The Changes of Water Distribution in Cytoplasm and Nuclear Sap during Division as Indicated by Changes in their Refractive Indices

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SUMMARY

The changes in refractive index of the cytoplasm and nuclear sap of the dividing spermatocytes of *Locusta migratoria* were measured by the refractometric technique first employed by Barer and Ross in 1952. It was found that, while certain restricted regions behaved slightly differently, the refractive index of the greater part of the cytoplasm fell during the first part of prophase from $1\cdot354$ in the resting cell to about $1\cdot346$, and rose again steadily after the beginning of metaphase until it approximated to its original value at the end of telophase. These results did not accord very closely with previous refractometric measurements, but showed a marked agreement with a previous record of changes in viscosity made by Carlson in 1946. It is suggested that this parallel behaviour is best explained by assuming that shifts in water distribution occur in the dividing cell, and that during prophase the cytoplasm and nuclear sap become correspondingly more aqueous as the chromosomes become denser.

INTRODUCTION

THE aim of the investigation was to try to determine the changes in refractive index that occur during cell-division by using the method of live-cell refractometry first employed by Barer and Ross in 1952, which involves the examination by phase-contrast microscopy of cells suspended in varying concentrations of isotonic protein. Since, however, as was pointed out by Barer in 1952, the great majority of the organic substances occurring in the cell have nearly the same refraction increment (that is to say, their solutions all exhibit nearly the same increase in refractive index for each 1 per cent. increase in their concentration w/v), such refractometric measurements can be regarded as giving a fairly close indication of the water and solid content of the cell and the changes occurring in it.

Previous work on the refractometry of dividing cells

Ever since 1882, when Flemming first recorded his impression that cell division was accompanied by alterations in the refractive index of the cell, this problem has, from time to time, aroused interest; but although several subsequent authorities confirmed Flemming's subjective impression, no actual measurements were attempted until 1921, when Vlès made a study of the changes occurring during the first cleavage division of the fertilized eggs of *Echinus*. His method of measuring the refractive index of the cells was to

regard them as spherical lenses and to measure their diameter and focal length in a parallel beam of light (the focal length depending on the refractive index of the sphere relative to its surrounding medium). The value he obtained for the refractive index of the egg just after fertilization was 1·385, which is approximately equal to a 28 per cent. solution of protein and is not an unduly high figure in view of the amount of granular yolk that these cells contain. During the subsequent division this value did not appreciably change until after metaphase, when, on the appearance of the cleavage furrow, he recorded a marked increase, to 1·405 or approximately 39 per cent. of protein, followed by a fall to about the previous value on the separation of the blastomeres. These results have, however, been criticized by Castle, who in 1933 used essentially the same technique for measuring the refractive index of the mycelial stalk of *Phycomyces* in connexion with an investigation of phototropism, and who expressed doubt as to whether the experimental error involved in measuring the focal distance permitted a refractive index measurement accurate to less than 2 units of the second decimal place. But the main disadvantage of this technique is that it can only give average values for the whole cell contents which are in fact rarely wholly homogeneous, least of all during division, and thus the values obtained may be quite unrelated to the actual refractive index of any one cell-constituent.

Another rather more extensive refractometric study of individual dividing cells was undertaken in 1951 by Pfeiffer, who used an entirely different method. This consisted of inserting into the cell cytoplasm a succession of fine glass micro-needles, each of known refractive index, and judging by the presence or absence under the microscope of the Becke line (which characteristically appears at the boundary of two media of different refractive indices), which kind of glass had a refractive index nearest to that of the cell cytoplasm. He obtained approximately the same values with four widely differing types of cell (*Glyceria* pollen mother-cells, *Tradescantia* staminal hair cells, *Nereis* eggs, and *Palaemon* glandular tissue), but these did not form any very easily interpretable pattern related to the successive stages of division, and were all very much higher than one might reasonably expect to get with rather watery cytoplasm. Their figures ranged from 1·44 at their lowest, which is equivalent to over 60 per cent. of protein, up to over 1·53, which is equal, or nearly equal, to the accepted values (quoted by Chamot and Mason, 1938) for the refractive indices of such almost completely dehydrated protein products as leather (1·53), wool, and dried casein (1·54). Consequently, although Pfeiffer states that in these cases the insertion of the micro-needles did not in any way interfere with the normal behaviour of the chromosomes, one can hardly avoid concluding that the technique must be at fault and probably damages the cells in some respect. The most likely suggestion, made by Dr. R. Barer, is that the cell contents in immediate contact with the glass may coagulate and form a thin protein precipitate round the needle, which, while amounting to no more than a very local injury, could very well considerably affect the appearance of the Becke line.

The material and method of the present investigation

Spermatocytes from the common locust—*Locusta migratoria migratorioides* —proved very suitable for this study because the resting cells were found to display considerable uniformity of refractive index, the value being equivalent to 11 per cent. of protein for both nucleus and cytoplasm in practically every case. The dividing cells, on the other hand, all gave, to a varying degree, lower values than this. They occurred sufficiently commonly for one to be reasonably certain of finding several stages in most preparations. In the living material the recognition of each stage with a 4-mm. objective is sometimes difficult— particularly the individual stages of prophase; but while no claim is made to have established them with complete certainty in every instance, this task was made very much easier through the loan, by Dr. J. R. Baker, of a very fine set of fixed preparations showing all the meiotic stages in the identical material (the spermatocytes of *L. migratoria*), which were used as a basis of comparison; and the existence of these demonstration slides was, of course, a further recommendation for using the material in question.

The technique itself, the theory of which is more fully discussed elsewhere, depends on the fact that when a cell immersed in a protein solution is examined by phase-contrast microscopy (with an ordinary positive, 90° phase-plate), its contents will give the normal darkish appearance only if the refractive index of the surrounding medium is less than that of the substances within the cell; and when a more concentrated medium is used with a refractive index greater than that of the cell contents, the latter will appear bright instead. Thus, when one finds a protein medium which approximately *matches* the cell contents in relative brightness, one can assume that the refractive indices of both are practically the same; or, in other words, the concentration of protein outside the cell is then about equal to the concentration of the organic solids comprising the cell protoplasm in question. It was found that by employing this criterion it was possible to detect quite easily refractive index differences of 0·0008, which is approximately equivalent to $\frac{1}{2}$ per cent. of protein.

An essential condition of this technique, of course, is that the protein medium must be isotonic with the cells in question, so that they do not swell or shrink and thus alter in refractive index. This was achieved by dissolving the protein in saline, the correct concentration of which had already been deduced from cell size-measurements on other material. It was found in the present study that a 20 per cent. solution of Armour's bovine plasma-albumin in distilled water caused almost exactly the same amount of swelling of the cells as a 0·2 per cent. solution of sodium chloride. A 20 per cent. solution of the plasma-albumin in 0·7 per cent. sodium chloride solution was therefore used, in order that the total osmotic pressure might be as close as possible to that of the blood of insects (that is to say, approximately of the same osmotic pressure as 0·9 per cent. sodium chloride solution). This albumin-saline solution was diluted with 0·9 per cent. sodium chloride solutions to the various concentrations required, their refractive indices being measured with a Bellingham and

Stanley pocket refractometer. After being dissected out in saline, fragments of the testis were teased in a watch-glass containing a relatively large quantity of the medium in question and preparations were made of the resulting cell-suspension. (These had the distinct advantage over ordinary wet microscope preparations in that they were self-sealing, the protein round the edge of the coverslip coagulating almost immediately and preventing further evaporation from taking place.) A 4-mm. phase-contrast objective was favoured as it facilitated searching and enabled the stages, when found, to be kept continuously under observation; while with a 2-mm. objective they were often moved out of the field through slight surges in the fluid.

Results and conclusions

The investigation was very greatly helped by the particularly fortunate find in one preparation of a cell actively dividing in a 9·5 per cent. protein solution, which was watched continuously over the space of 40 minutes as it progressed from late anaphase through to mid-telophase. This, in addition to providing an important extra piece of evidence in support of the contention that immersion in isotonic protein cannot greatly upset the cells, enabled all the successive changes in refractive index at these stages to be recorded, and these are fully indicated in the drawing in fig. 1. The remaining measurements, however, which included the much slower changes that occur in prophase, were derived from single observations and depended on finding and recognizing each stage.

The results obtained in the limited time available, which must be regarded as preliminary, are shown in table 1. It will be seen that in spite of a certain number of minor variations (which might be due either to incorrect recognition of the stage in question or to possible differences between the first and second divisions of meiosis, but which could also be due to individual variation in different cells), the results shown in the table indicate a reasonably consistent picture which can be stated as follows:

1. Cell division here is characterized by a marked fall during prophase of the refractive index of the cytoplasm. This reaches a minimum, apparently at pachytene, when the refractive index can be as much as 0·008 below that of the resting cell (1·3463). This represents a drop in protein concentration of about 4·5 per cent. During these stages the nuclear sap frequently appeared appreciably brighter than the surrounding cytoplasm. Ordinarily it is very difficult to make quantitive deductions about the refractive index of cell inclusions from their appearance under phase-contrast microscopy (see Barer and Tkaczyk, 1954). Here, however, a number of cases were observed in which the nuclear sap still appeared brighter than the background even when the refractive index of the cytoplasm was closely matched by that of the mounting medium, which suggests that such bright appearances may be truly indicative of a lower refractive index.

2. In the later stages—from metaphase onwards—the refractive index of the cytoplasm appears to rise again fairly steadily, until by late telophase it approximates to its original value, a rise of this kind being certainly observed

in the course of the observations covering late anaphase to mid-telophase described above. In this case also, a bright region of cytoplasm was found closely associated with the spindle. As this was observed first when the more peripheral cytoplasm approximately matched the background, it again seems probable that the refractive index of this region was lower, which would mean
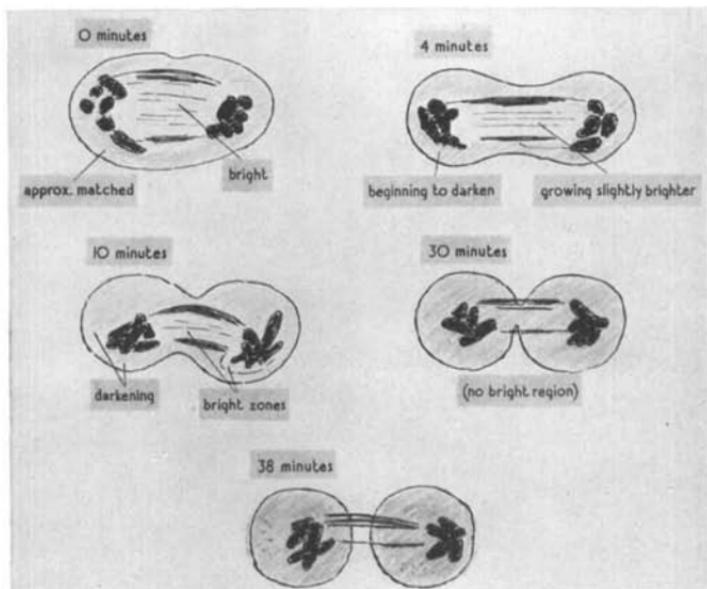

FIG. 1. Successive stages (anaphase to telophase) in a primary spermatocyte of *Locusta migratoria* actively dividing in 9·5 per cent. Armour bovine plasma-albumin, showing the relative brightness of the cell constituents when viewed with a 4-mm. 90° positive phase-contrast objective. (Semi-diagrammatic; from sketches by S. Tkaczyk.)

that the cytoplasm within the spindle was relatively more aqueous at this stage. (The fact that this region subsequently disappeared while the spindle structure persisted suggests that it was not just a halo effect due to the latter.)

3. The chromosomes themselves, right from the time of their first appearance, always appear as dark bodies in all the concentrations employed and must have a relatively much higher refractive index since they still appear dark even in a 31 per cent. protein solution (refractive index 1·390).

It will be seen that these results do not agree in any way with those of Pfeiffer; nor can they be strictly compared with the rather more credible figures obtained by Vlès, which are average values for the whole cell-contents including the chromosomes, although they accord with them in certain respects

TABLE I

The appearance, under a phase-contrast microscope, of the cell cytoplasm and nuclear sap of the spermatocytes of Locusta migratoria at different stages of division when immersed in various concentrations of isotonic bovine plasma-albumin.

Per cent. Protein	Resting cell	Leptotene/zygotene	Pachytene/diplotene	Diakinesis	Prometaphase/metaphase	Anaphase	Telophase
11 (R.I. 1·354)	Cytoplasm and nucleus both approximately matched		Cytoplasm and nuclear sap very bright		Cytoplasm very bright		Cytoplasm approximately matched or in some cases still faintly bright
9·5 (R.I. 1·351)	Cytoplasm and nucleus both dark		Cytoplasm and nuclear sap both bright	Cytoplasm faintly dark; nuclear sap bright		General cytoplasm matched or faintly dark → Cytoplasm within spindle bright Transitory brighter region round chromosomes	→ became progressively darker → became rather brighter before being occluded → disappeared } continuously observed for 40 minutes
8·5 (R.I. 1·349)	Cytoplasm and nucleus both dark	Cytoplasm dark; nuclear sap faintly bright	Cytoplasm matched; nuclear sap both bright	Cytoplasm and nucleus both bright	Cytoplasm faintly dark with a bright region round chromosomes		
6·7 (R.I. 1·346)	Cytoplasm and nucleus both dark	Cytoplasm and nucleus both matched	Cytoplasm and nucleus both approx. matched; in another instance both faintly bright		Cytoplasm dark except round chromosomes where it was approx. matched		Cytoplasm very dark

which will be discussed. They do, on the other hand, show a noticeable general agreement with the changes in cytoplasmic viscosity during mitosis recorded by Carlson in 1946 with the neuroblasts of the grasshopper *Chortophaga viridifasciata*. Using the amounts of observable mitochondrial movement as an index of relative viscosity, Carlson made a very careful study of all regions of the cytoplasm at every stage. His results showed that, in all the peripheral part of the cell, the viscosity of the cytoplasm falls during the latter part of prophase, and, after reaching its minimum at the end of metaphase, rises during anaphase and regains its original value in the early part of telophase. On the other hand he found that the viscosity of the cytoplasm within the spindle (which he calls the 'interzonal region') continued to fall throughout anaphase when the rest was rising. It can of course be argued that a parallel ought not to be drawn between this work on mitosis and the present results obtained from meiotic divisions, but there seems no reason why the general pattern of the *cytoplasmic* changes should be very different in each case.

One cannot avoid concluding that the most likely explanation for such parallel changes in both refractive index and viscosity would seem to be in terms of changes of water distribution within the cell: and indeed these changes are very much what one might expect to find as the chromosomes begin to assume the relatively dense form they have in division: if during prophase little or no water was exchanged between the cell and its surrounding medium, the condensation of the chromosomes would result in first, the nuclear sap, and then, as the nuclear membrane became permeable or broke down, the cytoplasm, becoming more aqueous; and at a later stage the formation of a relatively solid spindle may account for a related aqueous zone in the ground cytoplasm. After metaphase, on the other hand, the refractive index of the cytoplasm increases again before the chromatic material is to any appreciable degree redispersed, and so it looks as if true synaeresis must be involved at this stage; and it may be worth noting that it is in fact at about this stage that Vlès recorded a significant rise in total refractive index. It is also interesting that Vlès specifically mentions a few instances in which he found that his cells formed two images at different foci, and this of course is just what one would expect to find with his technique when the refractive indices of the cytoplasm and nuclear sap were different; and this may sometimes occur, according to the results of the present investigation.

Finally, it should be emphasized that although these investigations appear to form a coherent story, the conclusions are in fact based on relatively few findings and may quite possibly have to be modified when further work is undertaken.

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REFERENCES

BARER, R., and TKACZYK, S. 1954. Quart. Journ. Micr. Sci.
—— and Ross, K. F. A., 1952. J. Physiol. **118**, 38 P.
—— Ross, K. F. A., and TKACZYK, S. 1953. Nature, Lond., **171, 720.**
—— 1952. Ibid., **169,** 366.
CARLSON, J., 1946. Biol. Bul., **90, 109.**
CASTLE, I. S., 1933. J. Gen. Physiol., **17,** 41.
CHAMOT, E. M., and MASON, C. W., 1938. *Handbook of chemical microscopy*, vol. 1, 2nd edition. London (Chapman & Hall).
FLEMMING, W., 1882. *Zellsubstanz, Kern und Zelltheilung*, Leipzig (Engelmann).
PFEIFFER, H. H., 1951. La Cellule, **54,** 41.
VLÈS, F., 1921. C.R. Soc. Biol., **85,** 492 and 494.