

The use of the word 'dense' in microtechnique

By JOHN R. BAKER

(From the Cytological Laboratory, Department of Zoology, University Museum, Oxford)

Summary

The word 'dense' has been used in writings on microtechnique with 3 different meanings, to convey either (1) that a microscopical object is dense in the physical sense, or (2) that it is close-textured and therefore somewhat impermeable to the large, complex ions of certain dyes, or (3) simply that the object colours strongly with some particular dye. It is urged that the word should be used in microtechnique only in the first or physical sense.

THE word 'dense' has been used in 3 quite different senses in descriptions of dyed microscopical preparations, and considerable misunderstanding has resulted.

To clarify this matter, let us imagine a granule in a cell, consisting of nothing except a particular protein and some associated water (which will no longer be present in the finished preparation). If there is an excess of dibasic amino-acids in the protein, the granule will be acidophil, and we may therefore consider the reaction with an anionic dye.

If a dye that penetrates easily is used, the amount of it that can be taken up will depend on the number of basic groups that are present, and this—in granules of any particular size—will be proportional to the mass of protein in unit volume: that is to say, to the *density* in the sense in which that word is used in physics. This is the sense in which I have used the word density in my books on microtechnique (1958, 1960). A dense microscopical object, in this sense, will colour strongly with certain dyes simply because there is much matter in it: the more matter (of any particular kind), the more reactive groups there will be. If a smaller amount of the same kind of matter was associated with a larger bulk of water in a granule of the same size, it would obviously take up less dye.

Certain anionic dyes exist in aqueous or aqueous-alcoholic solutions in the form of large, complex ions. If such a dye be used, the intensity of colouring will depend largely on the *texture* of the granule. If it is close-textured, the dye will have difficulty in entering the granule. This will be particularly evident if two anionic dyes of contrasting colours are used in making the preparation, one of them existing in solution in the form of small ions (e.g. orange G), the other as large ions (e.g. methyl blue). If the small ions colour the granule strongly and the large ones weakly, the substance of the granule is close-textured. Certain authors describe such a granule as *dense*.

It must be noted that the denser an object in the first sense, the *more* strongly it will be coloured (if the dye can enter easily); the denser in the

second sense, the *less* strongly (unless the dye happens to penetrate close-textured objects very easily).

It is possible, of course, for an object to be dense in both these senses, and indeed an object that is very dense in the first sense is likely to be dense also in the second. The red blood-corpuscles of vertebrates provide an example of this. Chromatin, however, is dense in the first sense but easily permeable by nearly all cationic and anionic dyes. The results obtained with dyes that penetrate certain objects with difficulty will depend on the balance between the opposite effects of the two kinds of 'density'.

In descriptive papers in the fields of histology and micro-anatomy one often finds the word 'dense' used in a third or loose sense. The author simply states that an object is dense, without giving any clear indication of the meaning he attaches to the word. Careful study of such papers suggests that the intended meaning is simply that the object colours strongly with the particular dye used. Obviously an object cannot owe this kind of 'density' to its being particularly close-textured; but there is no reason why an object that is 'dense' in this third sense should necessarily be dense in the first or physical sense. If the substance of which the object is composed contains a very large number of reactive groups in unit mass, it will colour strongly with an appropriate dye even though its density in the first sense is low.

It seems undesirable to use the word 'dense' in different senses in physics and biology. The biologist must use a physical method, such as interferometry, if he wants to measure how much matter there is in a microscopical object, and when he has obtained his result he necessarily uses the word 'dense' exactly as the physicist does. It may be suggested that the expression 'close-textured' should replace 'dense' in the second sense. The third usage appears indefensible: it is better to say simply that an object is coloured strongly by a particular dye.

References

- BAKER, J. R. 1958. *Principles of biological microtechnique*. London (Methuen).
— 1960. *Cytological technique*. London (Methuen).