How Thin Should a Section Be? By V. E. Cosslett. (*From the Cavendish Laboratory, Cambridge, England.*)*

Ornstein (1956) has recently discussed the maximum useful thickness of a section for electron microscopy, as limited by the energy loss of the beam in traversing it and by the chromatic aberration of the objective lens. For working voltages of 50 to 60 kv. his conclusion is almost identical with that which we initially established experimentally (Cosslett, 1951) and later supported by theoretical arguments very similar to his (Cosslett, 1956): that the resolution cannot be expected to be better than one-tenth the thickness, for the normal run of biological sections. He proceeds, however, to discuss the special case of an openwork section observed at 100 kv., a voltage higher than usually used for sections; after removing most of the embedding material with the beam, he claims a resolution of 20 A from a thickness of 2000 A. We leave aside the question as to whether, on the definition of resolution basic to the treatment of chromatic aberration (point-point separation), 20 A has actually been resolved, as this cannot be judged from a reproduction. The important point is that the specimen observed is exceptionally favourable from the electron optical viewpoint as it consists of well separated features and these are line rather than point objects. Biological material is more usually of a ramified nature, with features of many shapes overlying each other.

The purpose of this note, whilst not denying that Ornstein's conclusions may be valid for certain special cases, is to warn against applying them to sections generally. A voltage of 50 to 60 kv. is usually more rewarding than 100 kv., as the objective aperture becomes inconveniently small at the latter voltage (especially from the contamination viewpoint), if contrast is to be preserved. It is also debatable whether it is desirable to remove most of the embedding material with the beam, from the standpoint both of artefact formation and of contamination. In any case it is more difficult to do so from sections embedded in araldite (Glauert *et al.*, 1956) or polyesters (Kellenberger *et al.*, 1956), which have certain advantages over methacrylate. For the general run of sections we see no reason

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to depart from the rule that a section should be no thicker than ten times the resolution sought. With specially favourable specimens, or working at 80 to 100 kv., this may leave a factor of 2 to 3 in hand; but this is very desirable since, in our experience, the ultramicrotomist's estimate of the thickness of his sections is as overoptimistic as is the electron microscopist's estimate of his resolution.

References

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