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Introductory Remarks

H. E. Huxley

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I. NEW DEVELOPMENTS IN INSTRUMENTATION

Introductory remarks

By H. E. Huxley, F.R.S.
M.R.C. Laboratory of Molecular Biology, Cambridge

The purposes we envisaged that this meeting might serve was to provide, especially for biologists, a review of the current position in electron microscopy and an estimate of what further developments are likely or feasible in the fairly near future. We are very fortunate that so many distinguished workers in this field have been able to come to the meeting, so that they can, in section I, describe the capabilities of the most advanced forms of electron microscope at present available, and the additional devices and techniques that can be used to improve the quality of the information that the instrument gives us about a specimen. Then, in section II, we will hear about various advances in specimen preparation techniques, especially ones depending on physical approaches to the problem and, in section III, about various ways of handling and manipulating the information recorded in the electron micrographs themselves so that we can extract from them the maximum amount of reliable information about the three-dimensional structures which are being examined.

I think that we are at a very interesting stage in this field and that this meeting may therefore be rather timely, for the following reason. For a long period now, since the early 1950s, the main preoccupation of most workers has been to explore the vast new areas of investigation opened up by the availability of reasonably reliable electron microscopes, by the development of methods for the adequate fixation of biological tissues, and by the advent of thin sectioning; and, later on, by the discovery of the negative staining technique. Although it was—and is—somewhat surprising that these techniques worked as well as they did, it fairly soon became apparent that the images they produced were moderately reliable, and, since there were so many interesting things to be looked at, the motivation to understand why the methods worked, and to develop the methods further, was, in general, not very strong. Furthermore, the microscope itself seemed very adequate—having extended the useful magnification which could be employed by a factor of about 100 over the best light-microscope, there was an enormous amount of new structural detail to examine and digest before one tried to push the useful instrumental resolution further still.

However, I think it is now true to say that a high proportion of the various principal working parts of different kinds of cells, down to the 2 nm level, have now been discovered; and quite a lot about their function is understood reasonably well at this limited resolution. Of course, there is a tremendous amount of work which now can be done, using this basic information and technique, to study and compare the behaviour of different cells in all sorts of different situations. But if we are to make substantial advances in our fundamental knowledge of the detailed structural basis of various biological processes, whether it be protein synthesis, muscular contraction, membrane permeability, or mitosis and cell division, it is becoming increasingly apparent that the 'classical' electron microscope techniques need to be improved quite substantially. Present methods of fixing biological material, and of staining it so that it gives

adequate contrast in the conventional electron microscope, give a stained representation of the original structure which is only faithful down to a resolution of about 2 nm at best. Thus, for example, the optical diffraction patterns given by electron micrographs of a large number of regularly repeating structures show virtually no reflexions further out than about 2 nm. Smaller scale details of structure are not reproduced in the stain distribution, and doubts about even larger structures exist in many cases. This limit of resolution is not set by the resolving power of the microscope, which nowadays may be better than 0.5 nm, but by the microscope's present requirement for a heavily stained specimen. It may also be—we do not really know this yet—that specimen damage in the microscope is going to be a limiting factor even if we are able to circumvent the staining difficulty. But, at all events, we very badly need electron microscopes which are better able to extract and record all the information possible about the interaction of the specimen and the electron beam, so that we can minimize or eliminate the necessity for staining, and so that we can get the best possible image information with the least possible damage to the specimen. It is clear that this is not done to best advantage in the usual conventional microscope, which relies on very crude and somewhat fortuitous mechanisms for producing contrast, namely the scattering of some electrons by the specimen at angles greater than the usable aperture of the objective lens, and the phase contrast effects introduced by defocusing.

It is fortunate—and not entirely coincidental, of course—that there are today a number of techniques appearing over the horizon which do indeed begin to show possible ways in which the situation might be improved. Section I will be concerned with a number of these techniques, and with their application in extending the useful resolution of the microscope, and in extracting as much information as possible about the specimen.

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