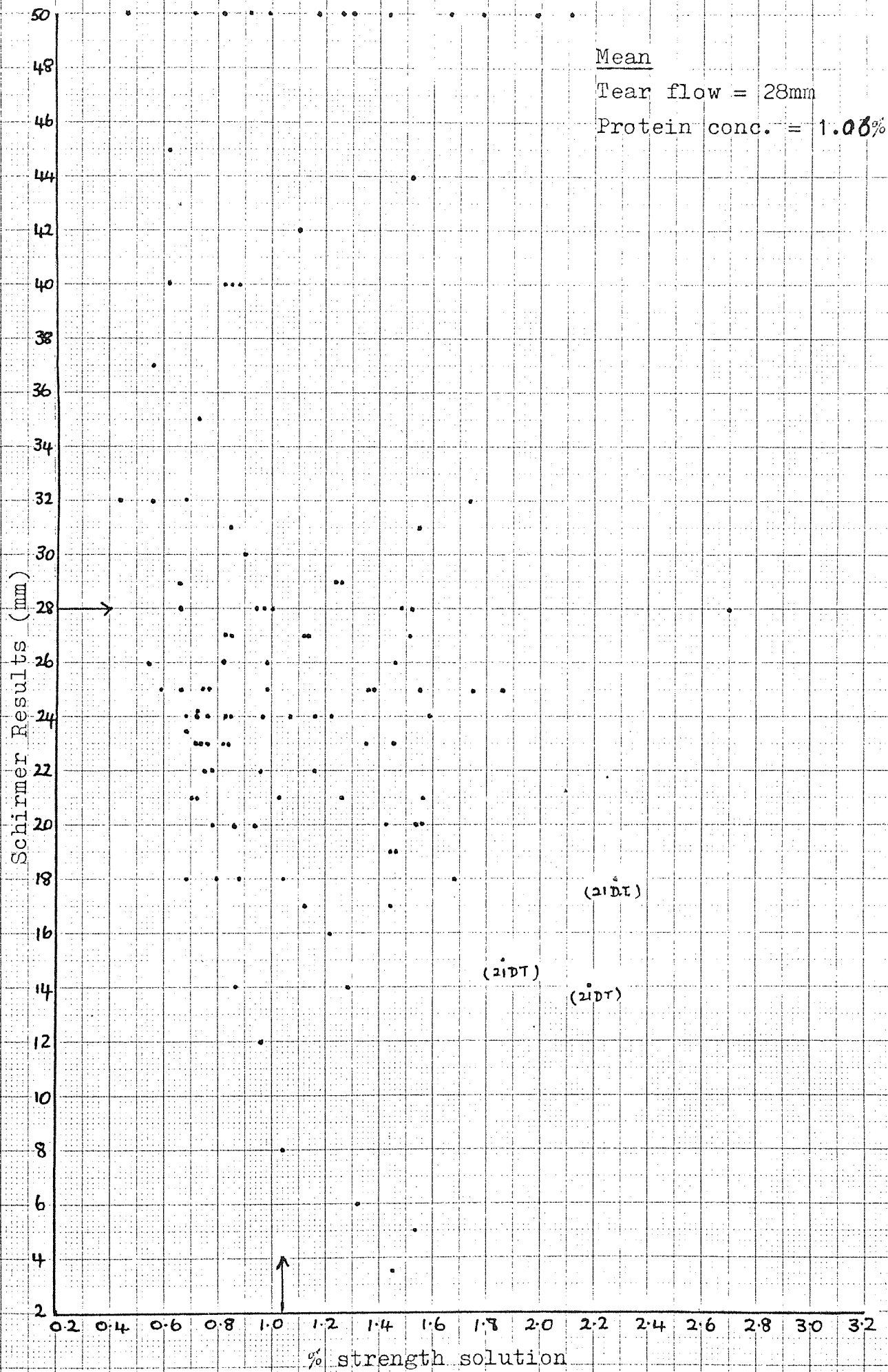
Figure 23. Total protein concentration. Lens adaptation period.

(ii) <u>Protein levels under the Influence of Medication.</u>

The electrophoretic results relating to the effects
of medication were obtained three months before those
supplying information about the basic protein levels. The
number of results available for each person varies, but is
never more than five. This is again due to the late
availability of the Chromoscan.

Comparison of results with those of the non-medication
period must therefore be considered on a very general level
because of the distortions, discussed previously, which may
be introduced into a very small sample by one abnormal
reading.

When the results of each medication group were considered
there was again found to be no correlation between tear volume
and the concentration of the albumin or globulin type
proteins. The positive correlation of lysozyme however,
was confirmed in all three groups.

At an individual level, the average concentration of
each protein group has been calculated for each subject during
both the medication and non-medication period. The difference
between these two mean concentrations is shown in table 4,
represented in terms of the % change in concentration during
the medication period compared to the basic control period.
A + sign indicates an increased concentration during the
medication period.

There is considerable variation in the results of all
three groups, as may be expected from results often averaged
from only 2 or 3 samples in which variation of tear volume

may cause considerable related protein differences.

Inspection of the control group results indicates
that the group 1 proteins are the most variable, and
lysozyme the most consistent fraction of tear fluid.
With the striking exception of subject 2EC it would appear
that a variation of $\pm$ 25% may be accounted for by normal
physiological variation on the part of the person, or
experimental method variation, e.g. power supply current
variation during electrophoretic separation or variable
age of amido black stain.

Variation greater than 25% therefore, outside the
range present in the control group, may be indicative of
definite alteration in the composition of the tear fluid.
In view of the limited number of results available in this
study however, an apparently large protein change has only
been considered to be of significance when inspection of
the original traces indicates that the alteration is
derived from all results and is not being unduly influenced
by one atypical result.

Control Group.

Within the six subjects comprising the control group,
only protein fractions I-III (i.e.group 1) and all results
for subject 2EC fall outside the $\pm$ 25% alteration level.
The reason for the altered results of subject 2EC is
uncertain. They cannot be attributed to the vitamin C
control medication. A possible reason may be that the
3 tear samples which provide the data for the non-medication
period are abnormally high for this subject. This subject

-

Table 4. Percentage change in protein concentration during medication period compared with the non-medication (Control) period.

Control Group

Protein Group	1HG	2EC	3KS	4AF	5HG	6BS	7JR	
1	+37%	+181%	+29%	+56%	+29%	–	Insuff. Data	
2	-16%	+92%	-17%	-20%	-12%	+15%		
3	-18%	+117%	–	-4%	+21%	-28%		
4	–	+55%	-9%	-12%	-24%	+36%		
L	-14%	+56%	-20%	-23%	-7%	–		
Total	-11%	+113%	-6%	-2%	-5%	+4%		

Progestogen Group

Protein Group	8JH	9PK	10RA	11CN	12SL	13WD	14JC	15AMcN
1	+100%	+92%	-5%	+18%	+17%	-36%	+5%	+83%
2	+37%	+73%	+17%	+20%	+20%	-28%	-20%	+54%
3	+51%	+22%	-29%	+22%	+30%	-40%	–	-51%
4	+28%	+70%	+13%	+36%	–	-13%	+3%	+32%
L	-4%	-8%	+7%	-28%	–	+16%	-4%	-37%
Total	+42%	+64%	-2%	+13%	+17%	-30%	-2%	+13%

Oestrogen Group

Protein Group	16MH	17WP	18GS	19VH	20AMcL	21DT	22YF	23CM
1	+184%	-29%	+6%	+20%	Eye Infection	Insuff. Data	-6%	+64%
2	+59%	-46%	+3%	+7%			-12%	-22%
3	+142%	-68%	-61%	+39%			+28%	+30%
4	+42%	+9%	+15%	+55%			-6%	-23%
L	–	-20%	-3%	+12%			–	-61%
Total	+90%	-44%	-6%	+21%			-2%	-12%

+ sign represents increased concentration during medication.

has a low tear volume level which, as has been indicated,
tends to be related to disproportionately high globulin
concentrations. The average difference of 4 mm (from
7 to 11 mm) which is present between the two results series
is likely to move the resultant globulin and total protein
concentrations out of the excessively high levels associated
with the dry 6 and 7 mm Schirmer results, into more average
concentrations associated with the reasonably moist 11-12 mm
readings.

The effect of such a fluid volume change is likely to
be much more pronounced in a dry eye, since it is proportion-
ately greater compared to the basic fluid volume, than a
similar numerical alteration would be in a moist eye.

The fluid alteration may therefore account for the large
discrepancy in protein concentrations between the two results
series.

Despite the variation in the five protein groups, the
total protein concentration in all other control subjects
is remarkably consistent.

Within the oestrogen and progestogen groups, protein
variation is considerably greater than among control subjects.

Progestogen Group.

Within this group of 8 subjects, lysozyme demonstrates
the overall reduction in concentration (in 6 patients)
which would be expected in view of the correlation of this
enzyme concentration with tear volume and the significant
decrease in Schirmer reading induced during the medication
period. The fact that the correlation is not perfect is

probably sufficient to explain the anomalous response of subjects 10 and 13.

Excluding the lysozyme faction, all other proteins and the total protein show an increased concentration during the medication period in five of the eight subjects. Two subjects demonstrate a mixed response and one (13WD) a definite reduction in protein concentration. The reason for this one dissimilar result is unknown. It cannot be related to a change in tear volume, and the patient was not apparently affected by the medication in any other more general way.

Oestrogen.

Within the oestrogen medication group, 2 patients, 16MH and 17WP, show a significant change over all protein groups except lysozyme. The change in these two patients is in opposite directions however, and this cannot be accounted for by a change in tear volume. No explanation of the difference can be suggested, other than the differing individual metabolic response to the medication. Patient 16 reacted badly to the oestrogen, feeling unwell and depressed, while patient 17 was quite unaffected by the treatment.

Other subjects within this medication group show a varied response, with protein group 3 being most affected. Two subjects, 18 and 22, show only minor alteration in all except group 3 protein, but the change in this band is again in opposite directions. Patient 19, who later experienced severe greasing of her contact lenses shortly after starting medication, shows an overall increase in protein concentration.

The remaining subject, 23CM, shows a mixed response at a fairly high level, although the total protein is reduced by only 12%.

Discussion.

The progestogen group shows a greater reaction to the medication than the oestrogen group, although as the control group illustrates some variation is inherent between the two small results samples taken three months apart.

Nevertheless, the greater scale and consistency of the reaction within the progestogen group suggests a definite medication-induced effect. This greater lacrimal fluid protein concentration during medication affects both the albumin and globulin-type factions. It is unlikely that the change is only due to the relatively decreased tear volume during the medication period, since all subjects except one in this group have normal or above normal Schirmer readings which do not directly correlate with albumin or globulin concentrations. The one subject, 14JC, with basically very low tear secretion of high protein concentration was the least affected by the medication.

This increased protein concentration may be provisionally explained by analogy with similar structures elsewhere in the body, for which steroidal hormone effects are known (see Chapter 5).

It has been found that 'Enovid' a combined oral contraceptive containing norethynodrel 5 mg. and mestranol 0.075 mg, suppresses sebum production and improves acne.[107,108]   It

is felt that oestrogen is responsible for the change, but
the authors indicate that the change is generally not evident
until the 3rd or 4th cycles of use. Progestational compounds
are considered to stimulate sebaceous secretion.[101,122]

These results concerning sebum production relate only
to possible effects on the Meibomian glands, which are
sebaceous glands. On the basis of the 'Enovid' findings,
a reduction in some of the globulin concentrations in the
oestrogen group and a corresponding rise in the progestogen
group might have been anticipated. No protein group
demonstrates this type of variation which may be due to
the limited time, approximately 8 weeks, for which the
medication was used, or to the fact that the predominantly
lipid nature of the Meibomian gland secretion is preventing
its accurate detection by the electrophoretic technique
used.

The conjunctival goblet cells form part of a typical
secretory mucus membrane similar histologically to the
cervical mucus of the reproductive tract. The effects of
contraceptive steroids on the secretion of the cervical
mucus have been extensively studied.[10,14]   Oestrogenic
compounds have little effect on the normal, low viscosity,
thin secretion. Progestational agents, however, induce a
thick, viscous secretion with a higher albumin and protein
content.

The electrophoretic results for tear fluid indicate
that a similar alteration in the nature of the mucus
secretion is occurring within the eye during progestin

medication. It also suggests the reason for the absence
of any regular effect on the oestrogen sample group.

(iii) Protein levels during the initial 4 week contact lens adaptation period.

Six sets of results were obtained for each subject during the initial 4 weeks of contact lens wear. In this period all day wear was achieved by the majority of subjects.

Results were plotted as a scattergraph related to Schirmer volume readings as had been done for the basic control period of the project. Comparison of the same protein groups under the two conditions illustrate the basic changes occurring during the adaptation period.

The average tear secretion level is higher, as may be expected, during the lens adaptation period. The mean Schirmer reading increased from 18 mm to 28 mm.

Lysozyme Levels.

The correlation between tear volume and enzyme concentration breaks down under the reflex lacrimation and irritant conditions of lens wear as may be seen by the different form of the scattergraph (fig.20 & 21).

The mean concentration of lysozyme is however slightly higher during the lens wearing period and this is probably a reflection of the higher concentration normally associated with higher tear volume levels.

Albumin.

The mean level of albumin concentration shows a considerable increase in the lens wearing period, from 0.18% to 0.29%. This alteration is most marked during the 3rd and 4th week of the adaptation period. It is unlikely to be a result of an alteration of the processing

treatment applied, since not all samples taken on the same
day show an increased albumin concentration. Also, traces
showing high albumin concentrations do not always demon-
strate the equally elevated levels of other proteins which
would be expected if the effect were due to a different
staining reaction.

Inspection of the results of the control medication
group suggest that not all subjects demonstrate elevated
albumin levels, but that where they do occur, the rise is
maintained for the next two months of wear at least. After
this there is a suggestion that the protein level may start
to fall again, see table 7, p.180.

The reasons for, and mechanism of, this protein rise
are unknown. It is possible that it might be a direct
response to irritation, and in this context it is relevant
to mention the results of subject 20. Before being supplied
with lenses she developed a pronounced swelling of the right
upper lid which was diagnosed as a herpetic infection. The
eye itself remained white and was not involved, although
there was profuse lacrimation. Electrophoresis of the
tear fluid during the course of this infection showed an
abnormally high albumin concentration, well in excess of
any other results at any stage for this patient. Lysozyme
was also slightly elevated, although this may be partially
accounted for by the increased tear volume. Globulin factions
did not fall outside the normal range for this subject.

Globulins

The three groups of globulin-type proteins react rather differently to the presence of the contact lenses. Group 2 proteins (scattergraph fig.14,15) show a slightly reduced concentration generally, the mean concentration being reduced from 0.38% solution to 0.32% solution. There is also a loss of the very high protein concentrations above 0.6% and conversely an increase in the number of low concentration values below 0.2% strength.

Group 1 and 3 proteins however show a very much greater reduction during the initial adaptation period, group 1 proteins showing a reduction of approximately 30% and group 3 a reduction of 50%. Group 3 protein (i.e. band VI) is occasionally totally absent.

Although no definite overall correlation between the globulins and tear volume could be established, the possibility of a greater individual relationship between the two factors cannot be disregarded. It is likely therefore that the alteration in the group 2 protein may relate purely to the increased tear secretion. Some proportion of the reduced concentration of the other proteins may also be accounted for on this basis. It is possible in fact that the entire 50% band VI reduction is attributable to the volume change, although an additional mechanism is possible.

Band VI is felt to originate from the lid margin or palpebral conjunctiva. A contact lens might be expected to stimulate the lid margins and produce an excess secretion from the affected glands. This does not occur, and it seems likely that the secretion actually diminishes.

A tentative explanation for this may be advanced.
There is some suggestion, when the results for band VI,
group 3, protein are examined, that after the initial drop
in concentration associated with early lens wear, the protein
continues to decrease in the second half of the adaptation
month. This tendency is not found in all patients, but
where present it cannot be explained on the basis of tear
volume, since this is starting to decline as adaptation
improves.

Neural sensibility of the lower lid margin is reduced,
due to continued physical contact with the lens, as
adaptation proceeds.[61] Sensory supply to the lower lid is
predominantly from the infra-orbital nerve (a branch of the
maxillary division of the trigeminal nerve) and to a lesser
extent from the lacrimal nerve (a branch of the ophthalmic
division). Motor supply from the facial and oculomotor
nerves controls the lid muscles, and an autonomic supply
is present to the involuntary muscles, the vessels and the
glands of the skin (p.533).[123] The autonomic system is
known to innervate the Meibomian glands, but detail of
innervation by the central nervous system of these or other
conjunctival or lid glands is vague.

If, however, there were some central innervation
involved in the secretion of protein band VI, then a
reduction in overall sensibility induced by the presence
of the contact lens could also result in a reduced stimulus
to glandular secretion, which would accord with the electro-
phoretic results obtained.

Summary.

The average total protein decreased slightly during this initial 4 week adaptation period, from 1.18% to 1.06% (scattergraph fig.22 & 23). The increased concentration of lysozyme and albumin tends to offset the reduced globulin concentrations, giving rise therefore to a solution containing a similar amount of protein, but of rather different balance.

(v) Protein levels during lens wear with medication.

After 4 weeks lens adaptation, when most subjects had
achieved all day wear, the hormone or control medication
was restarted for 8 weeks.

Control Group.

The results for this group provide an indication of
continuing changes to be expected, purely on the basis of
continuing adaptation to contact lenses once all day wear
has been achieved.

The tabulated results (table 5) again show the
difference, in terms of percentage change, between the
mean protein concentration of each group during the initial
4 week adaptation period and the following 9 weeks estab-
lished wear. For comparison purposes the initial adaptation
period has been taken as the reference level.

As may be seen, considerable alteration occurred in
the tear protein pattern during continued adaptation. The
tear albumin concentration continued to rise, while the
lysozyme concentration steadied or started to fall.
Globulin levels, which had been relatively low during the
early adaptation period, all show a rise in concentration
as lens wear becomes better established.

Reference to summary table 7 shows that by this stage
of wear, group 1 and 2 proteins (i.e. gel bands I - V)
have become re-established at concentration levels equal to,
or above, those which were normal for that individual prior
to lens wear.

The generally increasing globulin and albumin and decreasing lysozyme concentrations may be explained on the basis of tear volume alteration with increasing adaptation.

Tear secretion variation however, does not adequately explain the continuing high levels of tear albumin and low levels of the band VI, α-globulin.

The total protein level during this stage of lens wear is slightly higher than pre-fitting levels.

Oestrogen Group.

Three of the five patients for whom results are available, show an increase in concentration of all proteins during the medication period. Two of these (6 and 18) demonstrate changes of similar scale to those of the control group and which bears a similar relationship to their pre-fitting protein levels. The third patient, 19VH, had protein increases at a much higher level, outside the control group range. These results were obtained within the first 3 weeks of medication, by which time she was experiencing severe greasing of the contact lenses which made wear impossible. During this 3 weeks the protein concentration doubled, so medication was stopped in the hope that the greasing would clear itself. This did not happen during the next 6 weeks, although by the end of this time the concentration was reducing towards more normal levels and after a further 5 weeks, tolerance trials with large tetracurve lenses produced no problems. The protein concentration with lenses in place was then normal for this patient (see p.236, Appendix 2)

The remaining two subjects within this group, 17WP and 20AL, show a more mixed result during the medication period. They both have a continuing reduction of group 3 protein, with group 1 or 2 also reduced. The total protein concentration is similar (17WP) or reduced (20AL) in the medication period compared to the initial lens wearing period.

Progestogen Group.

In view of the finding of increased protein concentration in the progestogen group during the preliminary medication period, it was expected that protein values would show a steep rise in concentration during this wearing period. This did not occur, and the amount of protein increase over the 8 week medication period as a whole is less than that which occurred in the unmedicated control group during the same period.

Because of the University Christmas vacation, results are only actually available for the first 2-3 weeks and last 2 weeks of the medication period and the 2 weeks immediately following the end of medication.

Since medication effects do not cease abruptly when the actual drug use stops, it seems reasonable to include the first week after the end of medication, with the final 2 weeks results when making comparison with the control group. If then the initial and final medication periods are separately compared with the corresponding control group findings, a somewhat different pattern emerges (see table 6). The change in total protein concentration is shown separately for the two periods, compared with the

initial 4 week adaptation time.

This confirms that the control group proteins are undergoing a steady increase in concentration related to increasing adaptation and reducing tear flow.

The pattern is similar in the oestrogen group. In the progestogen group it is quite different. The total protein concentration drops in 4 of the 6 subjects during early medication. By the time the norestisterone acetate has been taken for 6-8 weeks however, this effect has been reversed and the amount of concentration increase between these two periods is then very similar in all three treatment groups.

It is this initial reduction in concentration in the progestogen group which gives the effect of a much lower level of protein generally when all medication data are taken together as in table 5.

General Discussion.

At the end of three months lens wear, protein concentrations with the exception of the very elevated albumin were similar to, or slightly above, pre-fitting levels in the control group. Oestrogen treated subjects were, broadly speaking, similar to the control group, although subject 19VH was a marked exception to this. An additional result is available for some patients after a further month. This does not suggest that the increasing protein trend continues in the control group and although there is insufficient information to definitely confirm this, it seems likely that the protein levels would stabilise approximately at their pre-fitting levels.

The position of progestogen however is far from clear.
The preliminary medication period prior to the supply of
contact lenses indicated that progestogen increased protein
concentration. It is significant however that these results
were obtained after 9 or 10 weeks of hormone use. The
initial ocular response, as previously explained, could
not be monitored. It is quite possible therefore that the
hormone effect is not constant, but gradually builds up -
as is found to be the case with 'Enovid' - induced effects
on sebum production.[197,108] This difference in the time
at which samples were taken may explain the apparent
discrepancy between the results of the two medication
periods, i.e. if the assumption is made that the protein
concentration would have continued to increase in the
progestogen-treated group had medication continued, then
the discrepancy may be resolved. It cannot be proved,
however, although it is relevant to point out that the
contraceptive effect of the orally active steroids is not
established until the second cycle of use, and that the
specific action of progestogen is directed towards the
cervical mucus.

The reason for the initial reduction in protein is
unknown. It cannot be explained by analogy with other
general physiological effects. It is possible that the
sudden introduction of progestogen is suppressing or
activating some other substance concerned with the activity
of the accessory glands - as is the case with this hormones
effect on the aldosterone system for example[28]- but until
the mechanism of secretion of the conjunctival glands is
known, the precise answer cannot be established.

Table 5. Percentage change in concentration during lens wear with medication compared with initial 4 week lens adaptation period.

Control Group Subjects

Protein Group	1HG	2EC	3KS	4AF	5HG	7JR		
1	+10%	+29%	+88%	+52%	−11%	+122%		
2	+26%	+42%	+68%	+80%	+3%	+25%		
3	−20%	+22%	+24%	+101%	+89%	+121%		
4	−1%	+30%	+46%	+41%	−7%	+27%		
L	−70%	+14%	+17%	−21%	−23%	−		
Total	+5%	+32%	+56%	+39%	−	+46%		

Progestogen Group Subjects

Protein Group	8JH	9PK	10RA	11CN	12SL	13WD	14JC	15AMcN
1	+45%	Lens Problems	+5%	−4%	+56%	Failed to take tablets.	+28%	+3%
2	−		+12%	+26%	+44%		+35%	−8%
3	−18%		+60%	−50%	+21%		−2%	−56%
4	+3%		−13%	+20%	+16%		+50%	−3%
L	−17%		−2%	+18%	+4%		+9%	+9%
Total	−		−3%	+1%	+30%		+23%	−4%

Oestrogen Group Subjects

Protein Group	16MH	17WP	18GS	19VH	20AMcL	6BS		
1	Lens Problems	−41%	−5%	+177%	+3%	+10%		
2		+7%	+36%	+122%	−22%	+63%		
3		−79%	+4%	+90%	−42%	+63%		
4		+37%	+64%	+50%	+3%	+71%		
L		+16%	+55%	+20%	−12%	+21%		
Total		+3%	+31%	+100%	−12%	+39%		

Table 6. Percentage change in total protein concentration
between preliminary lens adaptation period and
the beginning and end of the medication period.

Control Group

| Patient | Percentage change compared with 4 week adaptation period. | | |
	Initial Period	Final Period	Change
1	-18%	+24%	+42%
2	+185%	+27%	-158%
3	+40%	+74%	+34%
4	+38%	+57%	+19%
7	+30%	+65%	+35%

Progestogen Group

8	-13%	+26%	+39%
10	-11%	+1%	+12%
11	-22%	+16%	+38%
12	+20%	+39%	+19%
14	+6%	+23%	+17%
15	-35%	+20%	+55%

Oestrogen Group

17	-30%	+13%	+43%
18	+20%	+39%	+19%
19	+100%	Not wearing lenses	
20	+2%	-15%	-17%
		(discomfort)	
6	+32%	+45%	+13%

Table 7. Individual progression of protein concentration
changes, expressed in terms of percentage solution.

All figures = Mean of all available results for
that section.

Protein Group		Basic Level	With Medication	C.L.Adapt. Period	Estab.C.L. Wear + Medication
	1	0.160%	0.22%	0.184%	0.202%
	2	0.436%	0.366%	0.406%	0.511%
1HG	3	0.355%	0.276%	0.202%	0.161%
	4	0.257%	0.250%	0.450%	0.444%
	L	0.14%	0.120%	0.154%	0.143%
Total		1.378%	1.22%	1.392%	1.460%
	1	0.16%	0.45%	0.137%	0.177%
	2	0.25%	0.48%	0.287%	0.407%
2EC	3	0.24%	0.52%	0.134%	0.164%
	4	0.127%	0.20%	0.174%	0.228%
	L	0.053%	0.081%	0.105%	0.120%
Total		0.833%	1.78%	0.831%	1.096%
	1	0.17%	0.22%	0.120%	0.225%
	2	0.40%	0.33%	0.235%	0.396%
3KS	3	0.23%	0.23%	0.125%	0.155%
	4	0.176%	0.16%	0.173%	0.253%
	L	0.09%	0.076%	0.083%	0.097%
Total		1.026%	0.97%	0.728%	1.146%
	1	0.25%	0.39%	0.142%	0.216%
	2	0.50%	0.40%	0.288%	0.517%
4AF	3	0.35%	0.335%	0.122%	0.246%
	4	0.16%	0.178%	0.212%	0.299%
	L	0.133%	0.10%	0.166%	0.131%
Total		1.398%	1.392%	0.929%	1.392%
	1	0.17%	0.225%	0.196%	0.175%
	2	0.325%	0.28%	0.324%	0.333%
5HG	3	0.24%	0.29%	0.074%	0.140%
	4	0.27%	0.205%	0.432%	0.400%
	L	0.14%	0.130%	0.151%	0.116%
Total		1.155%	1.091%	1.169%	1.164%

Table 7 (continued)

Protein Group	Basic Level		With Medication	C.L.Adapt. Period		Estab.C.L. Wear + Medication
6BS 1	0.23%	Control	0.23%	0.118%	Oestrogen	0.130%
2	0.34%		0.39%	0.166%		0.270%
3	0.25%		0.18%	0.046%		0.075%
4	0.22%		0.30%	0.194%		0.332%
L	0.10%		0.10%	0.094%		0.114%
Total	1.158%		1.202%	0.660%		0.920%
7JR 1		No	0.293%	0.137%		0.302%
2		Results	0.520%	0.477%		0.596%
3			0.406%	0.173%		0.385%
4			0.320%	0.392%		0.496%
L			0.076%	0.141%		0.138%
Total			1.616%	1.318%		1.918%
Progestrogen Group						
8JH 1	0.16%		0.32%	0.117%		0.170%
2	0.30%		0.412%	0.358%		0.356%
3	0.257%		0.39%	0.096%		0.079%
4	0.18%		0.23%	0.540%		0.557%
L	0.13%		0.125%	0.201%		0.166%
Total	1.035%		1.468%	1.311%		1.327%
9PK 1	0.13%		0.25%			
2	0.26%		0.45%			
3	0.22%		0.38%			
4	0.17%		0.29%			
L	0.12%		0.11%			
Total	0.902%		1.48%			
10 RA 1	0.19%		0.18%	0.125%		0.131%
2	0.435%		0.51%	0.377%		0.423%
3	0.35%		0.24%	0.087%		0.139%
4	0.195%		0.22%	0.457%		0.398%
L	0.14%		0.15%	0.175%		0.172%
Total	1.262%		1.28%	1.220%		1.184%

Table 7 (continued)

Protein Group		Basic Level	With Medication	C.L.Adapt. Period	Estab.C.L. Wear + Medication
11 CN	1	0.17%	0.20%	0.160%	0.154%
	2	0.296%	0.355%	0.380%	0.477%
	3	0.27%	0.33%	0.198%	0.097%
	4	0.126%	0.17%	0.322%	0.287%
	L	0.14%	0.10%	0.128%	0.151%
Total		1.017%	1.152%	1.190%	1.197%
12 SL	1	0.265%	0.310%	0.163%	0.255%
	2	0.375%	0.450%	0.355%	0.510%
	3	0.285%	0.370%	0.150%	0.181%
	4	0.170%	0.170%	0.233%	0.270%
	L	0.107%	0.100%	0.123%	0.128%
Total		1.203%	1.403%	1.025%	1.332%
13 WD	1	0.335%	0.210%	0.123%	Failed to take medication
	2	0.420%	0.300%	0.233%	
	3	0.610%	0.360%	0.152%	
	4	0.230%	0.207%	0.321%	
	L	0.060%	0.077%	0.106%	
Total		1.648%	1.143%	0.937%	
14 JC	1	0.370%	0.392%	0.236%	0.301%
	2	0.450%	0.370%	0.416%	0.561%
	3	0.700%	0.705%	0.370%	0.363%
	4	0.160%	0.165%	0.174%	0.261%
	L	0.077%	0.074%	0.129%	0.141%
Total		1.745	1.703	1.324	1.627
15 AMcN	1	0.145%	0.265%	0.153%	0.158%
	2	0.325%	0.500%	0.375%	0.346%
	3	0.410%	0.200%	0.206%	0.090%
	4	0.170%	0.225%	0.224%	0.218%
	L	0.190%	0.117%	0.155%	0.169%
Total		1.151%	1.305%	1.111%	0.963%
Oestrogen Group					
16 MH	1	0.130%	0.378%	0.190%	Lenses dis continued Central Erosions
	2	0.27%	0.430%	0.300%	
	3	0.175%	0.430%	0.200%	
	4	0.19%	0.270%	0.202%	
	L	0.094%	0.100%	0.128%	
Total		0.358%	1.633%	1.020%	

Table 7 (continued)

Protein Group		Basic Level	With Medication	C.L.Adapt. Period	Estab.C.L. Wear + Medication
17 WP	1	0.31%	0.220%	0.216%	0.127%
	2	0.71%	0.380%	0.414%	0.442%
	3	0.63%	0.193%	0.358%	0.075%
	4	0.21%	0.230%	0.358%	0.490%
	L	0.123%	0.097%	0.125%	0.145%
Total		1.995%	1.118%	1.450%	1.494%
18 GS	1	0.160%	0.170%	0.168%	0.177%
	2	0.300%	0.310%	0.280%	0.380%
	3	0.155%	0.060%	0.070%	0.073%
	4	0.132%	0.150%	0.192%	0.313%
	L	0.150%	0.145%	0.125%	0.194%
Total		0.865%	0.837%	0.866%	1.131%
19 VH	1	0.176%	0.210%	0.132%	0.366%
	2	0.290%	0.310%	0.202%	0.450%
	3	0.153%	0.215%	0.038%	0.168%
	4	0.090%	0.140%	0.129%	0.194%
	L	0.083%	0.095%	0.107%	0.128%
Total		0.797%	0.967%	0.653%	1.303%
20 AMcL	1	Eye Injection No Results	0.276%	0.218%	0.224%
	2		0.522%	0.528%	0.411%
	3		0.206%	0.200%	0.116%
	4		0.276%	0.382%	0.395%
	L		0.091%	0.153%	0.134%
Total			1.375%	1.489%	1.303%
21 DF	1	0.313%	0.180%	0.246%	0.231%
	2	0.613%	0.393%	0.486%	0.775%
	3	0.403%	0.236%	0.224%	0.138%
	4	0.456%	0.213%	0.584%	0.628%
	L	0.098%	0.102%	0.133%	0.145%
Total		1.885%	1.1125%	1.664%	2.240%

(Oestrogen — With Medication column, group 21; Control — Estab.C.L. column, group 21)

Table 7 (continued)

Protein Group		Basic Level	With Medication	C.L.Adapt. Period	Estab.C.L. Wear + Medication
22 YF	1	0.170%	0.160%		
	2	0.260%	0.230%		
	3	0.180%	0.230%	Failed to adapt to	
	4	0.170%	0.160%	lenses	
	L	0.080%	0.078%		
Total		0.866%	0.852%		
23 CM	1	0.140%	0.230%		
	2	0.415%	0.330%		
	3	0.200%	0.260%	Failed to adapt to	
	4	0.220%	0.170%	lenses	
	L	0.230%	0.089%		
Total		1.205%	1.065%		

(v) Summary of Electrophoretic Results.

Modification of the acrylamide gel electrophoresis technique for lacrimal fluid has resulted in a considerable improvement in resolving power compared with all other existing techniques. Lacrimal fluid samples taken from the lid portion of a Schirmer strip may be potentially resolved into 10 protein fractions. These have been placed in 5 major groups, and considered quantitatively by reference to standardisation curves of purified serum proteins run under the same experimental conditions.

The average total protein concentration has been found to be 1.18% in the approximate ratio 2/3 globulins: 1/3 Albumin + lysozyme. Under normal, unstimulated conditions, lysozyme concentration correlates moderately well with the Schirmer volume result (correlation coefficient +0.67). Albumin is relatively unaffected by tear volume while the globulins have a general inverse relationship with fluid quantity.

The presence of a corneal lens produces a considerable reduction in globulin concentration and increase in albumin and lysozyme values. With the exception of albumin, all proteins return to near pre-fitting levels after approximately 3 months of lens wear.

There is a wide individual variation in all protein fraction levels as may be seen from the scattergraphs.

The effects of the two steroid hormones norethisterone acetate BP 2.5mg. and ethinylestradiol BP. 0.05mg have been discussed. Oestrogen appears to have little general effect

on lacrimal proteins although there are exceptions where certain individuals appear to be more susceptible to the drug.

Progestogenic effects also show individual variation, but it seems likely that its use leads to increased protein concentration, mainly of the globulin-type fractions.

7. DISCUSSION

(i) Introduction

We have considered the general effect of a corneal lens on an eye, the general effects of the relevant contraceptive steroids on the body and the actual experimental results obtained during the course of this project. It is necessary therefore to relate these three aspects together.

Of the 21 subjects who adapted well or adequately to corneal lenses during the initial 4 week adaptation period, 12 continued to the end of the project without experiencing any difficulties or problems with their lenses. Of the remaining 9 subjects, 2 experienced a typical overwear reaction (5HG, 13WD) but later achieved full wear, one suffered recurrent oedema symptoms finally cured by lens fenestration (9PK) and one wore erratically due to persistent reading problems (10RA). The remaining 5 patients all experienced a partial or total loss of tolerance during the medication period which is difficult to explain except in terms of a medication-induced response.

The overwear reactions both occurred early in the wearing programme, one (5HG) during initial adaptation, and the other (13WD) shortly after all-day wear had been achieved. In this latter case, progestogen medication had been started approximately 4 days before the attack, and although the possibility of a link cannot be completely ruled out, it is thought more likely to be coincidental since a characteristic of overwear attacks is their tendency to occur spontaneously even in well adapted wearers without any obvious provoking cause.

Both these patients were advised to discontinue lens wear until their eyes were quite comfortable again and then to restart the wearing schedule. They did this and readapted fully to their lenses without any further problems.

The 5 subjects who experienced trouble more directly attributable to the medication will be considered in greater detail as an illustration of the proposed mechanism of interference of the contraceptive steroids with the normal cornea-contact lens system. Two of these subjects were in the progestogen group and three in the oestrogen group. No subject in the control group produced symptoms in any way similar to these 6 patients. All control group patients, with the exception of the overwear attack (5HG) already considered, responded normally throughout the project. The remaining subjects in the oestrogen and progestogen groups (3 and 4 respectively) also remained trouble free.

(ii) Case Histories.

(a) Oestrogen treatment group

Patient 16MH

This patient reacted badly to the oestrogen medication on both occasions, becoming rather unsettled and depressed. She had reasonable motivation for lens wear, being dependent on spectacles for close work although not for general vision, and was a reliable project patient on the whole.

The corneal lens fit was a compromise, halfway between the corneal radii due to considerable corneal astigmatism, but patient response was excellent during both tolerance trials and full all-day wear was achieved easily within the 4 week adaptation period.

Objectively, during the medication/non medication period preceding lens supply, this subject had shown an appreciable reduction in Schirmer reading (22.3 to 14.6 mm average) with a definite increase in protein concentration as a result of the oestrogen tablets.

During lens adaptation, there was little reflex lacrimation but very substantial corneal steepening, especially in the right eye, which was however subsiding normally after 4 weeks wear. There was some slight central oedema of the right eye during the adaptation period, but minimal or no corneal staining.

After 2 weeks medication, the right cornea was again abnormally steep and showing mild oedema. Steepening of the left eye remained much less than the right, but again showed mild oedema and very faint central stain. The lens fit by this stage was again flat with excessive vertical edge stand-off as it had been during the early adaptation period. The subjective comfort now became less good although all-day wear was maintained.

During the succeeding 4 weeks of the Christmas vacation, lens wear was maintained, although her eyes were generally uncomfortable. The discomfort was worse when lenses were removed. A routine visit to an ophthalmologist for a spectacle refraction resulted in the advice to discontinue lens wear due to central abrasions on both corneas.

When I saw her approximately 10 days after discontinuing lens wear, both corneas were quite clear and the curvature had reduced to pre-fitting levels in both eyes.

She had been told however by the ophthalmologist that she would probably require different specification lenses, and was consequently quite determined not to try and re-adapt to the existing ones despite accepting that she had had no trouble until shortly after the start of the oestrogen medication, which had by then stopped. In addition, when finally persuaded to try and re-adapt to the existing lenses, any discomfort at all, of the type that had been quite happily ignored when first adapting to lenses, was now interpreted as a return of the corneal abrasion. She therefore stopped wearing the lenses regularly every few days until a slit lamp examination could be carried out.

Satisfactory wear was for these reasons never re-established. The patient was finally refitted with conoid lenses and when last seen had achieved 10 hours per day wear although she was still tending to discontinue wear at the first sign of the slightest discomfort.

This psychological rejection of the original lenses prevented the readaptation and monitoring of re-established wear which would have shown conclusively whether there was a different ocular response without the influence of oestrogen. One cannot therefore say categorically that the development of the corneal damage was a direct result of the oestrogen, since it is quite possible that this type of lens fit, i.e. a spherical lens on a toric cornea, will produce central pressure independently of any other factors.

Nevertheless, it is significant that the type of ocular changes attributable to oestrogen all make the occurrence of this type of response more likely.

Corneal Oedema: One expects to find the greatest corneal steepening occurring during the early stages of adaptation, when the osmotic tear effects and oxygen deprivation effects are maximal and additive. Once adaptation becomes reasonably established, the diminishing osmotic effect and adjustment of the corneal metabolism to the new state of affairs results in progressively less corneal steepening. This process may be seen to occur in this patient up to 4 weeks wear, and would normally be expected to continue, giving less and less likelihood of central corneal lens pressure as the cornea flattened to give a relatively steeper lens fit.

The presence of oestrogen, however, reversed this process at a time when all-day wear although achieved was not really consolidated, thus producing prolonged central pressure on a cornea which was still in a rather unsettled state.

This same situation of a steepened cornea and flat lens however, had caused no problems during the adaptation phase. On the contrary, adaptation had been rapid. The explanation of this difference may well be in changes in the pre-corneal film.

The effect of oestrogen on this subject had been shown to be a reduction in tear volume and a rise in tear protein.

The cushioning and oxygenation properties of the tear film are always of great importance in a corneal lens wearer. It is reasonable to suppose that they might be even more crucial when the lens fit is not as regular or "good" as usual.

Tear volume is elevated during initial adaptation,
it then declines as the foreign-body reaction subsides. In
this patient, therefore, there was a plentiful tear supply
during the period of initial corneal steepness. Allied
however to the second, oestrogen-induced, period of steepness
is a reduction and change in the tear film. It may be
suggested therefore that the cushioning and metabolic
properties of the tears were impaired to the extent that
corneal integrity in the presence of the contact lens could
not be maintained. Aggravation of the already present oedema
would then result in epithelial breakdown and erosion. The
continuing presence of the contact lens would produce a self
perpetuating situation which would be maintained even when
the initial corneal response to the oestrogen itself had
subsided.

Patient 19VH.

This patient was a moderately high myope, completely
dependent on a spectacle correction. She was keen to wear
contact lenses, although slightly apprehensive about the
procedure .

She showed no obvious general physiological response to
the initial oestrogen medication. Schirmer readings were
low, 8 mm, but they did somewhat atypically increase to
approximately 10.6 mm. during the medication period. During
this same time the protein concentration rose by approximately
25%.

All patients in this study were fitted with 8.50 mm
diameter tricurve lenses since this is more universally

acceptable than a large diameter lens. In this patient however, although a good curvature relationship was obtained with the cornea, the lens diameter was smaller than would otherwise have been fitted. This was not felt to prejudice the likelihood of wearing success in any way. Larger lenses were to be fitted at the end of the project only if unacceptable levels of peripheral dryness developed.

Tolerance trials were reasonable, with a tendency to high lacrimation. When lenses were supplied however adaptation and all-day wear was achieved much more easily than had been anticipated. Low levels of oedema and corneal stain were observed and there was moderate corneal steepening initially which was subsiding well by the end of the adaptation period. Tear secretion was much higher (average 28 mm) than during pre-fitting periods.

Ten days after starting oestrogen medication her lenses began to grease over. This got progressively worse until after another week the lenses became thickly coated with an oily, greasy secretion immediately upon insertion, reducing visual acuity to 6/36. Over this same 3 week period the Schirmer reading dropped radically to an average of 11 mm. and the tear protein concentration doubled. The protein change was predominantly in the globulin-type proteins groups 1-3.

The normal oestrogen-induced corneal steepening was not apparent in this case, the corneas being in fact marginally flatter after 6 weeks than at 4 weeks. There are two possible explanations for this, (a) the greasing had so reduced the

visual acuity that wearing time had been drastically reduced
to only a few hours in the evening. Under these conditions
the tendency of the cornea to revert to pre-fitting levels
would run directly counter to the oestrogen oedema effect.
Alternatively (b), the effect of the low volume, concentrated
tear film would be to produce an osmotic fluid flow out of
the cornea which would again tend to neutralise the medication
effect.

This patient is the only one among the oestrogen treat-
ment group in whom corneal steepening of one or both eyes did
not occur. It is probable however, that both the wearing
time and the osmotic pressure factors contribute to this
apparent anomaly.

Greasing of corneal lenses is uncommon and a problem
of this magnitude very rare. It is just not reasonable to
assume that it would have occurred spontaneously when there
had been no indication of such a problem at any stage in
the initial adaptation.

Seven sets of results are available for protein levels
during the pre-fitting control and oestrogen medication
periods. In 5 of these cases the total protein concentration
remained the same or was reduced during medication. Subjects
16MH and 19VH are the only two to demonstrate an overall rise
in protein concentration in this group, a point which is
potentially significant in view of their later lens wearing
problems.

In this patient (19VH) medication was discontinued
after a month in view of the severity of the reaction. The

effect however was prolonged, and it was not until approximately 8 weeks after the end of medication that the electrophoresis results showed a reducing concentration. At this point limited wear of the original contact lenses was again possible, although greasing was still a problem.

In view of the low tear flow and the early corneal stain however, it was thought advisable to consider larger diameter lenses. 9.50 mm tetracurve lenses were finally supplied when it was found that the different edge/lid relationship produced less tendency to the low level residual greasing which still recurred with the original lenses.

When last seen this patient was progressing well with the replacement lenses, electrophoresis results had returned to normal and greasing was only occasionally noticeable.

The reason for the increased protein in these two patients during the medication period is not known. It cannot be related directly to tear volume reduction since in other members of the group a corresponding reduction produced the opposite protein effect (see 17WP). Nevertheless, in subject 16MH, the higher Schirmer results during lens wear will tend to mask the protein change which is highlighted by the very reduced tear volume of the other subject.

A protein elevation with oestrogen is not explicable by analogy with other glandular structures. The two major parallels, the cervical and sebaceous glands, both support the principle of unchanged or reduced protein secretion as encountered with the other subjects within the treatment group. It must be considered to be an individual atypical

reaction to the hormone used. A very much larger sample size would be required to establish the frequency of this type of response among the population as a whole.

Patient 20AMcL

This final patient within the oestrogen medication group to experience trouble with her contact lenses potentially attributable to the steroid had a severe herpetic lid infection which prevented the collection of control period electrophoretic data. The medication-induced protein change is not definitely known therefore although the implication of the change during lens wear is that this subject conforms to the more usual pattern of a reduction in concentration during medication (see table 5).

This subject was a dental student with very demanding close range visual requirements. Lens tolerance was good however and no difficulty was foreseen. Adaptation was generally good, although lens movements produced difficulty with very fine close range vision, such that she would not wear the lenses in certain clinical practical periods. This produced an erratic weekly wearing pattern, although comfortable 14 hours per day wear was experienced at weekends and on some days during the week.

Corneal steepening was relatively low and a reasonable spectacle V.A. was retained throughout the adaptation period.

One week after starting oestrogen medication (after 6 weeks lens wear), this subject was ill with bronchitis and discontinued lens wear for 2 weeks, while still continuing to take the oestrogen tablets. When seen 4 weeks after restarting lens wear and while still taking the medication, wearing time

was still only 3-4 hours per day. The lenses were described
as "uncomfortable" with a hot, dry gritty sensation, quite
dissimilar to that experienced when first adapting to the
lenses. At this point the corneal curvature was considerably
steeper, especially in the right eye, than at any previous
stage in the adaptation period.

Four weeks after stopping medication, lenses were
subjectively very much improved, described as "excellent"
outdoors. Reading vision was improved and wearing time
back to all-day if desired. Corneal curvature had reverted
almost to pre-fitting levels.

This case is very similar to that of subject 16MH.
Both show considerable corneal steepening having already
adapted to all day wear and both had shown a definite tear
reduction during the initial medication period. The difference
in the severity of the corneal response, definite epithelial
breakdown in the first case and an intact cornea but with all
the symptoms of a badly fitting lens in the other, is likely
to be due to the differing wearing times and the very different
shape of the two corneas. In the case of patient 16, the very
astigmatic cornea allied to all-day lens wear would produce
prolonged pressure over a small central area. Patient 20 had
very little corneal astigmatism, which was reduced even
further under the influence of lens wear. The corneal
steepening would therefore produce a flat fitting, but
symmetrical lens, with a large bearing area on the cornea.
In addition the excessive edge stand-off induced by the
steepening (in conjunction with the high reflex lacrimation
which would itself aggravate the existing oedema) prevented

the re-establishment of lens toleration. Central corneal
disturbance would not be expected to develop under such
limited wearing conditions.

Removal of the oestrogenic hormone resulted in this
case in a rapid improvement in symptoms related directly to
the improving lens fit as the cornea flattened. Improvement
in comfort resulted in a more normal tear secretion, which
in turn reduced the osmotic steepening with further improve-
ment in comfort.

(b) Progestogenic treatment group

Patient 8JH

This girl was a very reliable project subject, not in
any way inclined to exaggerate or over re-act to transient
lens problems. She was not apparently generally affected
by the progestogen medication.

She had consistently high Schirmer readings which did
reduce during the initial medication period, although still
remaining well above average. During this medication period
also, total protein concentration increased by $\simeq 45\%$, with
the change predominantly occurring in the globulin-type
protein.

Lens adaptation proceeded well at first until an arc
stain on the left eye necessitated supply of a steeper lens.
All day wear was then rapidly achieved.

Two weeks after the start of the progestogenic medication,
a steep rise in curvature was present in both eyes, the
corneas having a steeper radius than at any previous stage
in the adaptation period. Lens comfort remained excellent

at this stage and for a further two weeks. The lenses
then started to get progressively less and less comfortable
for the first few hours of wear until after a further two
weeks they were discontinued completely. This patient was
not seen during this period being away from Birmingham
during the Christmas vacation. By the time she returned
to Birmingham, several days after discontinuing lens wear,
both corneas were completely normal and the curvature reduced
to near pre-fitting levels.

Medication was then stopped and all-day lens wear
rapidly re-established. Approximately a month after this
(i.e. at about the same wearing stage as during the initial
medication period) a very mild recurrence of the same problem
was experienced. On this occasion however, it took the form
of general discomfort starting about one hour after lens
insertion and lasting for approximately one hour. It did
not on this occasion deteriorate further and never produced
any desire to remove the lenses.

The lens fit was not in any way modified for a month
(although the same mild symptoms persisted) in order to
confirm the different level of reaction on this occasion.
A blending curve was then added to both lenses to spread the
lens weight more widely across the cornea. This eliminated
the problem in the left eye and considerably reduced it in
the right.

The patient was finally seen 3 months after the modifi-
cation and was quite happy with her lenses.

The progression of events described during the medication
period while on holiday are typical of corneal oedema, which

must have been of a fairly severe nature to cause so much discomfort that the lenses had to be abandoned. It seems likely in fact that some central stain or mild erosion also developed in view of the severity of the symptoms.

The tendency of the eyes to this type of response to the lenses is shown by the recurrence of the problem after re-adaptation. There is however a very considerable difference in the severity of the response under the two conditions.

In this patient, as in the oestrogen group patients, the flattening tendency of the cornea as adaptation proceeds was reversed following the start of medication, whereas the flattening was allowed to proceed normally during the second adaptation period. This is probably sufficient to explain the different level of severity of the ocular response on the two occasions.

Patient 14JC

This patient was notable mainly for her very low tear volume results, average 7.7 mm, which were reduced even further during the initial progestogenic medication period (average 6.5 mm). She showed little change in tear protein during medication although the basic levels of the globulins were high and those of lysozyme very low.

Both corneas were almost spherical, and reaction to the contact lenses excellent. There was no sign of an adverse general reaction to the hormone.

Initial adaptation produced little reflex lacrimation, Schirmer readings were almost unaltered as were protein

concentrations, with the exception of group 3 globulin which was reduced.

After all-day wear had been achieved, the corneas steepened following the start of the progestogen, producing a flat lens fit. There was no reduction in subjective comfort at any stage, but some 4 weeks after starting medication, the lenses became rather greasy and very marked conjunctival injection developed.

When the patient was seen on returning to Birmingham after 6 weeks hormone use, the cosmetic appearance of the eyes was very poor, although they remained quite comfortable. Cessation of the medication was accompanied by a rapid improvement, until after 2 weeks, the injection had completely subsided and general appearance was again good. The problem has not recurred. Full time lens wear continued throughout the medication period.

Inspection of the Schirmer test results indicates the probable source of the problem, namely, the fluid reduction during medication. The amount of superficial corneal stain was also greater during this period than at any time before or afterwards.

(iii) General Discussion

It may be seen from these five case histories that there are several ways in which the presence of the two hormones may interfere with the normal processes relating an eye and a corneal lens. Not all mechanisms are operative in every case, and it is important to recognise also that subjects who re-act badly to the hormones in a general way (see 12SL) may suffer no adverse effect on their lens tolerance.

The major effects would seem to relate to the fluid retaining properties of oestrogen and the alteration in the exocrine gland secretions induced by progestogen. It is not possible to supply specific answers to the question of the precise metabolic processes involved. This is due both to a lack of knowledge of the precise mode of action of hormones[125] and an absence of information regarding the actual secretory mechanism of the structures involved.

With reference to the question of hormone action, Tausk[125] states: "Numerous protein-like hormones and oestradiol have been shown to have a site of action in the external membranes of cells which are sensitive to these hormones. This can explain why hormones act precisely on those cells, which they can 'recognise' by the exterior. In these membranes an enzyme adenylcyclase is activated which very rapidly increases the level inside the cell of a characteristic substance known as cyclic A.M.P. (adenosine monophosphate). Cyclic A.M.P. imitates the actions of the hormones concerned as if it acted on behalf of these hormones as a 'second messenger'. It has been shown to be very likely that the final effect of various hormones is directed towards the genetic apparatus in the cell nucleus."

The secretory structures involved, namely, the lacrimal and lid glands, and the conjunctival goblet cells have been investigated anatomically, but very little is known about their metabolic and secretory activity.

(a) Lacrimal Gland.

Anatomically, the lacrimal gland is a serous gland of tubulo-racemose structure consisting of very small lobules made up of fine tubules. These tubules are composed of a thick layer of cylindrical cells lining the central lumen, outside which is a layer of flat basal cells lying on a basement membrane. The basal cells are myoepithelial and contractile, while the cylindrical cells are secretory containing fatty globules and showing definite histological signs of activity (Duke Elder[123] p.562).

The gland has a sensory, sympathetic and parasympathetic nerve supply and stimulation of any of these three may produce reflex lacrimation. Removal of the parasympathetic supply, e.g. with atropine, a parasympatholytic drug, reduces secretion. The increased secretion induced by early contact lens wear is readily explicable on the basis of trigeminal nerve stimulus in the lids and cornea forming the afferent portion of a reflex arc.

Hormonal control is not readily explicable. The direction of influence has been shown to be towards a reduced secretion with increased circulating levels of progestogen or oestrogen steroids.

Control of the electrolyte and fluid balance within the body is by aldosterone, a hormone secreted by the adrenal cortex under the influence of pituitary gland A.C.T.H., and possibly some substances produced by the renal system. In general, the greater the concentration of aldosterone in the tissues, the greater the retention of sodium and water within the cells. The system is by no means as simple as

this however, and involves the metabolites of aldosterone and their rates of excretion from the body[126,127]

Progesterone produces an increased secretion of aldosterone on a negative feedback basis by increasing diuresis of sodium. The low sodium levels then stimulate increased aldosterone secretion. It is of note also that progestone is itself an important precursor of aldosterone biosynthesis within the adrenal cortex[28]

Oestrogen by comparison alters the metabolitesof aldosterone, causing a reduced excretion of certain ones and an increased plasma-binding of the hormone[126] At higher doses, oestrogen is also thought to stimulate aldosterone secretion, although in what way and by how much is uncertain.

Since there is no information available about the precise metabolic or biochemical processes involved in the secretory action of the lacrimal gland - it is not known, for example, how the gland produces such an abnormally high lysozyme concentration - it cannot be established with any certainty at all whether the reduced secretion is related to altered adrenocortical steroid levels. Many other hormone systems and metabolic levels are affected by the sex hormones. Lipid, protein and carbohydrate metabolic processes are all altered and this might potentially disrupt the normal glandular activity[101]

(b) Corneal Response

The cornea of a contact lens wearer has been shown to respond to oestrogen medication with an increased water retention during the early stages of drug use. Some patients in the progestogen group are also affected in this way. No effect is produced on the corneas of non-lens wearers with either preparation (see p.114).

In the case of oestrogen this is felt to be the same sodium retention - aldosterone system effect which is responsible for general oedema tendencies throughout the body. The overall metabolic effect is most noticeable during the first cycle of hormone use and this trend is also present in the corneal response.

The same effect with progestogen is less readily explained. The initial diuresis of sodium results, as has been stated, in a stimulation of aldosterone secretion. Gláz and Vecsei[28] state however that the diuretic effect does not persist for more than 5-10 days. Thereafter there is a rebound phenomenon due to the increased aldosterone production. The steeper corneal curvature readings were obtained 10-14 days after the start of the progestogen medication. This corresponds to the 'rebound' period when the excess aldosterone would be expected to induce a relative oedema. In addition the tear protein concentrations have been found to be reduced during the early weeks of progestogen treatment. This will produce a lower osmotic pressure fluid film and thus make the development of oedema more likely.

The drug-induced steepening was found to have subsided

after 6-8 weeks medication. The absence of all subjects
during the Christmas vacation however prevented the time
scale of this flattening being recorded. It is not known
therefore how long the effect persisted.

(c) Goblet Cells

These mucous glands are absent near the lid margins
and limbus, and most numerous in the bulbar conjunctiva.
They are round or oval modified columnar cells which have
become swollen due to an accumulation of droplets. The
nucleus is displaced to the side, and the cells, which are
formed in the deeper layers of the conjunctival epithelium,
increase in size as they approach the surface. The cell
membrane finally ruptures, liberating mucin, whereafter the
cells degenerate. (Duke Elder[123] p.546).

It is felt that these cells form the protein classified
as band IV in the gel electrophoresis, which is of an
α-globulin, mucopolysaccharide nature. The concentration
was found to increase with progestogen medication (and
occasionally with oestrogen). It has been suggested (p.166)
that this effect is analogous with the well documented
progestogen-induced changes in cervical mucus.[10,14]

Again, however, the exact mechanism of action is
unknown. This is inevitable when it is not known what
endocrine-enzyme system is responsible for the initial
demarcation of the basic columnar cells into goblet cells.
The cells do not appear to possess a definite nerve supply
although the conjunctiva as a whole has a widespread supply
of both sensory and sympathetic nerves. Goblet cell control

has always been considered to be hormonal, but no mechanism
has ever been investigated.

(d) Meibomian Glands

The Meibomian (tarsal) glands are sebaceous glands,
30-40 in the upper lid and 20-30 in the lower lid. They
consist essentially of straight tubes, perpendicular to the
lid margin and embedded in the tarsal plate. Numerous lateral
ducts lead off the main duct into single or composite acini.
These acini consist of central polygonal fat cells and
peripheral, cubical, fat-free cells on a basement membrane
reinforced by connective tissue. The glands are surrounded
by lymph spaces and are supplied by blood vessels and nerves.

Strauss and Pochi[107,108] have described the suppression
of sebum production by Enovid, an oestrogenic combination
contraceptive. It is suggested that oestrogen suppresses
and progestogen increases sebaceous secretion, but no
mechanism of action is postulated.

The small tear protein band VI is felt to originate from
the lid margin. Its concentration might therefore have been
expected to vary in line with the skin sebaceous gland changes.
This was not seen to occur. However, the predominant secretion
of the Meibomian glands is lipid in nature and substances of
this type are not accurately detectable by the electrophoresis
technique used.

In conclusion, therefore, a limited explanation of the
observed medication effect on the lacrimal gland and corneal
structure may be attempted by reference to the hormonal
effects on the aldosterone system. The progestogenic effect
of increased tear protein concentrations in the non-lens

wearing eye may be elaborated, but not explained, by analogy with the cervical mucus and the sebaceous glands of the skin.

This same structural analogy clarifies the finding of unchanged or reduced oestrogen-induced protein concentration levels.

Certain results in both medication groups are however in direct conflict with the general guidelines established. Until greater knowledge is gained about hormonal action and the control mechanism of the three glands primarily involved, these anomalous results cannot be explained.

8. SUMMARY AND CONCLUSIONS

This study has collected a large amount of clinical data regarding the observable response of the human cornea and lacrimal system to two particular steroidal hormones, norethisterone acetate B.P. 2.5 mg and ethynylestradiol B.P. 0.05 mg.

It has further monitored the effect of these compounds and dosage levels in the presence of a corneal contact lens fitted by means of current normal fitting philosophies.

The development and quantitation of a new method for the electrophoretic separation of tear protein and its application to the above study has resulted in more accurate assessment of individual protein levels and the manner in which they may be affected by contact lens wear and generalised medication.

. The data collected during this study may be summarised as follows:-

(a) Electrophoresis of normal tear fluid.

1. Acrylamide gel electrophoresis at pH 3.9 has enabled microquantities of tear fluid to be differentiated into up to 10 protein fractions. These have been provisionally identified as -

> 2 fractions: macromolecules β or γ-globulin.
> 1 fraction : γ - globulin
> 3 fractions: glycoprotein, α -globulin
> 3 fractions: tear albumin
> 1 fraction : lysozyme.

2. Tear albumin has been found to differ significantly from serum albumin, being probably a smaller or more regularly shaped molecule which is separated into 2 or 3 isomers at this pH.

3. Average tear protein concentration has been calculated to be 1.18% solution, of which approximately 14% is lysozyme, 20% albumin and 66% combined globulins.

4. Lysozyme concentration has a correlation of +0.67 with tear volume.
   Globulin-type proteins are thought likely to bear an inverse relationship to tear volume in any given individual although no such relationship is demonstrable within the accumulated results of a large group of subjects.
   Albumin concentration is relatively unaffected by tear volume fluctuation.

(b) Medication effect on non-lens wearers.

1. Tear volume as demonstrated by a Schirmer test was significantly reduced ($p \geqslant 0.05$) by norethisterone acetate and reduced to a less marked extent by ethynylestradiol.

2. Corneal curvature was not affected by either hormone.

3. Norethisterone acetate produced a generalised increase in both globulin-type and total protein concentration after 9-10 weeks of use. The oestrogenic preparation caused little change or a reduction in total protein, although an apparently idiocyncratic response in two individuals gave rise to a considerable increase in total protein.

(c) The ocular response during initial adaptation to a contact lens.

1. Increased tear volume as recorded by a 3 minute Schirmer test from 18 mm to 28 mm.

2. Decreased tear globulin and total protein concentration with increased albumin and lysozyme concentration.

3. A steepening of corneal curvature related to tissue oedema.

(d) Continuing adaptation in a non-medication, control subject.

1. A return of tear volume towards pre-fitting levels.

2. A return of total protein towards pre-fitting levels but a continued increase in albumin concentration counterbalanced by lower levels of fraction VI α-globulin.

3. A reduction of corneal steepening from the high levels of the first 2 weeks of wear as neural adaptation reduces the stimulus to reflex lacrimation.

(e) Effect of norethisterone acetate on a newly adapted lens wearer.

1. Renewed corneal steepening in 4 of 7 subjects.

2. An initial decrease in tear protein concentration during the first 2-3 weeks of hormone use which was reversed by the end of an 8 week medication period.

3. Loss of tolerance in one patient (8JH) and severe conjunctival injection in a further patient (14JC).

(f) Effect of ethynylestradiol on a newly adapted lens
wearer.

1. Renewed corneal steepening in 5 of the 6 patients.

2. Resultant loss of wearing tolerance in 2 cases.

3. Abnormal greasing of lenses (19VH) associated with
reduced tear flow and substantial tear protein
concentration increase.

4. No change in the protein pattern of other patients
in any way different from the control group subjects.

Conclusions.

This study set out to try and establish the nature
of the effect of certain orally active contraceptive steroids
on the eye and relate this to contact lens tolerance. It was
and attempt to evaluate in a controlled and objective fashion
a clinically held opinion, namely, that oral contraceptive
use could adversely affect lens wear.

It has succeeded in placing the matter on a firmer
foundation and answered certain questions, while posing many
more.

Both sample steroids selected for the trial have been
found to be capable of inducing sufficient ocular change to
disrupt normal corneal lens wear. It would be erroneous to
conclude however, that the effect of a combined oral contra-
ceptive preparation is necessarily going to be a summation of
the two individual effects.

If there were a directly additative response then the
effect of taking such a medication would be to:

      (a) reduce tear volume

      (b) increase tear protein concentration

      (c) cause substantial corneal steepening.

It has not been possible, however, to explain the observed
effects, since no information is available concerning the
secretory mechanism of the structures involved. In addition
it would be hazardous to extrapolate from these results too
categorically, since "closely related compounds or the same
compound at different dose levels may have distinctly different
modes of action. Similarly, compounds and their combinations
may have multiple modes of action."[10] This is particularly

true of oral contraceptive preparations where the relative
oestrogenicity may differ radically from one formulation to
another.[94]

The basic drug-induced trends have been established.
The logical extension of this study is to investigate the
effects of combined oestrogen-progestogen preparations and
the dose relationships of the two compounds.

Another factor not fully elaborated in this work is the
duration of the ocular side effects. It is suggested that
the oestrogen is a more immediate, and the progestogen a more
long term effect, but it has not been possible to establish
this with any certainty.

The development of new electrophoretic technique for
tear proteins has made possible very much more precise
evaluation of protein patterns and pattern changes. This
does raise however the question of the role of the various
protein fractions and the significance of changes in their
concentration. This also requires further study in terms of
drug-induced and disease-induced changes and their effect on
corneal integrity.

It was outside the scope of this study to include the
inorganic constituents of the tear film, predominantly sodium,
potassium, chlorine and urea. These substances are present in
the lacrimal gland secretion and are likely to be affected
by the hormonal effects on that gland, particularly since
both hormones alter the sodium/potassium balance in the body.

Sodium is of great significance in the hydration control
mechanism of the cornea and is derived predominantly from the
lacrimal fluid. The concentration of these inorganic

constituents also influences the osmotic pressure of the
liquid film, which itself influences corneal water flow.

Any further study ought therefore to consider both
protein and inorganic components. A more comprehensive
picture of the inter-relationship of tear fluid and cornea
could then be compiled.

In conclusion therefore the present state of knowledge
of the mechanism of hormonal action combined with the
absence of detailed study of the secretory processes of
the ocular glands, prevents elaboration of the hormone
induced effects observed.

The nature of the effects on the cornea and lacrimal
system however have been established. It has been shown in
a controlled setting that these are of sufficient magnitude
to disrupt lens wear, thus confirming a previously
unsubstantiated clinical opinion that contact lens intolerance
is a valid adverse effect of oral contraceptive medication.

APPENDIX 1.

Calculation of the relationship between change in corneal thickness and change in corneal curvature.
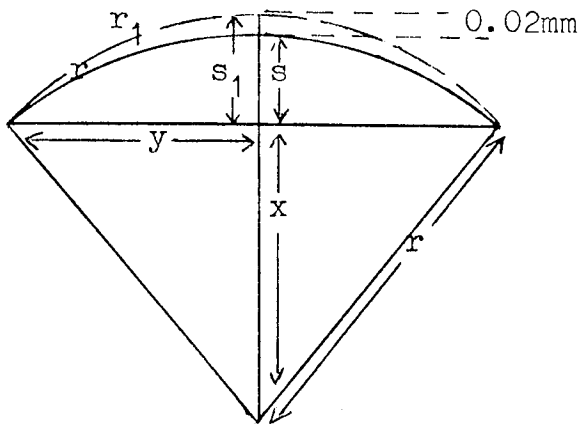
$$y^2 = 2rs - s^2$$
$$\text{and } r^2 = x^2 + y^2$$

Mandell. et al[64b] finds an average increase of 4% in central corneal thickness due to tear tonicity factors.

He further finds an average centre thickness of 0.506 mm.

4% of 0.506 mm = 0.02 mm change in thickness.

On the above diagram:

$2y$ = diameter of theoretical, round cornea, assumed fixed at the limbus but with the front surface free to swell outwards.

r = original corneal radius

r_1 = steepened corneal radius due to oedema

s_1 = s + 0.02 mm

(1) Assuming an average corneal diameter of 11.50 mm.[123] and an average corneal radius of 7.50 mm, r_1 may be calculated as follows:

$$y = 5.75 \text{ mm}$$

$$r = 7.50 \text{ mm}$$

$$x^2 = r^2 - y^2$$

$$x^2 = (7.50)^2 - (5.75)^2 = 23.19$$

$$x = 4.81 \text{ mm}$$

$$s = r - x = 7.50 - 4.81 = 2.69 \text{ mm}$$

$$\therefore s_1 = 2.71 \text{ mm}$$

$$y^2 = 2 r_1 s_1 - s_1^2$$

$$33.06 = 2 r_1 2.71 - (2.71)^2$$

$$r_1 = \frac{33.06 + 7.34}{5.42} = 7.45 \text{ mm}$$

Therefore at this average corneal curvature a 4% thickness change $\fallingdotseq$ 0.05 mm curvature change.

A similar calculation with steeper and flatter corneal radii gives:

At Basic radius 6.80 mm
4% thickness change $\equiv$ 0.02 mm curvature change

At Basic radius 8.40 mm
4% thickness change $\equiv$ 0.07 mm curvature change

(2) Obrig[82] states that, measured from eye impression casts, the average horizontal corneal diameter = 13.50 mm (p.23).

The above calculation with $y = 6.75$ mm gives:

Basic radius 6.80 mm
4% thickness change $\fallingdotseq$ 0.01 curvature change

Basic radius 7.50 mm
4% thickness change $\equiv$ 0.03 mm curvature change

Basic radius 8.40 mm
4% thickness change $\equiv$ 0.04 curvature change

APPENDIX 2

Summary tables of the electrophoresis protein concentration results.

Protein has been represented in terms of the percentage strength solution. Total protein concentration has been subdivided into the 5 major groups:

Group 1   -   Peaks I, II and III

Group 2   - α-globulin, peak IV

Group 3   - α-globulin, peaks V and VI

Group 4   -   Albumin, peaks VII, VIII, IX

Group L   -   Lysozyme, peak X.

Also shown on these summary sheets is the date of the sample, the Schirmer result in mm (measured from the end of the paper strip) and the actual number of protein bands present in the gel after electrophoresis.

The final column indicates the stage of lens wear and the presence of any unusual symptoms. Unless otherwise indicated, all samples after the start of contact lens wear were taken with the lenses in place.

Subject 1HG

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Control Medication									
20.6	0.22	0.30	0.32	0.21	0.13	1.18	7	16½	
22.6	0.27	0.42	0.30	0.36	0.095	1.445	7	12	
27.6	0.17	0.38	0.35	0.27	0.115	1.285	7	10	
29.6	0.22	0.38	0.27	0.23	0.085	1.185	7	11	
4.7	0.22	0.35	0.14	0.18	0.115	1.005	7	20	
Control Period									
10.10	0.14	0.57	0.35	0.34	0.16	1.56	7	15	
11.10	0.24	0.54	0.57	0.24	0.16	1.75	9	19	
17.10	0.14	0.32	0.12	0.17	0.10	0.85	6	6	
23.10	0.12	0.32	0.30	0.28	0.135	1.355	7	14	
Start Lens Wear									
31.10	0.12	0.30	0.19	0.34	0.115	1.065	6	24	
1.11	0.24	0.38	0.27	0.47	0.175	1.55	8	25	
7.11	0.14	0.40	0.19	0.49	0.16	1.38	6	25	1st a/c
15.11	0.20	0.38	0.22	0.45	0.16	1.45	7	23	
28.11	0.22	0.57	0.14	0.44	0.16	1.53	9	20	2nd a/c
29.11	0.10	0.40	0.09	0.24	0.13	0.96	7	12	
Control Medication with Lenses									
5.12	0.30	0.54	0.19	0.58	0.165	1.775	8	16	
6.12	0.14	0.35	0.12	0.22	0.13	0.96	7	24	
12.12	0.17	0.27	0.14	0.20	0.12	0.90	8	18	3rd a/c
16.1	0.44	0.82	0.24	0.61	0.14	2.25	7	19	
17.1	0.14	0.57	0.27	0.64	0.13	1.75	8	18	
23.1	0.20	0.50	0.14	0.38	0.16	1.38	6	21	
Normal lens wear									
31.1	0.17	0.61	0.14	0.49	0.14	1.55	8	20	
7.2	0.22	0.44	0.09	0.55	0.165	1.47	10	18	
22.2	0.14	0.61	0.19	0.53	0.15	1.62	7		
13.3	0.14	0.35	0.07	0.51	0.27	1.34	8	13	

Subject 2EC

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Control Medication									
13.6	0.27	0.47	0.40	0.27	0.11	1.72	9	8½	
14.6	0.47	0.47	0.54	0.17	0.09	1.74	7	8	
26.6	0.55	0.47	0.38	0.14	0.045	1.585	8	4	
27.6	0.50	0.50	0.73	0.22	0.08	2.08	8	4	
Control Period									
11.10	0.14	0.19	0.19	0.07	0.05	0.64	7	5½	
17.10	0.17	0.24	0.19	0.11	0.065	0.775	7	11	
23.10	6.17	0.32	0.35	0.20	0.045	1.085	8	14	
Start Lens Wear									
30.10	0.20	0.24	0.17	0.16	0.09	0.86	7	14	
31.10	0.10	0.27	0.12	0.13	0.12	0.74	6	25	
7.11	0.14	0.24	0.14	0.115	0.058	0.68	7	18	1st a/c
13.11	0.20	0.35	0.22	0.26	0.13	1.16	7	22	
15.11	0.10	0.30	0.09	0.22	0.13	0.84	6	20	
20.11	0.08	0.32	0.065	0.16	0.10	0.705	7	24	2nd a/c
Control Medication with Lenses									
27.11	0.12	0.47	0.18	0.27	0.135	1.175	7	17	
28.11	0.17	0.40	0.19	0.22	0.15	1.13	8	16	
5.12	0.14	0.47	0.14	0.19	0.11	1.05	7	20	3rd a/c
11.12	0.26	0.35	0.17	0.26	0.15	1.19	7	18	
13.12	0.30	0.40	0.19	0.15	0.08	1.12	8	15	
17.1	0.10	0.50	0.17	0.32	0.11	1.20	7	14	4th a/c
22.1	0.17	0.47	0.12	0.22	0.095	1.075	9	13½	
23.1	0.20	0.50	0.19	0.27	0.135	1.29	7	13	
Normal Lens Wear									
29.1	0.14	0.27	0.12	0.18	0.115	0.825	8	21	
30.1	0.17	0.24	0.17	0.20	0.12	0.90	8	17	
6.2	0.14	0.40	0.12	0.22	0.135	1.015	8	16	

Subject 3KS

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Control Medication									
13.6	0.22	0.38	0.27	0.19	0.07	1.13	8	17½	
15.6	0.08	0.27	0.22	0.16	0.06	0.69	6	21	
21.6	0.40	0.38	0.30	0.20	0.07	1.35	7	17	
28.6	0.40	0.70	0.34	0.32	0.23	1.97	7	13	
29.6	0.17	0.30	0.14	0.13	0.07	0.71	7	19	
Control Period									
10.10	0.22	0.50	0.38	0.21	0.09	1.30	7	16	
17.10	0.20	0.35	0.14	0.15	0.08	0.92	8	16	
23.10	0.08	0.35	0.17	0.17	0.09	0.86	8	13½	
Collect Lenses									
31.10	0.08	0.22	0.14	0.16	0.08	0.68	7	23½	
1.11	0.12	0.22	0.14	0.18	0.09	0.75	6	22	
6.11	0.10	0.14	0.09	0.13	0.08	0.54	6	26	1st a/c
14.11	0.08	0.40	0.14	0.21	0.10	0.93	7	20	
15.11	0.14	0.19	0.12	0.17	0.09	0.71	8	23	
20.11	0.20	0.24	0.12	0.19	0.06	0.76	6	23	2nd a/c
Control Medication with Lenses									
28.11	0.17	0.47	0.12	0.28	0.11	1.15	7	22	
29.11	0.17	0.27	0.04	0.16	0.09	0.73	7	24	
4.12	0.22	0.35	0.17	0.19	0.07	1.20	7	21	3rd a/c
12.12	0.17	0.30	0.14	0.19	0.13	0.93	7	26	
13.12	0.20	0.30	0.17	0.21	0.095	0.98	8	18½	
16.1	0.24	0.61	0.24	0.32	0.09	1.50	8	24	4th a/c
23.1	0.32	0.54	0.17	0.34	0.115	1.49	8	24	
24.1	0.32	0.30	0.17	0.32	0.09	1.20	7	19	
Normal Lens Wear									
30.1	0.14	0.32	0.06	0.25	0.11	0.88	9	25	
7.2	0.30	0.50	0.27	0.27	0.07	1.41	8	16	
19.3	0.10	0.37	0.14	0.115	0.08	0.805			

Subject 4AF

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Control Medication									
14.6	0.40	0.24	0.50	0.13	0.06	1.33	8	20½	
22.6	0.40	0.54	0.35	0.21	0.14	1.65	8	20	
26.6	0.32	0.38	0.19	0.16	0.08	1.13	7	17	
28.6	0.40	0.44	0.30	0.21	0.13	1.48	8	10	
Control Period									
10.10	0.35	0.54	0.30	0.17	0.16	1.52	8	24	
11.10	0.24	0.50	0.54	0.14	0.12	1.54	9	19	
16.10	0.40	0.57	0.50	0.19	0.13	1.79	7	18	
18.10	0.14	0.40	0.14	0.17	0.12	0.97	6	12	
23.10	0.10	0.50	0.30	0.13	0.14	1.17	8	18	
Collect Contact Lenses									
30.10	0.12	0.27	0.19	0.14	0.13	0.85	8	31	
31.10	0.12	0.27	0.12	0.13	0.12	0.76	7	25	
6.11	0.24	0.38	0.22	0.27	0.15	1.26	8	21	1st a/c
13.11	0.12	0.14	0.04	0.18	0.14	0.62	6	40	
15.11	0.08	0.27	0.04	0.27	0.25	0.92	7	50	
22.11	0.17	0.40	0.12	0.28	0.20	1.17	8	50	2nd a/c
Control Medication with Contact Lenses									
27.11	0.12	0.38	0.07	0.23	0.12	0.92	6	24	
29.11	0.12	0.40	0.07	0.23	0.15	0.97	7	24	
5.12	0.24	0.57	0.35	0.42	0.16	1.74	8	50	3rd a/c
13.12	0.20	0.74	0.24	0.21	0.12	1.51	8	23	
17.1	0.32	0.57	0.44	0.34	0.13	1.80	8	23	
24.1	0.14	0.35	0.09	0.26	0.12	0.96	8	25	
Normal Lens Wear									
29.1	0.12	0.40	0.30	0.22	0.13	1.17	7	23	
31.1	0.34	0.74	0.27	0.40	0.16	1.91	8	21	
7.2	0.34	0.50	0.38	0.24	0.10	1.56	8	24	
21.3	0.06	0.38	0.50	0.14	0.10	1.18	8		

Subject 5HG

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch mm	Comment
Control Medication									
15.6	0.12	0.14	0.09	0.15	0.17	0.67	6	22	
20.6	0.30	0.32	0.47	0.23	0.11	1.43	8	19	
21.6	0.22	0.38	0.32	0.24	0.11	1.17	8	20	
Control Period									
10.10	0.17	0.32	0.30	0.15	0.18	1.12	7	25	
14.10	0.20	0.32	0.14	0.42	0.12	1.20	8		
18.10	0.14	0.17	0.12	0.18	0.17	0.78	8	32	
23.10	0.17	0.50	0.40	0.36	0.10	1.53	8	16	
Start Lens Wear									
7.11	0.14	0.44	0.12	0.60	0.22	1.52	8	44	1st a/c
15.11	0.10	0.14	0.04	0.24	0.10	0.62	7	45	
27.11	0.30	0.40	0.14	0.38	0.09	1.31	8	17	)No lens
28.11	0.24	0.27	0.14	0.20	0.09	0.94	8	40	)overwear
4.12	0.17	0.24	0.12	0.26	0.10	0.89	7	40	4 hrs
5.12	0.30	0.50	–	0.80	0.19	1.79	6	50	
Control Medication with Lenses									
12.12	0.27	0.30	0.09	0.26	0.14	1.06	7	43	
13.12	0.47	0.47	0.82	0.21	0.04	.2.01	9	6	No lens
23.1	0.22	0.32	0.19	0.36	0.09	1.18	8	20	
24.1	0.12	0.30	0.09	0.30	0.12	0.93	7	32	
Normal Wear									
30.1	0.24	0.47	0.14	0.70	0.16	1.71	8	21	
7.2	0.12	0.24	0.14	0.24	0.09	0.83	6	24	
19.3	0.10	0.14	0.07	0.12	0.05	0.48	4	50	Heavy cold

Subject 6BS

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Control Medication									
27.6	0.20	0.38	0.19	0.32	0.10	1.19	7	16	
28.6	0.27	0.40	0.17	0.28	0.10	1.22	7	21	
Control Period									
11.10	0.34	0.35	0.27	0.32	0.13	1.41	8	15	
16.10	0.24	0.32	0.30	0.18	0.09	1.13	8	16	
17.10	0.20	0.38	0.17	0.19	0.07	1.01	8	14	
23.10	0.20	0.32	0.27	0.19	0.10	1.08	8	14	
Start Lens Wear									
30.10	0.14	0.14	0.05	0.17	0.07	0.57	7	32	
31.10	0.10	0.12		0.14	0.07	0.43	5	32	1st a/c
6.11	0.12	0.32	0.30	0.27	0.09	1.10	8	18	No lens
13.11	0.20	0.19	0.07	0.23	0.14	0.82	6	26	
14.11	0.05	0.19	0.12	0.20	0.10	0.66	5	25	
20.11	0.10	0.19	0.04	0.23	0.10	0.66	6	29	2nd a/c
Oestrogenic Medication with Lenses									
27.11	0.22	0.27	0.14	0.32	0.09	1.04	8	35	
28.11	0.12	0.27	0.04	0.20	0.07	0.70	6	50	
4.12	0.12	0.17	0.04	0.22	0.12	0.67	7	50	3rd a/c
12.12	0.14	0.30	0.12	0.34	0.18	1.08	6	50	
16.1	0.20	0.30	0.06	0.36	0.09	1.01	7	24	
17.1	0.08	0.38	0.09	0.40	0.13	1.08	7	50	
End Oestrogen									
23.1	0.10	0.22	0.09	0.32	0.12	0.85	6	29	
24.1	0.10	0.30	0.04	0.36	0.11	0.91	7	45	
30.1	0.12	0.22	0.06	0.46	0.12	0.97	7	35	
31.1	0.10	0.27	0.07	0.34	0.12	0.90	6	27	
6.2	0.08	0.14	0.06	0.34	0.10	0.72	7	45	

Subject 7JR

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Control Medication									
21.6	0.42	0.47	0.65	0.42	0.08	2.04	8	10	
22.6	0.22	0.65	0.40	0.34	0.05	1.66	6	16	
26.6	0.24	0.44	0.17	0.20	0.10	1.15	6	21½	
Control Period									
13.10	0.44	0.82	0.44	0.42	0.14	2.26	8	26	
Start Lens Wear									
26.10	0.12	0.47	0.14	0.57	0.14	1.44	6	26	
27.10	0.05	0.30	0.14	0.28	0.13	0.90	6	30	
2.11	0.09	0.54	0.09	0.36	0.16	1.24	6	29	
3.11	0.24	0.54	0.24	0.36	0.14	1.51	8	27	
9.11	0.12	0.47	0.19	0.34	0.13	1.25	8	29	1st a/c
17.11	0.12	0.54	0.24	0.44	0.15	1.57	7	21	
23.11	0.20	0.86	0.65	0.55	0.19	2.69	8	28	
24.11	0.44	0.74	0.38	0.34	0.10	1.86	7	25	2nd a/c
Control Medication with lenses									
1.12	0.30	0.47	0.17	0.27	0.16	1.27	8	16	
8.12	0.20	0.47	0.22	0.28	0.14	1.34	8	9½	
14.12	0.24	0.40	0.44	0.21	0.10	1.42	8	15	
15.12	0.27	0.82	0.30	0.47	0.15	2.06	8	24	Lost lens
18.1	0.30	0.70	0.35	0.72	0.16	2.23	8	27	
25.1	0.17	0.35	0.07	0.57	0.18	1.33	8	29	
26.1	0.30	1.10	0.47	0.90	0.20	2.97	8	20	
Normal Wear									
2.12	0.30	0.65	0.27	0.66	0.17	2.04	9	24	
8.12	0.40	0.44	0.57	0.42	0.07	1.90	8	12	
15.2	0.40	0.38	0.65	0.54	0.08	2.05	8	8	

Subject 8 JH

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Progestogen Medication									
20.6	0.24	0.39	0.30	0.23	0.11	1.27	7	32	
22.6	0.34	0.38	0.32	0.22	0.14	1.40	8	29	
27.6	0.40	0.44	0.70	0.21	0.07	1.82	7	50	
28.6	0.30	0.44	0.24	0.26	0.15	1.39	8	35	
Control Period									
11.10	0.22	0.30	0.17	0.18	0.17	1.04	8	50	
19.10	0.22	0.27	0.54	0.16	0.06	1.25	7	36	
23.10	0.10	0.32	0.07	0.20	0.19	0.83	6	50	
Start Lens Wear									
30.10	0.10	0.44	0.09	0.61	0.20	1.44	6	50	
1.11	0.11	0.19	0.09	0.20	0.12	0.71	6	50	
6.11	0.06	0.30	0.07	0.40	0.17	0.99	6	50	1st a/c
13.11	0.27	0.44	0.70	0.40	0.13	1.94	8	40	) No
14.11	0.10	0.50	0.17	0.67	0.30	1.74	8	30	) lens
20.11	0.20	0.50	0.14	0.95	0.32	2.11	7	50	2nd a/c
Progestogen Medication with Lenses									
27.11	0.12	0.50	0.09	0.32	0.13	1:16	7	50	
29.11	0.14	0.35	0.02	0.44	0.21	1.16	6	50	
4.12	0.20	0.35	0.12	0.58	0.16	1.41	7	43	3rd a/c
12.12	0.20	0.32	0.12	0.32	0.14	1.10	7	50	
13.12	0.20	0.22	0.04	0.27	0.14	0.86	6	50	
16.1	0.32	0.74	0.22	1.02	0.23	2.52	7	33	
17.1	0.24	0.50	0.09	0.98	0.20	2.00	7	50	
End of Progestogen									
23.1	0.12	0.27	—	0.77	0.16	1.32	6	50	
24.1	0.17	0.30	0.04	0.51	0.18	1.20	7	50	
30.1	0.14	0.35	0.18	0.63	0.16	1.46	7	50	
31.1	0.17	0.40	0.09	0.75	0.19	1.60	6	50	
6.2	0.10	0.30	0.07	1.22	0.15	1.84	6	50	

Subject 9PK

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch mm	Comment
Progestogen Medication									
13.6	0.14	0.40	0.35	0.30	0.10	1.29	7	13	
14.6	0.22	0.44	0.22	0.23	0.09	1.20	7	13	
20.6	0.40	0.50	0.57	0.34	0.14	1.95	9	24	
Control Period									
10.10	0.17	0.35	0.30	0.20	0.18	1.20	7	50	
16.10	0.12	0.17	0.17	0.14	0.14	0.74	7	18	
18.10	0.12	0.22	0.19	0.18	0.10	0.81	8	20	
23.10	0.10	0.30	0.22	0.17	0.19	0.87	8	15½	
Start Lens Wear									
31.10	0.10	0.30	0.22	0.26	0.10	0.98	7	21	No lens
1.11	0.20	0.38	0.19	0.44	0.18	1.39	9	25	No lens
7.11	0.14	0.24	0.19	0.26	0.11	0.94	7	28	1st a/c
14.11	0.12	0.24	0.06	0.24	0.09	0.75	7	24	
15.11	0.17	0.30	0.17	0.42	0.15	1.21	8	24	Oedema
21.11	0.14	0.35	0.09	0.15	0.12	0.85	7	24	2nd a/c
No medication due to lens difficulties									
27.11	0.20	0.38	0.38	0.30	0.11	1.37	7	27	
28.11	0.14	0.22	0.09	0.16	0.10	0.71	7	23½	
5.12	0.20	0.27	0.09	0.16	0.08	0.80	7	22	3rd a/c
13.12	0.10	0.19	–	0.16	0.11	0.56	6	24	
16.1	0.20	0.30	0.19	0.28	0.12	1.09	8	25	Bad Oedema
29.1	0.05	0.17	0.04	0.24	0.14	0.63	8	25	Clear
6.2	0.22	0.32	0.24	0.53	0.14	1.45	8	21	Clear

Subject 10RA

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mn	Comment
Progestogen Medication									
14.6	0.22	0.47	0.19	0.22	0.20	1.19	7	17	
20.6	0.24	0.47	0.19	0.17	0.14	1.20	7	20	
21.6	0.14	0.38	0.19	0.17	0.16	1.04	8	23	
28.6	0.17	0.74	0.38	0.32	0.14	1.75	8	15	
29.6	0.14	0.50	0.24	0.23	0.12	1.23	8	16	
Control Period									
10.10	0.17	0.47	0.32	0.16	0.14	1.26	7	17	
11.10	0.30	0.40	0.57	0.19	0.12	1.57	8	12	
16.10	0.22	0.40	0.24	0.24	0.18	1.28	8	24	
23.10	0.08	0.27	0.27	0.19	0.14	0.95	5	17	
Start Lens Wear									
31.10	0.24	0.19	0.09	0.19	0.11	0.82	6	40	
1.11	0.14	0.47	0.09	0.44	0.23	1.37	6	25	
6.11	0.05	0.38	0.09	0.60	0.18	1.30	6	50	1st a/c
13.11	0.10	0.17	0.04	0.34	0.17	0.82	7	50	
14.11	0.10	0.40	0.12	0.49	0.16	1.27	7	50	
22.11	0.12	0.65	0.09	0.68	0.21	1.75	6	29	2nd a/c
Progestogen Medication									
28.11	0.14	0.70	0.12	0.52	0.19	1.67	7	25	
29.11	0.10	0.17	0.12	0.24	0.13	0.76	8	22	
5.12	0.10	0.44	0.09	0.47	0.22	1.32	7	21	3rd a/c
6.12	0.08	0.19	0.07	0.13	0.12	0.59	7	30	
16.1	0.10	0.44	0.19	0.30	0.14	1.17	7	50	
17.1	0.08	0.44	0.07	0.34	0.18	1.11	6	24	
End of Medication									
30.1	0.27	0.54	0.24	0.80	0.18	1.31	7	29	Lens diff.
31.1	0.17	0.54	0.23	0.22	0.19	1.35	8	24	
6.2	0.14	0.35	0.12	0.57	0.20	1.33	7	23	
21.3	0.10	0.40	0.08	0.54	0.22	1.34	8		

Subject 11CN

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. peaks	Sch mm	Comment
Progestogen Medication									
13.6	0.17	0.40	0.30	0.23	0.12	1.12		21	
15.6	0.20	0.38	0.17	0.18	0.10	1.03			
20.6	0.20	0.24	0.30	0.11	0.11	0.96			
28.6	0.22	0.40	0.56	0.15	0.08	1.41			
Control Period									
10.10	0.17	0.35	0.19	0.15	0.26	1.14		21½	
11.10	0.14	0.32	0.22	0.15	0.16	1.03		15	
16.10	0.14	0.24	0.09	0.10	0.11	0.68		19	
17.10	0.30	0.38	0.47	0.13	0.10	1.38		21	
23.10	0.10	0.19	0.40	0.10	0.07	0.86		19	
Start Lens Wear									
30.10	0.14	0.24	0.12	0.18	0.14	0.82		14	No lens
31.10	0.12	0.24	0.12	0.22	0.14	0.84		24	
8.11	0.30	0.40	0.47	0.26	0.10	1.52		28	1st a/c
14.11	0.08	0.17	0.12	0.20	0.09	0.66		28	
15.11	0.08	0.54	0.14	0.49	0.17	1.42		20	
21.11	0.22	0.57	0.14	0.44	0.16	1.53		20	2nd a/c
Medication with Lenses									
27.11	0.08	0.54	–	0.27	0.15	1.04		24	
28.11	0.17	0.40	0.07	0.28	0.16	1.08		20	
6.12	0.12	0.30	–	0.15	0.12	0.69		24	3rd a/c
12.12	0.20	0.27	0.14	0.17	0.12	0.90		22	
16.1	0.17	0.47	0.14	0.55	0.14	1.47		45	
17.1	0.10	0.35	0.09	0.60	0.11	1.25		50	
22.1	0.14	0.38	0.14	0.38	0.16	1.20		31	
23.1	0.12	0.32	0.22	0.28	0.09	1.03		21½	
29.1	0.30	0.65	0.08	0.77	0.30	2.10		23	
30.1	0.14	0.40	0.09	0.42	0.16	1.21		23	
6.2	0.12	0.22	0.04	0.23	0.12	0.73		21	

Subject 12SL

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Progestogen Medication									
14.6	0.37	0.78	0.47	0.24	0.11	1.97	7	10	
15.6	0.30	0.38	0.30	0.13	0.10	1.21	6	9½	
20.6	0.22	0.30	0.30	0.14	0.14	1.10	8	13	
27.6	0.30	0.35	0.30	0.20	0.11	1.26	8	8	
29.6	0.37	0.44	0.47	0.14	0.06	1.48	7	10	
Control Period									
10.10	0.22	0.44	0.17	0.17	0.15	1.15	7	7	
17.10	0.32	0.38	0.38	0.17	0.08	1.33	7	14	
18.10	0.30	0.24	0.27	0.16	0.09	1.06	7	16	
23.10	0.22	0.44	0.32	0.18	0.12	1.28	9	12	
Start Lens Wear									
31.10	0.12	0.32	0.07	0.18	0.15	0.84	6	23	
1.11	0.18	0.30	0.24	0.17	0.11	1.00	7	28	
8.11	0.12	0.30	0.14	0.19	0.13	0.88	9	18	1st a/c
14.11	0.14	0.22	0.12	0.20	0.10	0.78	7	20	
15.11	0.20	0.38	0.19	0.32	0.12	1.21	8	16	
22.11	0.22	0.61	0.14	0.34	0.13	1.44	8	17	2nd a/c
Progestogen Medication with Lenses									
28.11	0.27	0.54	0.14	0.27	0.16	1.38	8	19	
29.11	0.12	0.57	0.12	0.23	0.13	1.17	7	18	
6.12	0.22	0.50	0.10	0.22	0.14	1.18	8	15	3rd a/c
13.12	0.22	0.54	0.14	0.21	0.10	1.21	8	17	
16.1	0.47	0.61	0.27	0.34	0.13	1.82	8	15	
17.1	0.18	0.40	0.24	0.30	0.12	1.24	8	21	
End of Medication									
23.1	0.32	0.54	0.22	0.32	0.12	1.42	8	13	
30.1	0.24	0.38	0.22	0.27	0.14	1.25	8	14	
19.2	0.22	0.27	0.19	0.19	0.10	0.97	8	11	
21.3	0.08	0.40	0.12	0.25	0.13	0.98	7		

Subject 13WD

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Progestogen Medication									
20.6	0.24	0.30	0.40	0.19	0.10	1.23	8	16½	
27.6	0.22	0.38	0.17	0.24	0.09	1.10	7	11	
29.6	0.14	0.27	0.44	0.20	0.06	1.11	8	12	
4.7	0.20	0.30	0.47	0.19	0.07	1.23	6	9	
5.7	0.24	0.24	0.32	0.19	0.07	1.06	7	12	
Control Period									
10.10	0.30	0.44	0.35	0.26	0.10	1.45	7	7	
11.10	0.32	0.44	0.74	0.17	0.07	1.72	8	19	
17.10	0.40	0.44	0.86	0.27	0.04	2.01	8	10	
23.10	0.32	0.35	0.50	0.22	0.04	1.43	9	16	
Collect Lenses									
30.10	0.10	0.19	0.12	0.21	0.09	0.71	7	24	
31.10	0.17	0.40	0.17	0.60	0.14	1.48	8	28	
7.11	0.11	0.24	0.22	0.28	0.11	0.96	6	28	1st a/c
13.11	0.11	0.18	0.17	0.26	0.20	0.82	6	27	
15.11	0.14	0.22	0.17	0.34	0.12	0.99	6	26	
20.11	0.11	0.17	0.07	0.24	0.09	0.68	6	24	2nd a/c
Medication with Lenses									
27.11	0.30	0.44	0.12	0.30	0.06	1.22	7	25	
29.11	0.22	0.60	0.24	0.77	0.12	1.94	8	23	
Overwear and medication not taken									
11.12	0.11	0.30	0.12	0.28	0.10	0.91	8	28	
22.1	0.14	0.27	0.12	0.20	0.07	0.80	7	23	
24.1	0.08	0.17	0.07	0.17	0.07	0.56	6	23	
29.1	0.10	0.17	0.09	0.15	0.07	0.58	8	21	
5.2	0.05	0.27	0.14	0.24	0.08	0.78	6	27	
19.2	0.08	0.19	0.12	0.22	0.08	0.69	7	27	

Subject 14JC

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Progestogen Medication									
21.6	0.38	0.30	0.65	0.11	0.09	1.53	9	12	
22.6	0.50	0.44	0.74	0.08	0.09	1.85	8	14	
4.7	0.47	0.35	0.61	0.11	0.07	1.61	9	4	
5.7	0.22	0.38	0.82	0.36	0.05	1.83	6	6	
Control Period									
11.10	0.30	0.35	0.57	0.14	0.09	1.45	9	6	
17.10	0.44	0.54	0.82	0.18	0.07	2.04	9	4	
23.10	0.57	0.91	1.36	0.22	0.09	3.15	10	3	
Collect contact lenses									
3.10	0.17	0.32	0.30	0.13	0.12	1.04	8	8	
1.11	0.27	0.35	0.57	0.16	0.10	1.45	8	$3\frac{1}{2}$	
8.11	0.24	0.47	0.47	0.23	0.12	1.53	9	5	1st a/c
14.11	0.20	0.40	0.32	0.19	0.18	1.28	10	14	
21.11	0.30	0.54	0.19	0.16	0.13	1.32	8	6	2nd a/c
Medication with contact lenses									
28.11	0.20	0.38	0.12	0.17	0.16	1.03	7	9	
5.12	0.55	0.82	0.65	0.38	0.11.	2.51	9	$3\frac{1}{2}$	No lens
6.12	0.27	0.74	0.47	0.22	0.18	1.88	7	8	3rd a/c
12.12	0.27	0.54	0.40	0.17	0.14	1.52	8	$6\frac{1}{2}$	
13.12	0.27	0.35	0.32	0.15	0.12	1.20	8	5	
17.1	0.42	1.00	0.44	0.58	0.14	2.58	9	4	) Conj.
22.1	0.32	0.47	0.35	0.26	0.13	1.53	9	8	)Infec-
23.1	0.24	0.35	0.30	0.21	0.13	1.23	7	15	) tion
End of Medication									
30.1	0.17	0.40	0.22	0.21	0.17	1.17	8	11	
6.2	0.24	0.47	0.44	0.30	0.12	1.57	7	9	
21.3	0.24	0.50	0.32	0.24	0.12	1.42	8		

Subject 15AMcN

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Progestogen Medication									
14.6	0.27	0.65	0.27	0.22	0.09	1.50	6	23	
15.6	0.17	0.40	0.09	0.19	0.12	0.97	6	17	
21.6	0.30	0.50	0.22	0.28	0.09	1.39	7	26	
28.6	0.32	0.44	0.22	0.21	0.18	1.37	7	40	
Control Period									
10.10	0.08	0.34	0.09	0.18	0.30	0.99	6	50	
11.10	0.22	0.32	0.34	0.13	0.06	1.07	8	12	
19.10	0.08	0.34	0.57	0.22	0.32	1.54	6	16	
23.10	0.20	0.30	0.65	0.16	0.09	1.40	8	9	
Collect contact lenses									
6.11	0.17	0.34	0.27	0.24	0.18	1.20	7	25	No lens
7.11	0.24	0.54	0.47	0.27	0.15	1.67	7	50	1st a/c
13.11	0.08	0.40	0.07	0.26	0.18	1.99	6	50	
21.11	0.12	0.22	0.02	0.13	0.11	1.59	6	24	2nd a/c
Progestogen Medication									
28.11	0.14	0.27	0.07	0.18	0.15	0.81	7	24	
29.11	0.10	0.22	0.07	0.15	0.13	0.67	5	50	
6.12	0.10	0.32	0.04	0.11	0.12	0.69	5	26	3rd a/c
24.1	0.05	0.27	0.04	0.18	0.14	0.68	6	24	
30.1	0.40	0.65	0.24	0.47	0.22	1.98	6	26	
19.2	0.02	0.14	0.04	0.15	0.09	0.44	6	23	

Subject 16MH

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Oestrogen Medication									
13.6	0.32	0.44	0.22	0.44	0.13	1.55	7	14½	
26.6	0.30	0.38	0.61	0.21	0.08	1.68	8	11	
27.6	0.32	0.44	0.27	0.23	0.12	1.38	8	13½	
29.6	0.57	0.47	0.61	0.20	0.08	1.93	9	13	
Control Period									
10.10	0.10	0.38	0.14	0.17	0.11	0.90	6	19	
17.10	0.17	0.22	0.17	0.20	0.07	0.83	6	18	
18.10	0.20	0.30	0.22	0.22	0.10	1.04	6	17	
23.10	0.05	0.19	0.17	0.16	0.10	0.67	6	13	
Collect Contact Lenses									
31.10	0.14	0.32	0.19	0.19	0.13	0.97	7	24	
1.11	0.20	0.30	0.38	0.16	0.12	1.16	8	24	1st a/c
8.11	0.32	0.47	0.74	0.26	0.13	1.92	8	14	No lens
14.11	0.17	0.32	0.22	0.27	0.14	1.12	9	15	
15.11	0.17	0.24	0.09	0.21	0.12	0.83	8	23	
28.11	0.27	0.32	0.12	0.18	0.14	1.03	8	21	2nd a/c
Oestrogen Medicated									
11.12	0.20	0.30	0.09	0.23	0.20	1.02	8	24	
Lens wear Stopped									
17.1	0.14	0.35	0.22	0.27	0.12	1.10	8	18)	No
22.1	0.17	0.38	0.27	0.20	0.13	1.15	7	13)	Lens
24.1	0.10	0.22	0.12	0.16	0.12	0.72	8	15)	
Stop Oestrogen - restart lenses									
29.1.	0.20	0.30	0.17	0.21	0.17	1.05	7	17	
7.2	0.22	0.35	0.17	0.29	0.15	1.18	7	21	

Subject 17WP.

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch mm	Comment
Oestrogen Medication									
21.6	0.27	0.40	0.32	0.23	0.09	1.31	8	25	
22.6	0.24	0.35	0.19	0.21	0.09	1.08	6	21	
26.6	0.14	0.40	0.07	0.24	0.12	0.97	6	20	
Control Period									
10.10	0.47	0.86	0.86	0.20	0.12	2.51	8	21	
11.10	0.24	0.70	0.44	0.22	0.14	1.74	8	30	
23.10	0.22	0.57	0.61	0.22	0.12	1.74	8	27	
Start Lens Wear									
30.10	0.30	0.40	0.78	0.76	0.10	1.74	8	32	
31.10	0.32	0.38	0.61	0.17	0.10	1.57	8	31	
13.11	0.20	0.57	0.22	0.75	0.19	1.93	7	24	No lens
14.11	0.12	0.32	0.09	0.47	0.14	1.14	7	27	
21.11	0.14	0.40	0.09	0.24	0.11	0.98	6	25	2nd a/c
Oestrogen Medication with Lenses									
6.12	0.10	0.40	0.05	0.26	0.13	0.94	6	42	3rd a/c
17.1	0.12	0.32	0.09	0.38	0.10	1.86	6	19	
21.1	0.17	0.40	0.07	0.56	0.19	1.39	7	44	
5.2	0.12	0.65	0.09	0.76	0.17	1.79	6	32	

Subject 18GS

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Oestrogen Medication									
20.6	0.17	0.32	–	0.16	0.16	0.81	6	22	
26.6	0.20	0.30	–	0.16	0.14	0.80	8	21	
27.6	0.17	0.22	0.12	0.12	0.14	0.78	6	26	
28.6	0.14	0.40	0.12	0.16	0.15	0.97	7	22	
Control Period									
10.10	0.24	0.44	0.27	0.13	0.17	1.25	7	50	
11.10	0.17	0.22	0.09	0.10	0.14	0.71	8	30	
17.10	0.10	0.24	0.09	0.10	0.12	0.64	6	24	
23.10	0.12	0.30	0.17	0.20	0.18	0.97	8	25	
Start Lens Wear									
30.10		0.19	0.09	0.14	0.12	0.54		31	
31.10	0.10	0.24	0.09	0.17	0.13	0.73	7	35	
14.11	0.20	0.30	–	0.19	0.14	0.83	6	27	
15.11	0.20	0.35	0.17	0.27	0.13	1.12	7	27	
21.11	0.17	0.32	–	0.19	0.12	0.79	6	22	2nd a/c
Oestrogen with Lenses									
27.11	0.17	0.35	–	0.23	0.19	0.94	6	50	
28.11	0.25	0.38	–	0.21	0.14	0.98	7	26	
4.12	0.12	0.27	0.04	0.23	0.25	0.91	7	50	3rd a/c
6.12	0.20	0.54	–	0.38	0.22	1.34	6	50	
17.1	0.20	0.27	0.09	0.24	0.16	0.96	8	20	4th a/c
22.1	0.24	0.47	0.12	0.34	0.20	1.37	7	28	
24.1	0.14	0.47	0.05	0.53	0.27	1.46	6	50	
End Medication									
29.1	0.10	0.32	0.17	0.30	0.14	1.02	7	50	
30.1	0.17	0.35	0.14	0.36	0.18	1.19	8	28	
5.2	0.17	0.30	0.12	0.27	0.14	0.99	8	26	

Subject 19VH

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Oestrogen Medication									
28.6	0.32	0.32	0.24	0.15	0.11	1.14	8	10	
29.6	0.10	0.30	0.19	0.13	0.08	0.80	6	5	
Control Period									
10.10	0.17	0.40	0.14	0.11	0.10	0.92	7	11	
11.10	0.27	0.32	0.23	0.12	0.07	1.01	8	7	
16.10	0.14	0.24	0.12	0.07	0.08	0.65	7	7	
17.10	0.20	0.27	0.14	0.09	0.09	0.79	7	9½	
23.10	0.10	0.22	0.14	0.07	0.08	0.61	8	10	
Start Lens Wear									
30.10	0.17	0.22	0.12	0.12	0.10	0.73	7	23	
31.10	0.08	0.12	0.04	0.13	0.10	0.47	6	50	
6.11	0.10	0.17	0.09	0.11	0.10	0.57	6	37	1st a/c
13.11	0.12	0.22	0.09	0.13	0.14	0.70	6	21	
15.11	0.16	0.24	0.12	0.18	0.10	0.80	7	18	
20.11	0.16	0.24	0.07	0.12	0.12	0.71	7	21	2nd a/c
Oestrogen Medication									
27.11	0.24	0.30	0.17	0.14	0.09	0.94	8	21	
28.11	0.27	0.38	0.14	0.13	0.10	1.02	7	18	grease
4.12	0.22	0.27	0.14	0.13	0.10	0.86	7	9	3rd a/c
11.12	0.32	0.44	0.12	0.23	0.14	1.25	8	9	
12.12	0.78	0.86	0.27	0.34	0.23	2.48	8	11	lens
End Medication									
16.1	0.32	0.40	0.27	0.21	0.09	1.29	8	3)	
17.1	0.22	0.32	0.17	0.19	0.09	0.99	8	5)	No
30.1	0.32	0.44	0.22	0.24	0.12	1.33	8	10)	lens
31.1	0.34	0.32	0.35	0.16	0.07	1.23	9	8)	wear
6.2	0.17	0.27	0.17	0.16	0.09	0.86	9	11)	
13.3	0.08	0.22	0.09	0.16	0.11	0.67	7		C4 lens

Subject 20AMcL

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Oestrogen Medication									
16.6	0.30	0.82	0.30	0.40	0.09	1.91	7	10	
19.6	0.24	0.38	0.12	0.22	0.10	1.06	7	13	
23.6	0.27	0.57	0.24	0.24	0.07	1.39	7	10	
26.6	0.27	0.44	0.22	0.30	0.12	1.35	6	10	
3.7	0.30	0.40	0.17	0.22	0.09	1.18	8	10	
Control Period									
6.10	0.60	0.96	0.57	0.60	0.15	2.88	8	10	
13.10	0.42	0.54	0.65	0.44	0.06	2.11	9	15	
Start of Herpetic lif infection									
26.10	0.30	0.57	0.27	0.70	0.18	2.02	7	40	
27.10	0.08	0.82	0.12	0.70	0.27	1.99	6	24	
2.11	0.37	0.78	0.30	0.72	0.18	2.35	8	14½	
3.11	0.20	0.54	0.04	0.74	0.27	1.79	6	18	
Start Lens Wear									
10.11	0.22	0.44	0.22	0.42	0.14	1.44	7	19	
16.11	0.17	0.54	0.17	0.42	0.14	1.44	6	19	1st a/c
17.11	0.12	0.57	0.17	0.32	0.17	1.35	6	23	
24.11									2nd a/c
Oestrogen Medication									
1.12	0.34	0.65	0.27	0.47	0.20	1.93	9	21	
8.12	0.24	0.44	0.17	0.32	0.13	1.30	6	15	
14.12	0.27	0.44	0.12	0.34	0.14	1.31	8	24	3rd a/c
25.1	0.14	0.38	0.06	0.38	0.14	1.10	7	24	)
26.1	0.20	0.44	0.14	0.40	0.18	1.36	7	17	)Mislaid
1.2	0.30	0.82	0.54	0.51	0.05	2.22	8	14	) lens
8.2	0.17	0.40	0.09	0.44	0.14	1.24	8	14	)Discom-
9.2	0.27	0.38	0.14	0.40	0.11	1.30	8	31	) fort
End Medication									
16.2	0.22	0.40	0.14	0.39	0.18	1.33	8	9½	
23.2	0.22	0.44	0.12	0.30	0.14	1.22	7	21½	
9.3	0.10	0.40	0.12	0.39	0.18	1.19	8	21	Good
16.3	0.14	0.32	0.12	0.20	0.12	0.89	7	16	wear

Subject 21 DT

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Oestrogen Medication									
15.6	0.12	0.44	0.20	0.20	0.09	1.05	6	23	
20.6	0.20	0.34	0.24	0.17	0.12	1.07	7	23	
27.6	0.22	0.40	0.27	0.27	0.10	1.26	7	26	
Control Period									
17.10	0.27	0.54	0.27	0.51	0.12	1.705	8	23	
19.10	0.50	0.94	0.82	0.42	0.07	2.77	7	6	
23.10	0.17	0.34	0.12	0.44	0.11	1.18	8	20	
Start Lens Wear									
31.10	0.24	0.24	0.17	0.28	0.11	1.04	6	18	
8.11	0.27	0.57	0.32	0.90	0.14	2.18	7	14	1st a/c
14.11	0.30	0.70	0.27	0.85	0.16	2.28	9	18	
15.11	0.30	0.54	0.27	0.63	0.14	1.87	8	15	
21.11	0.12	0.38	0.09	0.26	0.12	0.97	7	22	2nd a/c
Control Medication									
28.11	0.20	0.82	0.17	0.44	0.16	1.79	7	16	
6.12	0.22	0.74	0.17	0.44	0.14	1.71	7	22	3rd a/c
13.12	0.37	0.96	0.22	0.72	0.14	2.41	9	16	
29.1	0.22	0.57	0.09	0.57	0.14	1.58	7	13	4th a/c
30.1	0.14	0.65	0.14	0.60	0.15	1.68	6	19	
6.2	0.44	0.91	0.34	2.00	0.19	4.27	8	21	

Subject 22YF

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Oestrogen Medication									
20.6	0.08	0.17	0.22	0.13	0.08	0.68	8	22½	
21.6	0.17	0.19	0.19	0.09	0.06	0.70	8	16	
28.6	0.24	0.32	0.27	0.26	0.10	1.19	7	11½	
Control Period									
10.10	0.20	0.19	0.14	0.12	0.07	0.72	7	17	
17.10	0.22	0.27	0.22	0.21	0.08	1.00	7	15	
23.10	0.10	0.32	0.18	0.19	0.09	0.89	6	14	
Start Contact lens wear									
6.11	0.08	0.12	0.12	0.16	0.11	0.59	6	25	
8.11	0.12	0.57	0.23	0.62	0.14	1.68	7	18	
13.11	0.11	0.09	0.14	0.16	0.08	0.58	6	25	L only
15.11	0.09	0.36	0.09	0.47	0.09	1.10	6	42	
20.11	0.24	0.27	0.32	0.19	0.05	1.07	7	16	No lens
27.11	0.14	0.24	0.15	0.20	0.06	0.79	7	20	L only
4.12	0.31	0.24	0.19	0.15	0.06	0.95	8	17	No lens

Failed to adapt to contact lenses

Subject 23CM

Date	Gp.1 %	2 %	3 %	4 %	L %	Total %	No. Peaks	Sch. mm	Comment
Oestrogen Medication									
14.6	0.34	0.40	0.35	0.16	0.09	1.34	8	16	
20.6	0.27	0.32	0.19	0.17	0.08	1.03	8	15	
22.6	0.17	0.44	0.24	0.21	0.12	1.11	7	20	
28.6	0.27	0.24	0.24	0.16	0.07	0.98	9	17	
4.7	0.09	0.27	0.27	0.16	0.10	0.89	8	10	
Control Period									
10.10	0.20	0.44	0.30	0.17	0.18	1.29	8	22	
11.10	0.12	0.27	0.09	0.13	0.15	0.76	7	27	
17.10	0.14	0.38	0.17	0.19	0.21	1.09	7	20	
23.10	0.10	0.57	0.24	0.40	0.37	1.68	7	50	
Start Contact Lens Wear									
6.11	0.20	0.17	0.14	0.21	0.11	0.83	6	40	
8.11	0.17	0.17	0.12	0.14	0.08	0.68	7	32	
15.11	0.14	0.50	0.17	0.49	0.19	1.49	7	18	) No
20.11	0.10	0.44	0.04	0.36	0.18	1.12	7	19	) Lens

Failed to adapt to contact lenses

APPENDIX 3.

<u>Complete case record for subject 20AMcL.</u>

(a) Schirmer reading - keratometry reading record sheets,
p.242 - 244.

(b) Contact lens fitting record, p.245 - 261.

Project Record Sheet.

Name: 20AMcL

Date	Schirmer Reading	Keratometry Reading R. Eye	L. Eye
1.12.71	6	43.37 x 180 — 7.78 42.62 x 90 — 7.92	43.62 x 180 — 7.74 42.62 x 90 — 7.92
19.1.72	20	43.37 �⎫ 43.37 ⎭ — 7.78	43.75 x 180 — 7.72 43.37 x 90 — 7.78
24.1.72 28.1.72	16 22	43.37 x 180 — 7.78 43.12 x 90 — 7.83	43.50 x 175 — 7.76 42.75 x 85 — 7.90
31.1.72 4.2.72	17 9	43.25 x 180 — 7.80 42.87 x 90 — 7.87	43.75 x 180 — 7.72 42.87 x 90 — 7.87
7.2.72 11.2.72	23 7	43.00+ x 180 — 7.84 42.62 x 90 — 7.92	43.37 x 5 — 7.80 42.37 x 95 — 7.96
14.2.72 18.2.72	21 12	43.37 x 180 — 7.78 42.87 x 90 — 7.87	43.75 x 180 — 7.72 42.87 x 90 — 7.87
22.2.72 25.2.72	21 21	43.62 x 175 — 7.75 43.12 x 85 — 7.83	43.50 x 180 — 7.76 42.75 x 90 — 7.90
28.2.72 2.3.72	20 18	43.37+ x 180 — 7.77 43.12 x 90 — 7.83	43.62 x 180 — 7.74 43.00+ x 90 — 7.84
6.3.72 10.3.72	17 14	43.37 x 180 — 7.78 43.12 x 90 — 7.83	43.50 x 180 — 7.76 42.75 x 90 — 7.90
17.3.72	16	43.37 x 180 — 7.78 42.62 x 90 — 7.92	43.37 x 5 — 7.78 42.75 x 95 — 7.90

Project Record Sheet.

Name: 20AMcL.   Oestrogen Medication

Date	Schirmer Reading	Keratometry Reading	
		R. Eye	L. Eye
8.5.72 No Medication	9	43.50 × 180 – 7.76 42.62 × 90 – 7.92	43.37 × 180 – 7.78 42.75 × 90 – 7.90
Begin tbs. 12.5.72 15. 5.72	9½	43.37+ × 180 – 7.77 42.75 × 90 – 7.90	43.50 × 180 – 7.76 42.75+ × 90 – 7.89
25.5.72 30.5.72	21 7½	43.25 × 180 – 7.80 43.00 × 90 – 7.85	43.25+ × 180 – 7.79 42.62+ × 90 – 7.91
1. 6.72 Last tbt 7.6.72	12	43.37 × 180 – 7.78 42.87 × 90 – 7.87	43.25+ × 180 – 7.79 42.50 × 90 – 7.94
M. Period 8.6.72 {12. 6.72 {1st tbt	16		
16. 6.72 19. 6.72	10 13	43.37 × 180 – 7.78 43.00 × 90 – 7.85	43. 37 × 175 – 7.78 42.87 × 85 – 7.87
23.6.72	10	43.50 × 180 – 7.76 43.00 × 90 – 7.85	43.50+ × 180 – 7.75 42.87 × 90 – 7.87
26.6.72	10	43.37+ × 180 – 7.77 43.00 × 90 – 7.85	43.50+ × 180 – 7.75 43.12 × 90 – 7.83
30.6.72	9	43.37+ × 180 – 7.77 43.00 × 90 – 7.85	43.62+ × 180 – 7.73 43.00 × 90 – 7.85
{3. 7.72 {Last tbt 7. 7.72	10 14	43.25+ × 180 – 7.79 42.87 × 90 – 7.87	43.37+ × 180 – 7.77 43.00 × 90 – 7.85

Project Record Sheet.

Name: 20AMcL.

Date	Schirmer Reading	Keratometry Reading R. EYE	L. EYE	Slit lamp/ lens wear.
6.10.72	10	43.25 — 780	43.75 — 7.72	No Stain
13.10.72	15	43.00 — 7.85	43.12+ — 7.81	No stain
20.10.72	5	43.62 — 7.73 43.25- — 7.81	43.75 — 7.72 43.12 — 7.82	T.F. very greasy R.E. Sore corneas clear COLLECT LENSES
26.10.72	40 LE	43.12+ — 7.81	43.37 — 7.78	Herpes lesion R eyelid.
27.10.72	24 LE	42.28 — 7.87	43.00 — 7.85	Swollen + Sore No lens Wear
2.11.72	14½ LE	43.00+ — 7.84	43.37 — 7.78	Herpes improved
3.11.72	18 LE	43.00 — 7.85	42.75+ — 7.88	Lenses in. Comfortable.
9.11.72	16½	✓	✓	L. Eye Sore
10.11.72	19			Lenses in
16.11.72	19	44.00 — 7.67	44.00+ — 7.66	1st a/c
17.11.72	23	43.62 — 7.74	43.62+ — 7.73	No staining
24.11.72	10			10-12 hrs wear
30.11.72	16	44.00- — 7.68	44.00 .— 7.67	2nd a/c
1.12.72	21	43.50+ — 7.75	43.50+ — 7.75	No Staining
Begin tbs. 3rd 8.12.72	15	43.62 — 7.74	44.00 — 7.67	Mild P.E.E. 6%
14.12.72	24	43.25+ — 7.79	43.62 — 7.74	3rd a/c
25.1.73	24	44.37 — 7.61	44.25 — 7.63	4th a/c considerable
26.1.73	17.	44.25 — 7.63	43.62 — 7.74	discomfort
1.2.73	14			No lenses.
8.2.73	14	43.62 — 7.73	43.62 — 7.73	
9.2.73	31	43.25 — 7.80	43.25 — 7.80	4hrs wear
END MEDICATION 16.2.73	9½	44.12 — 7.65 43.50 — 7.76	44.00+ — 7.66 43.37 — 7.78	corneas clear Lenses more comfortable
23.2.73	21½	43.75 — 7.74 43.12 — 7.82	43.62 — 7.73 43.00 — 7.85	"
9.3.73	21	43.50 — 7.76 43.12 — 7.82	43.62+ — 7.72 43.25 — 7.80	5th a/c All-day wear
16.3.73	16	43.50 — 7.76 43.00 — 785	43.50+ — 7.75 43.00+ — 7.84	All-day wear.

THE UNIVERSITY OF ASTON IN BIRMINGHAM
CONTACT LENS CASE NOTES

CONFIDENTIAL

SECTION 1. (To be completed by patient)

Full Name: ~~Mr.~~ ~~Mrs.~~ Miss Annette McLoughlin.

Date ..1.12.71..........

Age ...19.............

Home Address	Local Address (if different) 4 Dovedale Ct Abdon Ave. Selly Oak B'ham 29
Tel:	Tel:

Occupation (if student, state course and institution)
Dentistry Univ B'ham.

Name of Regular Optician (if any) Address Vera	Family Doctor Univ. B'ham Health Centre Address

Reason for wanting Contact Lenses	Wearing at Present:		Spectacles	✓
			~~Contact Lenses~~	
Appearance	Date of supply of last pair		~~Both~~	
Sport	If spectacles, worn		If contact lenses, worn	
Work	Full time		Every day	
Vision	Part time	✓	Intermittently	
Medical	All distances	✓	Infrequently	
Any other:-	Long distance		Discontinued	
	Close work			

At what age were your first lenses prescribed ....

How long ago was your last eye examination: 12 Months ~~Years~~

Have you any complaints on the performance of your present lenses? NO

Family History. Are there any instances of eye diseases* in a

Grandparent		e.g. *Glaucoma
Parent		Retinal Detachment
Brother/Sister		Cataracts
None known	✓	Blindness or very poor sight of unknown origin

- 2 -

Personal History

General Health:		Any past:		Any eye treatment other than glasses
Good	✓	Eye disease		
Indifferent		Eye injury		Yes
Poor		Neither	✓	No ✓

Do you suffer from any of the following conditions:

Frequent colds		Red eyes	
Catarrh		Red eyelids	
Sinus trouble		Scaly eyelashes	
Hay fever		Styes	
Asthma		Sore or gritty eyes	
Food allergies		Itching eyes	
Drug allergies		Watering eye(s)	
Boils, abscesses		Sticky eyes	
Pimples, Acne		Discharging eyes	
Lip cold sores		Intolerance to light	
Headaches/Migraine		Double vision	
Dandruff		Intermittent "steamy" vision	

Are you at present taking any <u>regular</u> pills, tablets or medicines prescribed by your doctor?

Yes

No ✓

Name: McLOUGHLIN

SECTION 2. (To be completed by Practitioner)

REFRACTIVE STATE.

For full details
see preliminary
examination record

$\frac{6}{12}$ R. $-0.75|-0.50 \times 90$ V.A. $\frac{6}{5}$

$\frac{6}{18}$ L. $-1.00|-0.50 \times 80$ V.A. $\frac{6}{5}$ -

General Examination. By........H.F......... Date...1. 12. 71.........

FACE.
LIDS.
CONJ.
palp.
bulb.
CORN.
IRIS.
PUPIL.
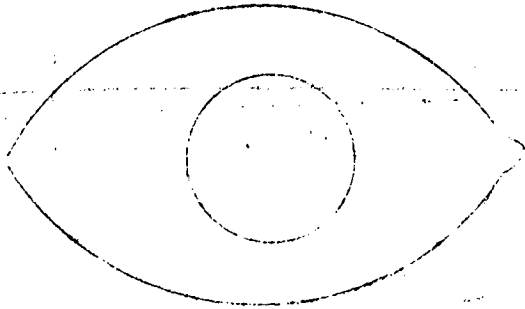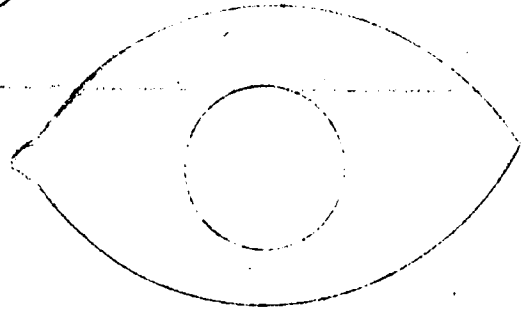
R.   L.

Slit Lamp

RE. Minor punctate stain
LE clear.

Schirmer test. V dry.
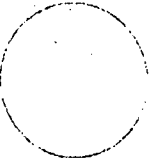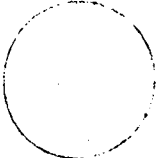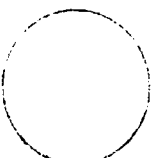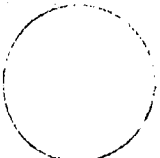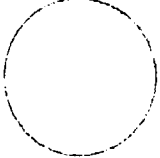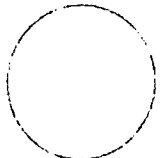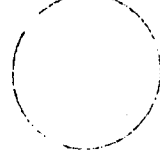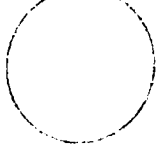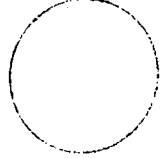
4.

CORNEAL LENS ASSESSMENT.

Prelim. K.   By............1+8f........ Instrument......B+L.......... Date.1.12.'72.

R.   7.78   L.   7.74
      7.92   7.92.

Trial Lenses		Set No	
○	7.80 9.20 C3. Large O/S. Flat Excess S/o	○	
○	7.80. 850 C3. Flat.	○	7.80. 850 C3. Flat.
○	7.75. Good clearance & edge s/o	○	7.75. Pos. shends flat. but OK for trial
○		○	
○		○	
○		○	
○		○	

Lenses for first reaction trial:

R.   7.75   850 C3   L.   7.75   850 C3
                                        Ser 23

Prelim. K.   By................1ff............ Instrument............β-h............. Date..1.12.'72..

R.   7.78   L.   7.74
      7.92   7.92

Trial Lenses		
Set No		Set No
◯	9.20 C3. 7.80 Large OIS. Flat Excess S/o	◯
◯	7.80. 8.50 C3. Flat.	◯ 7.80 8.50 C3. Flat.
◯	7.75. Good clearance & edge S/o	◯ 7.75. Pos. shends flat but OK for trial
◯		◯
◯		◯
◯		◯
◯		◯

Lenses for first reaction trial:

R.   L.   7.75   8.50 C3
      7.75   8.50 C3   Ser 23
      Ser 21.

Sheet No **3**

Reaction Trial No. **1**   By..... **IHF**   Name..............

                                                          Date..... **1.12.74**

Pre-Insertion
Slit Lamp

Minor
punctate
stain

R   L

NAD
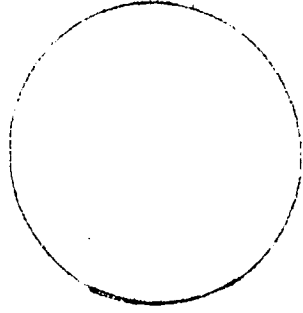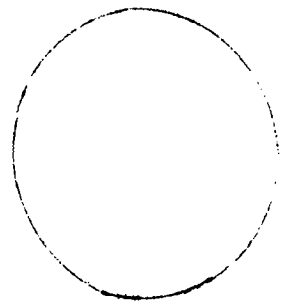
Insertion
Lenses.
R. **7.75   8.50 Se 21**   L. **7.75   850 C3   Set 23**
         C3   **1/4   2/4**

Time: From.......... To.......... Duration..... **1/4** Hrs....... ~~Mins.~~

On Return
Riding Position, Motility, Fluor. Fit.   Comfort equal. quite
                                                            good.
R. **Flat.**   L. **Flat..**

V shallow central pool, but little edge s/o.
pos. wants rel. flatter periph. curves.

Post-Wear Examination:
General, Fluor.,Keratom., Slip Lamp, Additional

R.   L.

Punctate
Stain
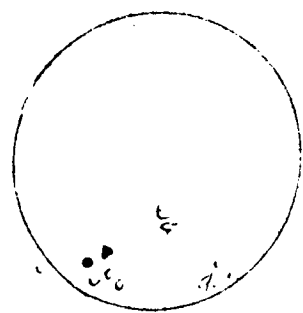
Scratches
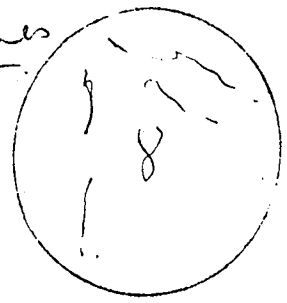
		Steeper		Steeper by		
K.Change	R. 7.78 → 772	0.06	L. 774 → 7.72	0.02	B+L
	7.92 → 7.76	0.16	792 → 7.76	0.16	

Action: Further Trial.   Refract.   Order

Checked by:

Reaction Trial No. 2   By.................   Date.... 6.3.72 ....

Pre-Insertion
Slit Lamp

clear   clear
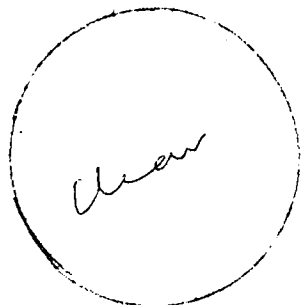
R   L

Insertion
Lenses
R. 7.70   850C3   L. 7.70   850C3
Time: From.... 1.45 .. To.. 4.45 ... Duration ... 3. Hrs ...... Mins.

On Return
Riding Position, Motility, Fluor. Fit.   Both comfortable.

R.   L. aligned centrally.

aligned
but good s/o. this time. (1mm drift BCOR ⇒ BPoR)
                                now seems OK.

replace by
7.65   Fit good. Power R — 1.75 ⊃ 6/6 +   [Vision c̄ specs after 1 hr]
7.65   required L — 1.75 D. 6/6+   [R 6/9+ L 6/9. 1 line blur]
                                              [on 3 hrs   ]

Post-Wear Examination:
General, Fluor.,Keratom., Slip Lamp, Additional
R.   L.

faint
scratch-type   NAD
stain
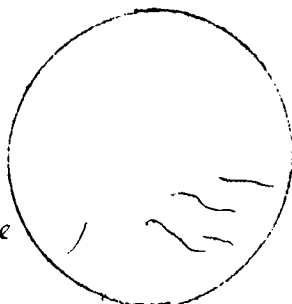
steeper
                                            steeper by

K.Change   7.74 ⇒ 7.65   0.09   7.73 ⇒ 7.63   0.10   Zeiss
R.   7.75 ⇒ 7.67   0.08   L. 7.81   7.67   0.14

Action: Further Trial.   Refract.   Order

3/7.65 : 700/865 : 800 / 12 25 .850   — 1.75   ct 0.19.

Checked by:

Page No. **4**

Prescription Lenses Received

Date.... **10.10.72**..........

Checked by... **HPF**....Date.......

	R.	L.
Polish	✓	✓
Markings		
B.C.O.D.	✓	✓
Transitions		
O.S.	✓	✓
Power & Quality		
B.C.O.R.	✓	✓
T.C.		
Edges		

Tick if satisfactory

Record findings if unsatisfactory.

Name **A. McLaughlin**....

Tuition of Patient

By.... **HPF**...... Date.. **20.10.72**

Insertion Method

Fingers

Removal Method

Fingers / suction holder

Recentering

✓

Comments

✓

Clinical Check   By..... **HPF**......... Date.. **20.10.72**

V.A. with C.Ls.   Refraction with C.Ls.   Resultant V.A.

R.E.   6/6   Duochrome Red. -0.25 D → Equal 6/5-

L.E.   6/5-   Equal

Power Modification - Immediate - Delayed   None.

Lens Fit: Equal.

R.E.   1 step steeper K.   L.E.

good edge s/o.

Wearing Schedule: 2 hrs/2 hrs. increase ½ hr/day

Cleaning/Storage Instructions: Information sheet.

Accessories Dispensed: ✓

Lenses Issued (Date) .... **20/10/72**....

First Follow-up on ...... **4/11/72**......

Checked By:

Staff Initials

No: 1   Name: A. McLoughlin   Date: 16.11.72

Follow-up Questionnaire (To be completed by patient - please tick appropriate boxes, Y = Yes, N = No)

Lenses used every day: [Y ✓]   [N]

Lens comfort: Excellent   [ ]
                  Fair   [✓]
                  Poor   [ ]

Both lenses equal comfort   [Y ✓]   [N]

If not: R better   [ ]
              L better   [ ]

Do you see well with lenses:

              For distance   [Y ✓]   [N]
              For reading   [Y]   [N ✓] improving

Do you see well with spectacles after   [Y]   [N ✓]
      removing lenses.

If No, for how long is spectacle vision disturbed:

              Less than ½ hour   [✓]
              ½ to 2 hours   [ ]
              2 hrs - 8 hrs   [ ]
              Several days   [ ]
              Don't know   [ ]

Do your lenses fall out.   [N ✓]   [Y]

Do your have difficulty inserting   [N]   [Y ✓]
                         removing   [N ✓] [Y]

any other complaints or problems?   [N]   [Y]

                                              hrs.
wearing time without removal   6 hrs.
Maximum wearing time to date   1½ hrs.
How long have you worn your lenses today?

Sheet No: 5

<u>Follow Up</u> No. 1. By H.P.F. Date: 16.11.72

Time since last ......1.... months orn today ......1½.. hrs.

---

c/o Reading difficulty

General Appearance

Good

Riding Central > low

Mobility Moderate RE
Steady LE.

Fit R aligned, w/de s/o.
L. one step steeper than
K. w/de s/o.

Vision
Binoc b/5.
R b/9 + Red −0.50D.
L b/5 Equal.

Slit Lamp Examination

R. ~~s/o~~ cornea clear.
upper lid still swollen
with after-effects of
herpes infection.

L. cornea clear.

Lens Condition

good

K. Instrument... B.r.L......

| 44.00 | 7.67×180 | 44.00+ | 7.66 |
| 43.62 | 7.74×90 | 43.62+ | 7.73 |

Action:

Doesn't like transol.
Much more comfortable with
water only.

Name: Annette McLoughlin

Address: 4 Dovedale 4.
Abdon Ave. Selly Oak
B'ham 29.

Tel.

AFTER-CARE QUESTIONNAIRE (To be completed by patient - please tick
appropriate boxes. Y - Yes, N = No).

Lenses used every day: [Y✓] [N]

Lens comfort: Excellent
                 Fair
                 Poor

See more over →

Both lenses equal comfort [Y✓] [N]

If not: R better
           L better

Do you see well with lenses:
                    For distance [Y✓] [N]
                    For reading [Y] [N✓]

Do you see well with spectacles after
removing lenses [Y] [N✓]

If No, for how long is spectacle vision
disturbed
           Less than ½ hour [✓]
           ½ to 2 hours
           2 hrs - 8 hrs
           Several days
           Don't know

Do your lenses slip off corneas [N✓] [Y]

Do you have difficulty inserting [N✓] [Y]
                        removing [N] [Y]

Any other complaints or problems? [N✓] [Y]

close work 6-7 hrs

Wearing time without removal   11 hrs

Maximum wearing time to date   2 hrs

How long have you worn your lenses today?

Have you had any necessity to seek advice
urgently about your eyes since your last
after-care examination? [N✓] [Y]

R ↓ 7·66 (:7·61)

c/o wearing time limited by
uft. focussing r. close at work
(ental - drilling etc). DV. good

Also feels eyes go through periods
when moist, then periods when
dry. Comfort vgd last week (10-12hrs)
this week much less comf.
(menstrual period due).

Riding central → low.

Mobility slow.

Fit good. slightly lge sto-
one step too flat for ideal

Vision
R. 6/5- Red
L. 6/6+ Equal.

Examination

Ophthalmoscopy

R

no stain

L

no stain

'73
'81

K   R   Instrument .... B/L   L
44·00 — 7·67 × 180   44·00 — 7·67 ·06
43·50 + 7·75 × 90   43·50+ — 7·75 ·06

Lens

Case

Other Investigations

...a check   Maddox wing
th lenses. dist 1 eso
           near 5→7 eso,
th specs. near 8-9 eso.
hout specs near 6 eso.

th lenses Mallett Unit h2 shp
sp RE. Corrected by ≠2 OUT
spectively   EE
th lenses easier to read detail
th 2ms, and D.V. reasonable

Action
1 eso · Dist
5-7 eso

BBLOAT   2 out EE
          Mallett Unit

Glaze own
plano safety
lean specs with → to wear while
treating patients.

No. **3** Name **Annett McLoughlin** Date **14.12.72**

<u>Follow-up Questionnaire</u> (To be completed by patient - please tick appropriate boxes, Y = Yes, N = No)

Lenses used every day: [✓Y] [N]

Lens comfort:
Excellent [ ]
Fair [✓]
Poor [ ]

Both lenses equal comfort [✓Y] [N]

If not:
R better [ ]
L better [ ]

Do you see well with lenses:
For distance [✓Y] [N]
For reading [Y] [N] *as before*

Do you see well with spectacles after removing lenses [✓Y] [N]

If No, for how long is spectacle vision disturbed:

Less than ½ hour [ ]
½ to 2 hours [ ]
2 hrs - 8 hrs [ ]
Several days [ ]
Don't know [ ]

Do your lenses fall out. [✓N] [Y]

Do you have difficulty inserting [✓N] [Y]
      removing [✓N] [Y]

Any other complaints or problems? [✓N] [Y] *only C.W.*

Wearing time without removal   hrs   *Varies depending*

Maximum wearing time to date   **10 — 12** hrs   *on job*

How long have you worn your lenses today?   *0 ½ hr* hrs   *requirements*

Sheet No..........

Follow Up   No....**3**...........   By....**HP**.........   Date..**14.12/72**..

Time since last ....**2 WKS**...months   Worn today .... hrs.

C/O **close work**	**Slit Lamp Examination**

General Appearance

✓

R.
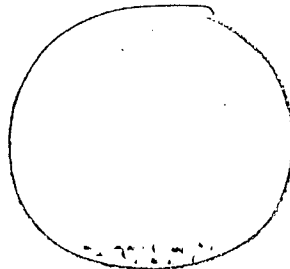
General   6%

Riding   **cent → low**

Mobility   **falls slowly.**

Fit   **Good ≯ poor than previously still free edges s/o**

L
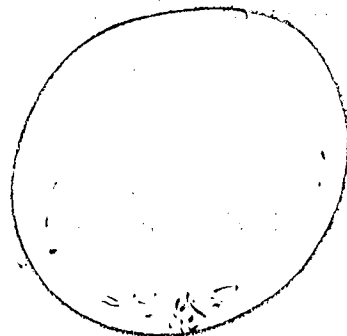
Vision

R   **6/6 b£** }
L   **6/6 b£** } **4/5**

6% one more defined area

Lens Condition	Action:
	None .

K.   Instrument..**SL**.........

43 62   774   44.00   761

45.25+ 779   45.62 · 774

Name: Annette McLaughlin   Address: 4, Divedale Court   Tel.

Abden Avenue Selly Oak.

<u>AFTER-CARE QUESTIONNAIRE</u>   (To be completed by patient - please tick
appropriate boxes. Y = Yes, N = No).

Lenses used every day: [Y ✓]   [N]   Occasionally miss a day.

Lens comfort: Excellent   [·]

Fair   [✓]

Poor

Both lenses equal comfort   [Y ✓]   [N]

If not: R better

L better   [ ]

Do you see well with lenses:

For distance   [Y ✓]   [N]

For reading   [Y]   [N ✓] Fair only.

Do you see well with spectacles after removing lenses   [Y]   [N ✓]

If No, for how long is spectacle vision disturbed

Less than ½ hour   [✓]

½ to 2 hours

2 hrs - 8 hrs

Several days   [ ]

Don't know

Do your lenses slip off corneas   [N ✓]   [Y]

Do you have difficulty inserting   [N ✓]   [Y]

removing   [N]   [Y]

Any other complaints or problems?   [N ✓]   [Y]

Wearing time without removal   3 - 4 hrs

Maximum wearing time to date   16 hrs

How long have you worn your lenses today?   3 hrs

Have you had any necessity to seek advice urgently about your eyes since your last after-care examination?   [N ✓]   [Y]

c/ ℓ general low grade discomfort
BE. puffy feeling; rather hot
and dry. Can only tolerate
for 4-5 hrs. Was wearing lenses
quite well; started oestrogen project
for then after only few days developed
bronchitis and discontinued C/L wear
w .wks; while still taking pills
has been attempting to re-adapt
w amost 4 wks, with little success.
Discomfort dissimilar to that experienced
in initially adapting to lenses, and is not
improving.

Examination

R

$S \ell$. clear

L

$S L$. clear.

Riding   central → low.

Mobility   free.

Fit   cent. pool, wide S/o a
previous checks.

Vision   6/5
            6/5

Ophthalmoscopy

K   R   Instrument B + L

| 44.37 | 7.61 × 180 | 44.25 | 7.63 × 180 |
| 44.25 | 7.63 × 90 | 43.62 | 7.74 × 90 |

Mires   clear BE

Lens   NB steeper K than on
any previous visit.

Case

Other Investigations

R C/6 - add — 0.50 6/5
L $^6/_{9bc}$ — 0.75 4/5

ie no corneal distortion
present

Action

Tbt. atropine Sut — Day 1. 25/1
Continue tablets for further
10 days. Then see

No. **5**   Name **Annette McLoughlin** Date **9·3·73**

<u>Follow-up Questionnaire</u>   (To be completed by patient – please tick appropriate boxes,
Y = Yes, N = No)

Lenses used every day: [Y ✓]   [N]

Lens comfort: Excellent   [ ] *improving* *Excellent outside*
                Fair
                Poor   [✓]

                         [ ]

Both lenses equal comfort   [Y ✓]   [N]

    If not: R better   [ ]
                L better   [ ]

Do you see well with lenses:
                For distance   [Y ✓]   [N]
                For reading   [Y ✓]   [N]

Do you see well with spectacles after removing   [Y]   [N ✓]
  lenses

If No, for how long is spectacle vision disturbed:
                Less than ½ hour   [✓]
                ½ to 2 hours
                2 hrs – 8 hrs   [ ]
                Several days
                Don't know

Do your lenses fall out.   [N ✓]   [Y]

Do you have difficulty inserting   [N ✓]   [Y]
                 removing   [N ✓]   [Y]

Any other complaints or problems?   [N]   [Y]

Wearing time without removal   *varies*   hrs   *all day except during patient*
                          clinics
Maximum wearing time to date   **8**   hrs   *(dental student)*

How long have you worn your lenses today?   **2**   hrs

Sheet No.........

Follow Up No......... By.............. Date 9.3.73....

Time since last ...6.wks.months.... Worn today ....2.... hrs.

C/O *Stopped estrogen tbs. wks ago subjectively more comf. last 2 wks reading improved. Comfort excellent outside.*

Slit Lamp Examination

General Appearance

Good.

R. clear

Riding Centred → low

Mobility free

Fit central
~~much~~ clearance
full edge S/O

L. clear

Vision

Schirmer 21mm.

Lens Condition

~~Action:~~
16/3/73 Comfort still improving
K 43.50 (R²)   43.50+ (L)
43.00   43.00+.
slight distortion,
Tear film greasy & disturbed lipid layer
However, patient now starting
full oval contraceptive for
dysmenorrhoea. Will
have to see what happens.

K reduced again (see last sheet.)

K. Instrument...............
43.50+ 776 43.62+ 773
43.12- 782 43.25 780

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