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Experiments on the function of the eye in light microscopy*

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SYNOPSIS

Since the rays of light can be traced from the lenses of the microscope to the retina, and since discoveries by light microscopy are nearly always made directly by the eye, this organ may be regarded as an integral part of the instrument. Nevertheless, it would not appear that experiments have previously been performed to establish certain important facts about the function of the eye in microscopy.

The experiments described in this paper have given the following results.

In microscopy the eye is generally focused for closer-than-distant vision. In diagrams showing the eye as well as the microscope, the rays from a point in the primary image produced by the objective should diverge between the eye-lens and the eye.

The author questions the validity of the method commonly used to determine visual acuity. His experiments suggest that in conditions simulating those of microscopy, visual acuity is only about $\frac{1}{2}$ (i.e. lights subtending an angle of 2′ at the eye can usually be seen separately, but those subtending 1′ usually cannot). It follows that an eyepiece magnifying at least 8 times is required to render visible all the details in the primary image produced by a first-rate oil-immersion objective.

Visual acuity does not rise progressively, as is usually supposed, with increase in the luminance (brightness) of the object viewed. It is highest with quite moderate luminance.

The mean diameter of the pupil of experienced microscopists, while actually using the microscope, is about 2·8 mm.

* The substance of this paper was delivered as the Presidential Address on 5th January, 1966.

[illegible] in which it may be said that the eye is an integral part of the light microscope. First, the rays of light may be traced all the way from the object to the retina. Secondly, discoveries are nearly always made directly with the eye, except when cinematography is used. As a general rule the photomicrograph is only used by the discoverer to exhibit his findings to others. In both these respects there is a strong contrast with electron microscopy, in which a new set of rays originates in the phosphor particles in the screen, and discoveries are nearly always made by the study of micrographs.

Although such a wealth of knowledge exists about the optics of microscopy and about ophthalmology, little has been done to bring the two subjects together by investigations of the function of the eye while it is engaged in microscopy, or is acting in circumstances simulating those of microscopical vision. Problems of considerable interest have hitherto remained unsolved, and indeed it would seem that no one has ever previously performed any experiments in an attempt to solve them. Experiments on four such problems will be described in the present paper. These are (1) the focus of the eye, (2) visual acuity, (3) the relation between the luminance (brightness) of the object and visual acuity, and (4) the diameter of the pupil.

Some of the best-known studies of visual acuity have been carried out on very small numbers of persons. For instance, König (1897), Wilcox (1932), and Shlaer (1936) each investigated the eyes of only two persons; Berger (1942) worked with three. Hecht (1928) based his calculations on König's data. In the present study 100 were subjected to the principal experiments, namely those on focus, acuity, and the effect of luminance on acuity. A smaller group (15 persons) was used for the study of the size of the pupil.

In the present paper the persons who were subjected to the tests will be called the "subjects". Sixty-two of them were male, 38 female. For the sake of brevity, all of them will be referred to by the use of the words "he", "him", and "his". They were a selected group in the sense that they were all (with the exception mentioned on p. 251) persons who did not wear spectacles or contact lenses, whether for distant or near vision or to correct astigmatism, though a few had done so in the distant past. The ages of the subjects varied from 9 to 50 years. When their ages were arranged in 5-year groups, 5–9, 10–14, 15–19, etc., the mode fell in the 20–24 year group. For certain purposes the subjects were classified as non-microscopists (24 persons), occasional microscopists (54), and experienced microscopists (22).

In a first attempt of this kind it seemed right to simplify the experiments so far as possible, and for this reason only monocular vision has been studied.

THE FOCUS OF THE EYE

Introduction

In text-books of optics and microscopy, diagrams are often shown in which the virtual image of the object is seen to be situated at or near the minimum distance for distinct vision (fig. 1). The rays of light from a point in the object, having converged from the objective to a point in the plane of the eyepiece diaphragm, must therefore *diverge* between the eye-lens and the eye, and the latter must be accommodated for close vision. In diagrams in other books, however, the rays from a point in the plane of the eyepiece diaphragm *pursue a parallel course* between the eye-lens and the eye, and it follows that the ciliary muscle must be relaxed to give a focus for distant vision. In Michel's (1964) work on microscopical optics there are 7 ray-diagrams

parallel course is represented. Mollring (1963) says that microscopes are inclined for the normal eye focused at infinity, and he gives a ray-diagram in accordance with this statement. It would not appear, however, that anyone has ever published a description of an experiment designed to discover what is actually the focus of the eye in microscopy.

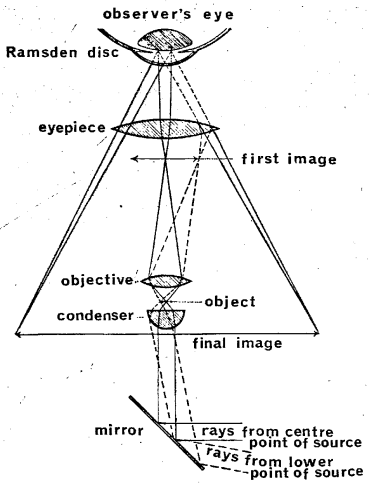

Fig. 1. Diagram illustrating the opinion that the eye of the microscopist is focused for close vision. (From Marshall and Griffith (1928). Reproduced by permission of Messrs. George Routledge and Sons Ltd. New lettering has been provided.)

Method

In the method here adopted to find the degree of accommodation of the eye in microscopy, the subject was required to focus a microscopical preparation by altering the distance between the objective and the eyepiece, as though the instrument were a telescope.

The principle of the method adopted is indicated in fig. 2. The object throws an image at the plane indicated by the broken line at the bottom of the diagram. The eyepiece, here represented as a single lens, is placed with its lower focus at the plane of the image thrown by the objective (fig. 2A). It follows that parallel rays pass from a point in the image in this plane to the eye. If the eye is a normal one, with the ciliary muscle relaxed for distant vision, a sharp image will be formed on the retina.

If the focus of the objective remains unchanged and the eye is accommodated for nearer vision, it will be necessary to lower the eyepiece, so that the rays from a

downward movement of the eyepiece (fig. 2B).

It is convenient to regard an eye accommodated for near vision as though it were a normal eye accommodated for distant vision, with a converging lens in front of it. The converging lens converts the rays diverging from the eyepiece into parallel rays (fig. 1C). The degree of accommodation of the eye may then be represented by stating the power of the converging lens (e.g. in dioptres).

It is now necessary to describe the method adopted in greater detail.

It was decided to use a Ramsden eyepiece, partly because the experiment would be simpler if the image produced by the objective was outside the lens system of the

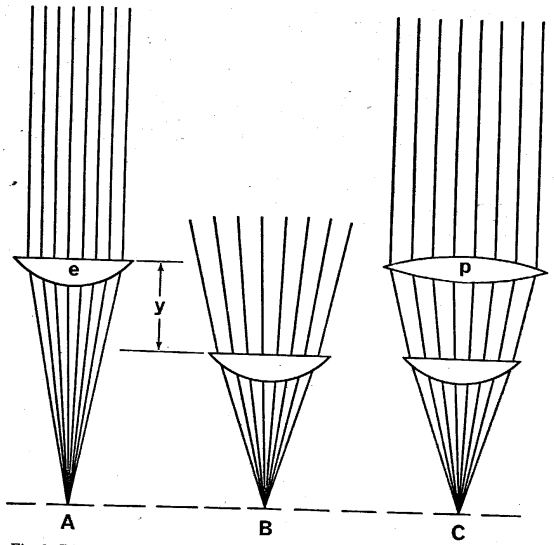

Fig. 2. Diagram illustrating the principle underlying the author's method for determining the focus of the eye in microscopy. The eyepiece is represented by a single lens (*e*). The dotted line at the bottom of the diagram represents the plane in which the image is produced by the objective. In A the eyepiece is placed in the position it would necessarily assume if the microscopist's eye were focused for distant vision. In B the microscopist is supposed to have focused his eye for close vision, and has therefore brought the eyepiece downwards through a distance *y*. In C the eyepiece has been left in the same position as in B, and a lens (*p*) has been placed above it, of such power that parallel rays are produced. The power of this lens (expressed in dioptres) is the measure of the extent to which the microscopist has accommodated his eye for closer-than-distant vision.

the eyepiece diaphragm would therefore be unlikely to attract the attention, and hence to affect the eye-focus, of the subject. A Ramsden × 10 eyepiece was used throughout.

The lower focal plane of the eyepiece was accurately determined. It was found to lie 6·5 mm (to the nearest 0·1 mm) below the flat lower surface of the lower lens of the eyepiece (fig. 3A).

A scratch was made with a diamond in a circular glass plate, and arrangements made whereby this could be readily slipped into the eyepiece in such a position that the scratch on its upper surface lay 6·5 mm below the lower lens (fig. 3A), and readily slipped out again.

A Watson "Van Heurck" microscope was used in the experiment, because it has a rack-and-pinion by which the distance between the objective and the eyepiece can be accurately controlled without danger of changing the focus of the objective. A Watson "Holoscopic" 12 mm objective, of high N.A. (0·45) for its power, was used. The condenser iris was set to fill the aperture of the objective with light. The objective was intended for a mechanical tube-length of 200 mm, and the draw-tube was set in exactly the correct position to give this tube-length. The plane of the flat lower surface of the lower lens of the eyepiece, when the tube-length was 200

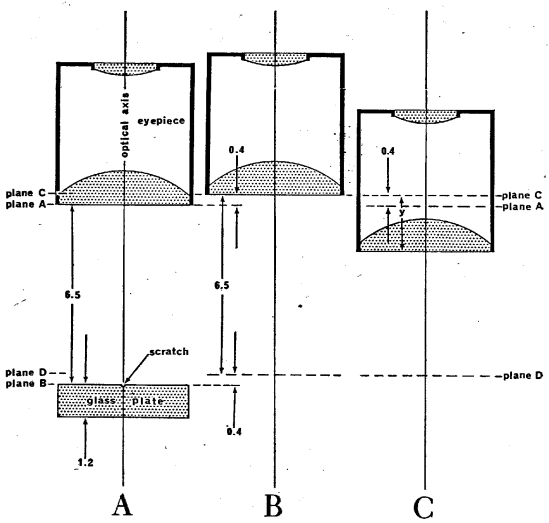

Fig. 3. Diagram illustrating, in greater detail than fig. 2, the author's method for determining the focus of the eye in microscopy by finding the distance *y*. The diagram is not drawn to scale; the numbers represent distances in millimetres.

[illegible] plane D (fig. 3A).

After trials of various test-objects, a chromosome preparation was chosen for the experiment, namely a section of the testis of the newt, *Triturus* sp., fixed in Bouin's fluid and dyed with iron hæmatein. In a particular first metaphase of maturation the tips of two chromosomes, beyond the centromeres, lay in exactly the same plane, and these were selected as the objects to be focused (fig. 4).

Fig. 4. The object used in the experiments on the focus of the eye in microscopy. It is a section of the testis of the newt, *Triturus* sp., fixed in Bouin's fluid and dyed with iron hæmatein. A side-view of the metaphase of the first maturation division is seen. The arrows point at the ends of chromosomes, beyond the centromeres. It was on these two ends that the subjects were asked to focus. Both ends could be brought into exact focus at the same time. The scale represents 20 μ.

The image thrown by the objective was brought exactly into the plane of the diamond-scratch by use of the fine adjustment of the microscope. This was done by myself. I was aged 64 and therefore had scarcely any power of accommodation, and I wore spectacles designed for distant vision. As a result the scratch was sharply in focus, and it was only necessary to bring the tips of the two chromosomes into the same focus.

The glass plate was then removed, since the scratch would attract the focus of the subject's eye. Allowance for the thickness and refractive index of the glass now showed that the image would be thrown by the objective 0·4 mm higher, in plane D on fig. 3B. If any subject focused his eye for distant vision, he would have to place the draw-tube in such a position that the reading on the scale would be 200·4 mm. If he focused his eye for nearer vision, he would have to lower the draw-tube from this position through the distance designated as y (fig. 3C).

Each subject focused the eyepiece twice. It was thought possible that the subject

down the microscope. The draw-tube was therefore put well above the focus before the subject's first test, and well below before his second. He was allowed to move the draw-tube both directions until he was satisfied that he had obtained a sharp focus on the tips of the two chromosomes.

The position of the draw-tube, when the subject had focused accurately, was read off with a hand-lens, by estimation, to the nearest 0·1 mm. An error of 0·1mm, or perhaps slightly more, was possible in the estimation.

The relation between y and the focus of the eye was determined experimentally. It was necessary to know, for each distance of y, how powerful an accessory lens placed in the eyepoint of the eyepiece must be, to cause rays diverging from a point in plane "D" to be rendered parallel. This might have been done by use of a source of light placed below the eyepiece, but in fact the experiment was done in reverse. A parallel beam of light was directed along the optical axis of the eyepiece from its upper side. Accessory lenses of various powers (spectacle lenses of +1, +2, +3, etc., dioptres) were put successively in the plane of the eyepoint of the eyepiece, and the distance from the flat surface of the lower lens to the focal point was measured. This distance was $6{\cdot}5 - y$ mm. A graph was drawn, relating the power of the accessory lenses to y. The graph, which only required slight smoothing to eliminate experimental error, was a straight line. Thus for any value of y the degree of accommodation of the eye could be read off in dioptres.

Since it was possible that some subjects might be hypermetropic, the experiment was extended by the use of negative lenses (−1, −2, −3, etc., dioptres). The value of y was now negative (i.e. the focal point was more than 6·5 mm from the lower surface of the lower lens). The graph continued in the same straight line.

Results

The measurements taken in the tests, initially expressed as values of y, were now converted into dioptres, to the nearest dioptre in each case. The results are shown graphically in fig. 5. It will be noticed that in only 7 of the 200 tests did the subjects accommodate their eyes for distant vision (i.e. to 0 dioptre, to the nearest dioptre). One subject, in one of two tests, accommodated his eye for hypermetropic vision. (In the other test he also showed himself hypermetropic, but the result fell into the category of 0 dioptre, to the nearest dioptre.) *In all the remaining 192 tests, the subjects accommodated their eyes for closer-than-distant vision.*

It had been supposed that the experienced microscopists might have developed the habit of using distant vision in their work with the microscope. It will be noticed that they showed a tendency to focus the eye for less close vision than the non-microscopists. The latter showed a strong tendency to focus the eye for very close vision. Many of them were young persons, easily able to obtain a sharp view at less than 250 mm. It is probable that they were not accustomed to place the eye very close to any such object as an eyepiece, and that the involuntary result was an attempt to focus on it.

A remarkable feature of the results recorded in fig. 5 is that all three groups of persons show a dip to a low figure in the histogram just where one would expect a high one. It seems that the subjects tended involuntarily to make some attempt at close or distant rather than intermediate vision.

The possibility suggested itself that there might be a stronger tendency towards distant vision if the microscope were placed horizontally. A small test of this was made. Ten persons participated (5 experienced and 1 occasional microscopists, 4 non-microscopists). Each did the test four times with the vertical microscope and four times with the horizontal. The mean focus of the eye was 4·0 dioptres with the

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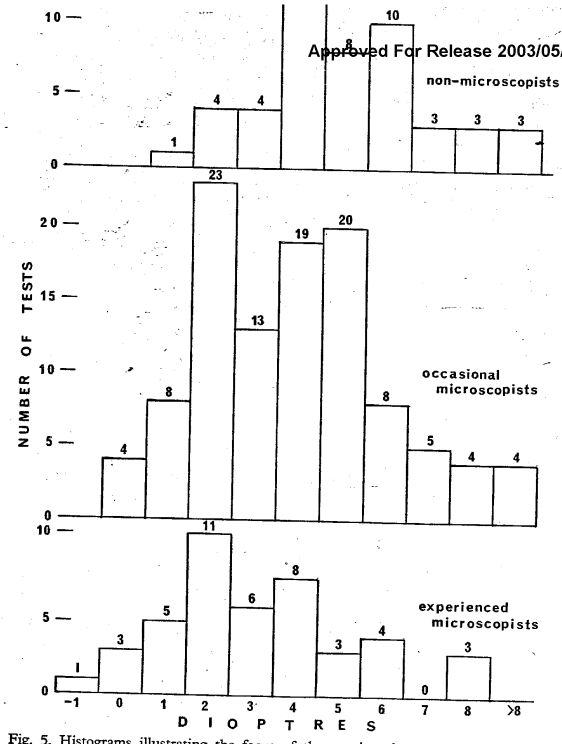

Fig. 5. Histograms illustrating the focus of the eye in microscopy. The abscissa represents the power (in dioptres) of the accessory lens that would be required to convert a normal eye, focused for distant vision, into the eyes of the subjects when they focused the microscope.

vertical microscope and 4·6 dioptres with the horizontal. Analysis showed that the difference between the means was not statistically significant.

Comment

The results of this investigation of the focus of the eye in microscopy indicate (1) that in diagrams showing the path of light from the object through the microscope and the cornea, and (2) that the eyepiece diaphragm should be placed closer to the eye-lens than the lower focal plane of the latter.

VISUAL ACUITY

Introduction

The microscopist is primarily concerned with the *resolving power* of the integrated instrument, microscope-plus-eye.

Berger (1942) sought to draw a distinction between the true resolving power of the eye on one hand and visual acuity on the other. He evidently regarded the latter as an ill-defined but empirically useful idea ("a complex function in which many factors, at first not thought of, enter").

The mere recognition of the presence of a single point of light or of a dark dot does not mean that it has been "resolved" by the eye, in the sense in which the word "resolve" is used in optics; or, to put the same fact in other words, *Punktensehshärfe* (Hofmann, 1919) is not the same as visual acuity. Hartridge (1923) claimed what amounts to a visual acuity of about 6 (that is to say, the ability to distinguish lights subtending at the eye an angle of about 1/6′), but this very high figure appears to be open to the objection that he was not measuring visual acuity in the strict sense (by determining the *minimum separabile*), but was relying instead on the subject's *Punktensehschärfe*.

Hofmann (1919) based his conclusions about visual acuity on the ability to see separately bright points, lines, or larger spaces, separated from one another by a dark area. He remarked, "To avoid all misunderstanding it must be emphasized that in what follows I use the convenient expression *Sehschärfe* . . . solely for the capacity to separate points, and therefore naturally also lines or larger surfaces. The limit of *Sehschärfe* corresponds, then, to the *minimum separabile*." In the present paper the expression "visual acuity" is used in exact accordance with Hofmann's *Sehschärfe*.

The ability to see separately bright stripes or points on a dark background has been used from time to time by various other authors as a test of visual acuity (e.g. by Lister (1913, in tests carried out in 1831–2), Wilcox (1932), and Berger (1942)), but most workers on the subject seem to have regarded Landolt's (1904) broken ring as the most critical test. It was used in the elaborate and well-known experiments of Lythgoe (1932), and is widely quoted in authoritative text-books as the best (see, e.g., Enoch, 1963). It is therefore necessary to consider in some detail the reliability of Landolt's broken ring as a test-object for acuity of vision.

A test-object is shown in fig. 6A. The bright area enclosed by the black is seen to be situated at the right side of the object. Suppose that at a certain distance, and with a certain luminance ("brightness") of the bright area, the subject is just able to recognize the fact that this bright area is to the right. Now present him with the test-object shown in fig. 6B. The bright area is smaller, but provided that there is a compensating increase in the luminance, there is no reason why the subject should not still be able to recognize the fact that it is on the right side. It could be made much smaller, and the subject would still be able to recognize its position in relation to the whole object, provided that the luminance was sufficient. This test would not provide evidence as to whether the subject could distinguish the light at the top of the bright area from the light at the bottom of it. The angle subtended at the eye by the top and bottom of the bright area would not be relevant to the problem of visual acuity. Indeed, the test would not seem to solve any specific problem connected with the subject's vision. The threshold of sensitivity of his eye to light would

way. One might possibly record the distance between the bright area (perhaps the centre of it) and the centre of the whole test-object, but a statement of the angle subtended at the eye by these two points would only give a rough and uncertain impression of visual acuity.

It is true that one may be said to "see" a black (or very dark) object, if one has just looked at a bright one (for instance, the bright surroundings), because of the *change* in the nerve impulses from the suddenly-darkened area in the retina; and in this sense it might be claimed that one could "see" the black limits of the gap, so that the angle subtended by them at the eye would be relevant; but any such "vision" of blackness would presumably be outlasted and outweighed by the real vision of the light in the gap.

If the diameter of the black area is now progressively reduced and its centre hollowed out (fig. 6C, D), the same arguments still apply. With sufficient luminance,

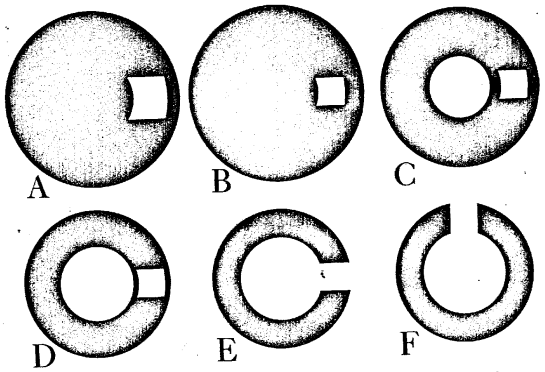

Fig. 6. Diagram illustrating the author's criticism of Landolt's broken ring as a test-object for visual acuity. For full explanation see text.

the subject will be able to recognize the position of the bright area in relation to the whole object. Next, present him with the test-object shown in fig. 6E. Further reduction in diameter combined with the hollowing out of the centre has resulted in the production of Landolt's broken ring, which is exactly represented in the figure. The ring is shown to the subject in 8 positions separated from one another by 45°, in random order, and he is required to say whether the gap is to the N. (fig. 6F), N.E., E. (fig. 6E), etc.

When Landolt's test is applied, the angle subtended at the eye by the gap is measured in minutes or fractions of a minute, and smaller and smaller copies of the broken ring are presented to the subject until he can no longer recognize the position of the gap. The smallest angle at which he can still state its position is recorded, and the reciprocal of the figure obtained is given as the numerical statement of his visual acuity. Landolt himself and many others (e.g. Lythgoe, 1932) have recorded

Landolt's broken ring seems to be subject to the same adverse criticisms as those directed against the test-objects shown in fig. 6, A–D. The angle subtended at the eye by the gap does not appear to be relevant. No proof is afforded that the subject can distinguish the light at one side of the gap from that at the other. The *minimum separabile* is not measured. The position of a very small gap could be recognized, if the luminance were sufficient.

The familiar tests with printed letters as the test-objects for visual acuity are convenient for clinical use, but it was well understood long ago (e.g. by Guillery, 1891) that there are valid objections to them. The various letters differ in the ease with which they can be read, and the recognition of their shapes involves not only the act of seeing but also a mental process (*Denkact*). It is realized that the *minimum cognoscibile* is not the same as the *minimum separabile*, which constitutes the true test of acuity.

Method

For the reasons just stated, it was decided to discover the *minimum separabile* by the use of test-objects consisting of bright parallel stripes on a dark background (fig. 7). The width of each bright stripe (left-hand pair of arrows in the figure) in

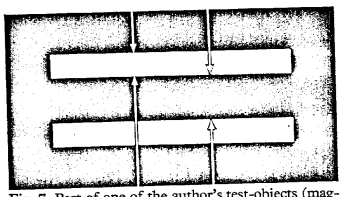

Fig. 7. Part of one of the author's test-objects (magnified). The arrows indicate the widths of the bright stripes and of the intervening dark area.

all cases subtended an angle of $\frac{1}{2}'$ at the eye. The distances between the bright stripes (right-hand pair of arrows) were 4′, 2′, 1′, and $\frac{1}{2}'$. Fig. 8 represents one of the test-objects. Four groups of bright stripes are seen. The subjects were asked to count the number of stripes in each group.

To simulate as well as possible the usual conditions of microscopy, transmitted light was used. The test-object was prepared by making a large drawing in negative contrast: that is to say, the stripes were drawn in black ink on a white ground. The drawing was then photographed to make a much smaller negative, in which the stripes were nearly transparent on a nearly black background. The negative (a 2 in. × 2 in. plate) constituted the test-object.

It was thought undesirable to reduce the original drawing to such an extent that the stripes would subtend angles of 4′, 2′, 1′, and $\frac{1}{2}'$ at the standard distance of close vision, namely 25 cm. It would be difficult to make satisfactory photographic negatives on such a minute scale and it would also be troublesome to hold the subject's head sufficiently firmly in the right position, for a small change in position would have a large effect on his capacity to count the lines. For this reason everything was done on a scale of × 10: that is, the test-object was placed 2·5 metres

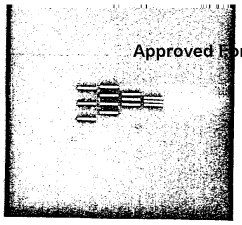

Fig. 8. Text-object Z. The bright stripes subtend angles of 4′, 2′, 1′ and ½′ at the eye, if the test-object is held 2½ metres away. (The small triangles are included in the test-object to ensure that its size is correct. The distance between their upper angles should be 20 mm.)

away. Since the tangent of 1′ at 2·5 metres is 0·727 mm, the test-object was made approximately to this scale. Fig. 8 is made as exactly as possible of the correct size.

It is worth remark that Landolt (1904) made a serious error in stating that the tangent of 1′ at 5 metres is 14·5 mm and at 33 cm is 0·96 mm. These figures for tangents are approximately 10 times too great. They may have misled other workers, who have reported astonishingly high visual acuities.

The optical density (extinction coefficient) of the test-objects was measured in both the bright and dark areas (see Appendix I).

The test-objects were illuminated from behind by a 12-volt, 100 watt Philips "mirror-condensor" lamp, placed 12 cm behind the test-object. The lamp can be obtained from photographic dealers. To diffuse the light, two pieces of ground glass were fixed between the front of the lamp and the test-object, 28 mm and 4 mm from the latter.

A variable resistor allowed the luminance of the ground glass behind the test-object to be controlled by the subject.

There is evidence that the surroundings of a test-object affect visual acuity. The test-object was therefore placed at the centre of a circular area of white paper, surrounded by black (fig. 9). The circular area represented the microscopical field of view, limited by the eyepiece diaphragm. It was made 1·54 metres in diameter, and thus subtended the same angle at the subject's eye as the mean field of view of 8 typical eyepieces of medium power made by four well-known British and Continental manufacturers. The white paper was illuminated by the daylight of the room. It was so placed that sunshine could never fall directly upon it. Its luminance was commonly about 1·1 log. foot-lamberts, but naturally this varied with the weather.

When the eye is placed at the eye-point of an eyepiece for microscopical vision, light strikes the eye not only from the microscopical field of view, but also—very obliquely—from the general illumination of the room, between the top of the eyepiece and the eye. It was thought necessary to simulate the conditions of actual microscopy in this respect.

diameter of top of eyepiece	28 mm
diameter of aperture for eye-lens	8·6 mm

A board was cut to represent the top of the eyepiece, 10 times this size; it was blackened on the side on which the subject sat. The subject looked through the hole with one eye (chosen by himself), the other eye being covered by an eye-shield or hand (fig. 9). It was necessary to place the eye at the correct distance from the board. The mean height of the exit-pupil (Ramsden circle) above the top of the eyepiece, in the 8 examined, was 8·1 mm: it was therefore necessary to place the eye about 8·1 cm from the board, since everything was on the scale of 10:1. This was achieved by asking the subject to hold his head still in such a position that two white dots, one of which is marked by an arrow in fig. 9, formed the limit of his field of view.

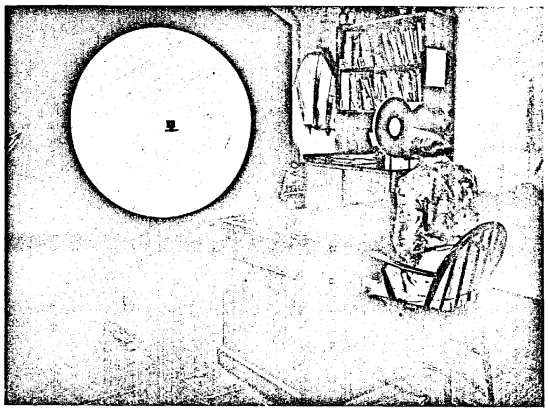

Fig. 9. A subject undergoing the test of visual acuity. She sits in front of a black ring representing the top of the eyepiece, magnified 10 times. The test-object is in the centre of the white circle that represents the microscopical field of view. The arrow on the left points to one of the two white spots that serves to ensure that the subject's eye is 2·5 metres from the test-object and about 8·1 cm from the black ring. For full explanation see text.

It was arranged that when this was so, his eye was as nearly as possible 8·1 cm from the circular board representing the eyepiece, and 2·5 metres from the test-object.

The test was carried out as follows.

The subject was shown one of the test-objects from close up, and the nature of the test explained. He was briefly shown the 9 test-objects, without being given an opportunity to study them; he was told that they were all different from one another in the arrangement of the lines. He was told that there would be four tests, and that one of the test-objects might be shown twice. He had no means of knowing that in fact every one of the 100 subjects was shown the same three test-objects, designated

stripes horizontal. X was shown on the first occasion with the stripes horizontal and on the second vertical, to allow astigmatism to reveal itself. In the description of the results, the symbol Xv is used to mean the test-object X placed with the stripes vertical.

In all three test-objects there were 3, 4, or 5 bright stripes in each of the four groups of stripes, but the subject had no means of knowing this, apart from his ability to count them correctly during the test. Details of the three test-objects are given in Appendix I.

The subject took up his position as described above, with one hand on the control of the variable resistor. He was allowed to move the control as often as he wished. He counted the easiest group of stripes first (4′ apart), and then proceeded to the more difficult ones (2′, then 1′, then ½′). He was allowed as long a time as he wished. The position of the pointer on the scale of the resistor was noted when he had finished counting all the stripes on a particular test-object, so far as he was able. All his counts were recorded.

Results

Since each subject was presented with four tests (test-objects X, Y, Z, and Xv), and in each test there were four groups of lines, it was theoretically possible to count correctly 16 groups. The maximum possible score, 16, was not obtained by anyone. A girl aged 11 and a man aged 20 achieved the highest score, 14. A man aged 50 counted 13 groups correctly. It will be remembered that the subjects were a selected group, since all of them were persons who did not use spectacles or contact lenses for any purpose.

The results obtained in the test of visual acuity are briefly summarized in Appendix II.

The figures show that the proportion of correct counts was lower at all four separations of the bright lines (4′, 2′, 1′, and ½′), when the test-object was Y than when it was X, Z, or Xv. Test-object Y has 5 bright lines in each group, whereas the others have 4 or 3. The facts summarized in statistical form in Appendix III leave no doubt that there is a highly significant difference between the results obtained when there are 5 bright stripes and those obtained when there are 4 or 3. The experiment was not planned in such a way as to allow a definite answer to the question whether it was easier to count 3 than 4 stripes, but the figures obtained do not suggest that this was so.

The fact that a small number of lines is more easily resolved by the eye than a larger number was attributed by Conrady (1913) to circumstances arising from the undulatory theory of light, but the possibility that the higher centres of the brain may be concerned must not be overlooked. One might suppose that difficulty in making the necessary eye-movements to cover the larger number of bright stripes was the cause, but if this were so, one would expect that the difficulty in counting 5 stripes would be more evident with the more widely-spaced stripes. With the stripes at separation of ½′, little or no movement of the eye might be necessary to count 5 stripes. Whatever the explanation may be, it seemed best to exclude the results obtained with test-object Y from the graphical representation of the results (fig. 10), since the results with test-objects X, Z, and Xv appear to be more reliable as indicators of visual acuity unaffected by other factors.

Fig. 10 reveals that a group of bright stripes separated by 4′ can nearly always be counted accurately, and the 2′ group in 81 p.c. of tests. The 1′ group can usually not be counted, and when the separation is only ½′ the number of correct counts is less than 9 p.c. It seems right to put the visual acuity at ½ (in the sense that reliable it is 1′).

Several subjects (who were presumably astigmatic) found it much easier to [illegible] object X when it was turned through 90° into the Xv position, but no general tendency of this sort was observed.

Comment

The visual acuity revealed in this experiment is much less than has been recorded by several other investigators. This may be due in part to the simulation of the circumstances of microscopical vision, but the main cause must be that nothing was sought in the test except the discovery of the *minimum separabile*. The reasons for rejecting the validity of the Landolt and similar tests has been considered in some detail on pp. 239–241.

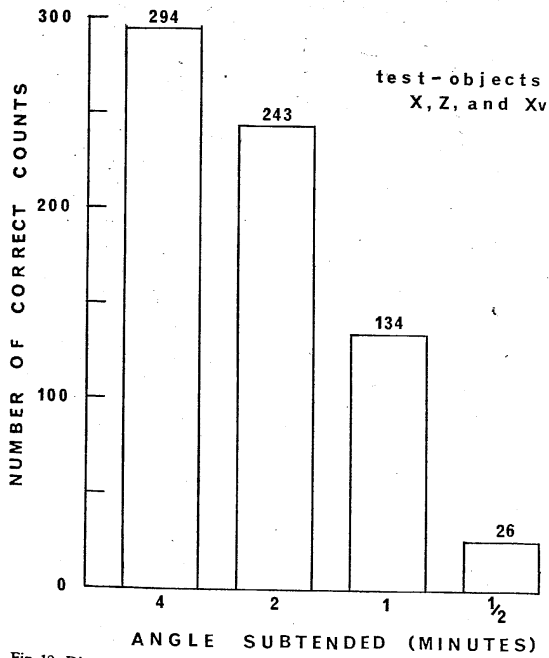

Fig. 10. Diagram illustrating the results of the tests of visual acuity with test-objects X, Z, and Xv.

[illegible] (a separation of 2'), it follows that he might be expected to count them at the standard distance of near vision (25 cm) if they were separated by 145 μ.

The distance apart (d) of two points of light that can just be resolved as separate by a microscope objective is given by the familiar equation

$$d = \frac{0{\cdot}61\,\lambda}{n.\sin.\alpha},$$

where λ is the wave-length of the light, α is the half-angle of acceptance by the objective, and n is the refractive index of the medium in which the angle is measured. An ordinary oil-immersion objective of N.A. 1·30, used with blue-green light of wave-length 500 mμ, is therefore just capable of resolving as separate two points 0·235 μ apart. If the objective magnifies 100 times, the corresponding points in the primary image will be 23·5 μ apart. In order that these may be seen by the eye, it follows that the eyepiece must magnify at least $\frac{145}{23{\cdot}5} = 6{\cdot}3$ times. Somewhat greater magnification would probably be justifiable to relieve the "strain" that is supposed to exist when the eye is used at or near the limit of visual acuity.

The equation given above shows that an objective of N.A. 1·40, used with violet light of wave-length 415 mμ, resolves 0·181 μ. It follows from the results recorded in the present paper that if the objective magnifies 100 times, a $\times$8·0 eyepiece would be required to resolve every detail in the image thrown by the objective. Once again, it would presumably be justifiable to use an eyepiece of somewhat higher power.

Although a person with normal sight would not actually need eyepieces of higher power than these, except to reduce "strain", and could not increase the resolving power of his microscope by using such eyepieces, yet it would be perfectly justifiable for persons with low visual acuity to use eyepieces of higher power. A curious outcome of this line of reasoning may be mentioned. If animals were to become sufficiently intelligent to be able to design and make optical instruments, they might well construct objectives similar to our own, but very different eyepieces. The square-lipped or "white" rhinoceros, which has very poor vision, might make eyepieces of very high power, or even use an accessory compound microscope to view the image thrown by the objective; but it seems almost certain that many species of birds would use eyepieces of lower power than those needed by man. There is little direct evidence on the visual acuity of birds (Rochon-Duvigneaud, 1943), but the slenderness and close packing of the cones in the fovea suggest high acuity. Rochon-Duvigneaud counted 81 cones in a distance of 100 μ across the central fovea of the common buzzard, *Buteo buteo* (L.), in comparison with 39 to 50 in man. He considered that the short-toed eagle, *Circaetus gallicus* (Gmelin), may have even closer packing of the cones than other Accipitres. On the basis of the number of cones in each square mm of the fovea, Walls (1942) claimed that some members of this group must have a visual acuity at least 8 times that of man. If this were true, such birds could discard the eyepiece without loss of resolving power. They could use the objective as a simple microscope, though they would have to re-design it slightly for the production of divergent or parallel rays (and they would need to devise a new way of correcting apochromatic objectives and high-power achromats for the chromatic difference of magnification).

It is perhaps legitimate to express the different eyepiece requirements of different observers by the aphorism, "The objective is objective, the eyepiece is subjective."

If the conclusion of Lythgoe (1932) were correct, and were applicable to microscopical vision, very surprising results would ensue. With a visual acuity of 2 (i.e. [illegible] 36·36 μ apart at a distance of 25 cm. There would, therefore, be no necessity for him to use an eyepiece magnifying more than 1·55 times with a $\times$100 objective of N.A. 1·30 $\left(\frac{36{\cdot}36}{23{\cdot}5} = 1{\cdot}55\right)$. It is unlikely that a single microscopist could be found who would accept such a proposition.

THE EFFECT OF LUMINANCE ON VISUAL ACUITY

Introduction

In this section the word "illumination", in inverted commas, will be used wherever it is convenient to have a comprehensive word to include all the various technical terms, with precise meanings, that authors have used to represent the amount of light used in their investigations (illumination in the strict sense (lux), luminance (foot-lamberts, etc.), luminous flux (lumens), luminous intensity (candelæ), and retinal illumination (photons)).

It has long been supposed that visual acuity rises with increased "illumination" in such a way that if a graph be drawn with visual acuity as ordinate and the logarithm of the "illumination" as abscissa, a straight line is produced. It follows, on this supposition, that there is not an optimum "illumination" for maximum acuity, beyond which acuity falls off.

König (1897) was one of the first to put this belief in concrete form. His graph consisted of three straight lines. There was first a gradual rise in acuity with increase of "intensity", but this part of the graph must have related to scotopic vision, which is irrelevant to the microscopist. The line then turned sharply upwards till it reached a maximum, beyond which it passed on parallel to the abscissa. Hecht (1928) re-analysed König's data and showed that a somewhat S-shaped curve, with a long, nearly-straight central portion, would represent the facts more exactly, but he accepted König's main conclusions.

Lythgoe's graphs show the same general form, except that there is no terminal part parallel to the abscissa. He stopped increasing the "illumination" because he thought the intense light would damage his test-objects. Lythgoe's conclusions have been accepted by most writers on the subject, including the author of the standard work on photometry (Walsh, 1958). Reasons have already been given for supposing that Lythgoe's result was almost inevitable from the use of Landolt's ring as test-object.

Lythgoe lays great emphasis on his contention that in studies of this sort the "illumination" should be presented on the abscissa in logarithmic form. He reaches the surprising conclusion that those investigators who have reported an optimum visual acuity with rather low "illumination", and a fall-off in acuity beyond this point, have been led to incorrect results by using non-logarithmic abscissæ.

Method

The experiment formed part of the test of visual acuity, which has already been described (p. 241). It differed from those of other investigators in that the subject was permitted to choose the luminance best suited to his vision, by control of the resistor included in the electrical circuit supplying the source of light.

The variable resistor was provided with a arbitrary scale. To calibrate this arbitrary scale, the luminance of the ground glass immediately behind the test-object was measured by an S.E.I. photometer, with the pointer of the resistor in various

in log. foot-lamberts, for a particular setting of the resistor, did not vary even if considerable changes in distance were made. The readings were graphed as shown intermediate values. The luminance could be increased by the subjects from 0 to more than 4·0 log. foot-lamberts.

It is to be noted that the values obtained represent the true luminance of the ground glass screen just behind the test-object. Reliable figures could not have been obtained if the photometer had been held at the distance of the subject's eye (2·5 metres), since the area of the ground glass was not sufficient to give readings unaffected by distance when the photometer was held so far away. It follows that although the luminances were measured by the appropriate method for the purpose intended, they do not provide a direct indication of the flux of light entering the eyes of the subjects.

It was soon noticed that many of the subjects, after first experimenting with various degrees of luminance, used a fairly bright light for counting the groups of stripes separated by 4′, but cut down the light when they were trying to count the more difficult groups, especially those separated by only 1′ or ½′. Indeed, a considerable number of them said aloud that they found it necessary to do this. The luminance was recorded (by noting down the position of the pointer on the scale of the resistor) as soon as the subject had *finished* his attempt to count the bright stripes on each test-object, i.e. when he had been trying to count the more difficult groups. Care was taken to avoid error arising from thoughtless movement of the pointer by the subject after he had finished announcing his counts.

Results

These are recorded in fig. 11. It will be noticed that, in accordance with Lythgoe's strong recommendation, the abscissa is logarithmic. Nevertheless, the results obtained do not bear out his contention that there is a continuous rise in visual acuity with increase of luminance. On the contrary, the modal luminance chosen by the subjects when trying to count the closer stripes was only 2·5 to 2·75 log. foot-lamberts (i.e. from about 316 to 562 foot-lamberts), though by the mere turning of a knob they could have used any amount of luminance up to 4 log. foot-lamberts and beyond (i.e. to well over 10,000 foot-lamberts).

It might be supposed that the subjects chose the luminance more or less at random. The experiment was conducted in such a way that this possibility could be tested, although the subjects had no knowledge that this was being done. The optical density of the bright stripes in test-object Z was twice that in test-object X, though in all other respects the two test-objects were closely similar (see Appendix I). It was impossible for the subjects to guess this, because they had their attention fixed on test-object Y after attempting to count the stripes on X and before attempting to count those on Z. It was therefore relevant to notice whether the subjects would use a stronger illumination with Z than with X.

The result of this investigation is set out graphically in fig. 12. The reader will at once notice the marked difference between the two histograms. There was a strong tendency for the subjects to use more light when the test-object was Z. Statistical study confirms this conclusion. The mean chosen luminance with X was 464 foot-lamberts; with test-object Z, 792. Thus the difference between the means was 328. The standard error of this difference was 83·34. The difference between the means was 3·94 times the standard error of the difference, and therefore highly significant statistically. *It follows that the subjects did not make random choices of luminance, but adjusted the luminance to their needs.*

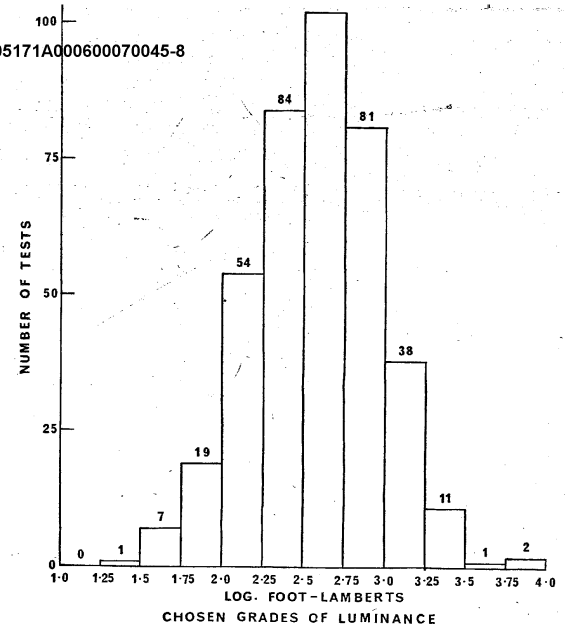

Fig. 11. Histogram showing the grades of luminance chosen by subjects in the tests of visual acuity. (The luminance is that of the ground glass plate placed 4 mm behind the test-object.)

Comment

The facts presented here prove that the subjects chose rather weak luminance of the test-object when they were trying to count the bright stripes subtending small angles at the eye. This result is contrary to the prevailing opinion, but the reason for the difference has already been made clear.

It is a remarkable fact that three independent observers (besides myself) who used *transmitted* light to illuminate their test-objects, found that the highest acuity was not obtained with the strongest light. Lister (1913), who used light reflected by a mirror from a white cloud on sunny days, sometimes found it necessary to interpose thin "gauze paper" between the mirror and the test-object, to moderate the light. Wilcox's (1932) bright stripes consisted of metallic mirrors, and although these were illuminated from the subject's side, yet the virtual image of the source of light was

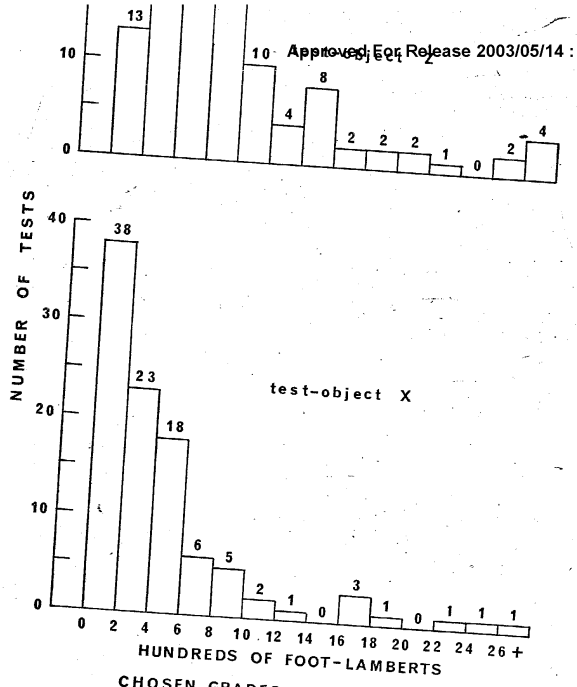

Fig. 12. Histograms showing that in the tests of visual acuity, the subject tended to choose different grades of luminance according to whether the test-object was X or Z.

necessarily behind the stripes, so that in effect transmitted light was used. It was Berger (1942) who first noticed that transmitted light differed from incident light in this respect. He did not use the expression "transmitted light", but wrote of "self-luminous objects". Actually, however, he illuminated transparent spaces in dark backgrounds from behind.

It is scarcely necessary to point out the significance of these findings for the microscopist, since transmitted is more commonly used than incident light in

[illegible] however, that Siller (1937), who used a complicated type of transmitted light, did not find any decrease in visual acuity with increase in "illumination".

THE DIAMETER OF THE PUPIL

Introduction

If the refractive properties of the component parts of the eye were such as to provide a perfect image-forming system, it would follow that a large pupil would give high resolving power. In fact, however, as is well known, the marginal rays are less well corrected than the intermediate and paraxial, and as a result the visual acuity scarcely changes when the diameter of the pupil increases beyond 2·0 mm (Stenström, 1964). It is perhaps for this reason that Michel (1964), in his discussion of the visual acuity of the eye in microscopy, assumes a pupillary diameter of 2·0 mm. The diameter is of interest, however, not only for its influence on visual acuity, but also because the diameter of the Ramsden circle of the eyepiece should be related to it. It was therefore regarded as important to obtain measurements of the pupillary diameter in persons actually using the microscope, partly because the diameter might be found to be less than 2·0 mm (in which case the visual acuity would be less), partly so as to provide reliable information on which to base the design of eyepieces.

Method

The diameter of the pupil was found by reliance on the consensual reaction, which ensures that in all normal persons the diameters of the two pupils are the same, even when they are differently illuminated (Steinach, 1887; Heddæus, 1904; Duke-Elder, 1932).

The microscope was set up in a vertical position. The object was a section of the testis of the newt, *Triturus* sp., dyed with iron hæmatein. A 4 mm objective and × 6 eyepiece were used. Köhler illumination was provided. The iris diaphragm of the substage condenser was set to give a cone of light filling about ¾ of the aperture of the objective.

Each subject chose for himself the intensity of the light by moving the control wheel of the variable resistor forming part of the microscope lamp.

The subjects of these experiments were all experienced microscopists. This limitation of the subjects to a particular group was imposed because others would not have had sufficient experience in the adjustment of the intensity of the light when making microscopical observations. In this set of experiments visual acuity was not directly concerned, and experienced microscopists who sometimes wore spectacles were therefore not excluded, provided that they were persons who were not accustomed to wear them when using the microscope. No one wore spectacles when the eye was photographed, since this would have altered the apparent size of the pupil.

A Zeiss-Ikon "Colora" 35 mm camera was used to photograph the eye that did not look down the tube of the microscope. The lens of the camera had a focal length of 45 mm. In front of it was placed a Zeiss "Proxar" double lens of focal length 10 cm. With the camera focused at infinity and the Proxar in position in front of it, the object to be photographed must be 105 mm from the front of the mount of the Proxar. A block of wood was provided, on which the camera was placed, facing vertically upwards. The thickness of the block was such that the front of the mount of the Proxar was 105 mm below the plane of the eyepoint of the eyepiece. The camera was placed with its optical axis separated from that of the microscope by

was taken. To calibrate the photographs obtained, an accurate ruler was placed horizontally in the plane of the eyepoint and photographed with the camera in the same position as when used to photograph the eyes of the subjects.

The photograph of the scale was projected on to a screen placed at such a distance that the magnification was exactly 10 times. The photographs of the eyes were projected with the same arrangement. The diameters of the images of the pupils were read off on the screen with a ruler, and divided by 10. Since the pupil approximates closely to a circle, the diameters were read in whichever direction the photographic image was clearest. In some cases reflections from the bench interfered with part of the image; in others the edge of the iris was only sharply defined in a particular direction, especially when it was dark brown.

Each recorded figure represents the diameter of the "entrance-pupil", that is to say, the apparent diameter of the pupil as viewed through the cornea. In what follows, the expression "diameter of the pupil" must be taken to mean the diameter of the entrance-pupil.

Sufficiently satisfactory photographs were obtained of the pupils of 15 subjects. Three of them provided two photographs each. Eighteen measurements were thus available.

If the head was not held in such a position that both eyes were at approximately the same distance above the bench as the eyepoint of the eyepiece, the photograph would not record accurately the size of the pupil; but the depth of focus of the lens-system of the camera (with Proxar lens) was very small at the apertures used, and photographs that were obviously out of focus were rejected.

Results

The mean diameter of the pupil in the 18 recorded measurements was 2·84 mm (standard deviation 0·35).

A certain amount of doubt was felt about the reliability of the measurements taken on the screen, because there were cases in which the exact limit of the pupil was not perfectly distinct, and an error of more than 1 mm (in the magnified image) was possible. All the photographs were therefore projected a second time, and measurements were made without any reference to the direction or result of those made earlier. The mean of the second set of measurements was 2·82 mm (standard deviation 0·32). It is clear that the method of measuring the photographs was capable of giving sufficiently accurate results, and that one may have some confidence in saying that the pupil has a mean diameter of about 2·8 mm when an experienced microscopist, using monocular vision, controls the illumination of a microscopical image. The extreme range was 2·2 to 3·5 mm in the first set of experiments and 2·3 to 3·4 mm in the second.

Comment

It is a familiar fact that emotional states may affect the size of the pupil (see e.g. Kuntz, 1934). Even the sight of an interesting picture may cause a change in the diameter of the pupil (Hess & Polt, 1960; Hess, 1965). However, there is no reason to suppose that the field of view exhibited to the subjects in this experiment was more interesting than any other ordinary microscopical preparation, and it did not appear that any subject was in an emotional state during the test.

Presumably there must be some ideal relationship between the diameter of the exit-pupil (Ramsden circle) of the eyepiece and that of the entrance-pupil of the eye; but it would not appear that any precise study of this subject has been published

measured). If the exit-pupil of the eyepiece were the smaller, some of the effective aperture of the eye might be wasted, to the detriment of the resolving power of the eye. If the exit-pupil of the eyepiece and entrance-pupil of the eye were of exactly the same size, the slightest movement of the head would cut off on one side some of the aperture of the lens-system of the microscope. In the entrance-pupil were the smaller, some of the aperture of the lens-system of the microscope would necessarily be wasted.

The diameters of the exit-pupils of the 8 eyepieces of moderate power, already mentioned, were measured. The mean diameter was 1·7 mm (extremes 1·0 and 2·0 mm). It is evident that with such eyepieces as these, no part of the aperture of the microscopical lens-system would be lost, but it is probable that with most of them the full resolving power of the eye would not be used.

ACKNOWLEDGMENTS

The work could not have been done without the unselfish co-operation of the subjects, more than 100 in number.

Mrs. Elizabeth G. M. Williams gave me a lot of help in different ways, as usual. I am also very grateful to the following: Mr. James M. McCrae (for help in setting up the apparatus used in measuring visual acuity); Mr. John Haywood and Mr. David Turner (photography, especially making the test-objects for visual acuity); Mrs. Sheila Bradbury (measuring the optical density of the test-objects); my wife (help in analysing the results); Professor E. B. Ford and Mr. G. J. Draper (advice on statistical methods); Dr. A. Pirie (organizing a discussion with authorities on vision); and Miss Gillyan Bennett (skilful secretarial help).

Appendix I. Details of the three test-objects for visual acuity.

Symbol for test-object	Number of bright stripes present				Optical density	
	1st group (4′ apart)	2nd group (2′ apart)	3rd group (1′ apart)	4th group (½′ apart)	of bright stripes	of background
X	3	4	3	4	0·5	3·4
Y	5	5	5	5	0·6	2·9
Z	3	4	3	3	1·0	3·5

It will be noticed that X and Z very closely resemble one another, *except* in the optical density of the bright stripes.

The symbol Xv is used to mean the test-object X placed with the bright stripes vertical.

Appendix II. Test for visual acuity: summary of results.

Test-object	Number of bright stripes	Number of persons (out of 100) who counted the bright stripes correctly			
		Stripes 4′ apart	Stripes 2′ apart	Stripes 1′ apart	Stripes ½′ apart
X	3	97		56	11
Z	3	98		39	10
Xv	3	99		39	5
X	4		87		
Z	4		76		
Xv	4		80		
Y	5	87	58	22	5

Appendix III. Comparison of the results of the test for visual acuity when the number of bright stripes was 3 or 4 with the results when the number was 5.

Test-objects	Separation of bright stripes	Number of bright stripes	Percentage of correct counts	Standard error of percentage	Differences between percentages	Standard error of differences between percentages	Number of times the differences between the percentages exceeds the standard error of the differences
X, Z, & Xv	2′	3	81 p.c.	2·26	23	5·43	4·2
Y	2′	5	58 p.c.	4·94			
X, Z, & Xv	1′	4	44⅔ p.c.	2·87	22⅔	5·04	4·5
Y	1′	5	22 p.c.	4·14			

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