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PREFACE

The diversity of fields in which exponents of immunology may be found continues to surprise even its most enthusiastic adherents. It is not just the exquisite sensitivity and precision of immunological techniques that have served as the impetus for this diffusion. More significant appears to be the recognition of the broad biological impact of the relevant events that precede and follow an immune stimulus. Volume 11 exhibits such diversity unusually well, with physicist to parasitologist represented. The volume also includes three chapters by English scientists illustrating once again the continuing strong contribution of this group to immunology.

The first chapter deals with the exciting contributions of electron microscopy to the analysis of antibody molecules. Dr. Green initiated the use of small bivalent haptenes to link antibody molecules which has proven so successful in their characterization. The concept of a three-armed molecule with a flexible hinge region is well documented for γG globulin through the surprisingly clear electron micrographs obtained by this procedure. The ameboid appearance of γM molecules is most esthetically satisfying.

Dr. McDevitt and Dr. Benacerraf review the recent important findings concerning "immune response genes" in the second chapter. The fact that genetic factors are involved in the response to antigenic stimulus has long been known. However, the credit for establishing this on a firm scientific basis in terms of modern genetics must be given to the authors of this chapter. The use of synthetic polypeptide antigens played a major role in elucidating the multiple genes which are described. The intriguing question of at what level in the immune response these genes act remains to be determined. It appears clear that they do not represent the structural genes for the antibody molecule.

The third chapter has been contributed by Dr. Humphrey and Dr. Dourmashkin and deals with that most important of all complement questions, the terminal phase of cellular injury. Their most elegant electron microscope pictures of the holes in the cell membrane produced by complement have intrigued all immunologists. Considerable progress in the understanding of the underlying mechanism involved has been gained although the final answer is not yet in. Is an enzyme attacking lipid

moieties in the membrane primarily involved? Many unpublished studies of the authors relating to these questions are included in this fascinating review.

The fourth chapter by Dr. Perlmann and Dr. Holm deals with the complex problem of different types of cytotoxic effects of lymphoid cells. These outstanding workers in the field have managed to present a cohesive picture of the various effects on target cells. The role of "nonspecific" factors is particularly well clarified. The interrelationships among contact lysis, release of pharmacologically active substances, and the terminal components of the complement system are given special consideration. There is little question that significant developments concerning *in vivo* events will stem from these *in vitro* findings.

In another chapter Dr. H. S. Lawrence reviews the extensive and confusing literature on various factors involved in cellular immunity. Transfer factor, which he first described, is placed in perspective with the various substances under active current investigation in the guinea pig. This is a very enlightening review of an area of immunology from which much will be heard in the future. The methodology has been partially worked out for obtaining transfer factor, as well as some of the other materials, in sufficient purity for chemical analysis, and further results in this area are awaited with great interest. The assay systems remain difficult but the shift to *in vitro* systems has been a major achievement.

The last chapter by Dr. Ivor Brown deals with immunity in malaria, an old subject that has suddenly become of considerable current interest. New methods for the study of the relevant antibodies and a new appreciation for a role for cell-mediated immunity are responsible for this development. The very diverse contributions to this subject present unusual difficulties for a reviewer. However, a clear and interesting summary of the subject has emerged which should prove of considerable value as a reference for all immunologists.

The complete cooperation of the publishers in all aspects of the work involved in the production of Volume 11 is gratefully acknowledged.

H. G. KUNKEL
F. J. DIXON

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Electron Microscopy of the Immunoglobulins

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I. Introduction

Chemical techniques have provided extensive information about the structure of the constituent peptide chains of immunoglobulin molecules and the way in which they are linked to each other (reviewed by Cohen and Porter, 1964; Cohen and Milstein, 1967). They have also shown how the chains interact to give the compact Fab and Fc fragments which are produced by splitting of a few peptide and disulfide bonds. A different approach is required to determine the overall layout of the molecule and the spatial relationships of the fragments to each other. Before electron microscopy and X-ray crystallography had reached their present level of development the only approach to this problem was through hydrodynamics. Sedimentation, diffusion, and viscosity measurements consistently showed that the IgG molecule was either highly hydrated or asymmetric (Neurath, 1939; Oncley *et al.*, 1947). A plausible hydration of 0.2 ml./gm. was usually assumed, from which the axial ratio of about 6:1 was calculated. IgM has an even higher frictional ratio ($f/f_0 = 1.9$) (Miller and Metzger, 1965a), and in these terms would have an axial ratio of about 10:1. The asymmetry of IgG was supported by the early electron micrographs and a rod-shaped or ellipsoidal model was accepted for some time. However, in 1965, Noelken *et al.* pointed out that there were other possible interpretations of the high fractional ratio and intrinsic viscosity, which were more consistent with the chemical evidence. The Fab and Fc fragments showed normal fractional ratios and viscosities and so were not unusually hydrated or asymmetric. It was suggested that these three fragments were joined in Y formation by a relatively flexible

region of peptide chain, of which the extensive hydration could explain both the high frictional ratio and the susceptibility to enzymatic attack.

The contribution of electron microscopy to the solution of the problem provides the main subject for this review. Brief reviews of the subject have appeared elsewhere (Horne, 1965, 1968; Stanworth and Pardoe, 1967). The use of ferritin-labeled antibody as a specific marker for cellular constituents has been treated elsewhere (Andres *et al.*, 1967) and is also the subject of a forthcoming article (Rifkind, 1969), so it will not be considered here.

II. Electron Microscopy at the Molecular Level

The recent application of electron microscopy to the study of structure of macromolecules followed the exploitation of negative contrast methods for the study of viruses (Brenner and Horne, 1959).

The limiting factor both now and in the earlier work was not the resolving power of the microscope (about 5 Å.) but the difficulty in obtaining sufficient contrast with specimens of molecular dimensions (Valentine, 1961). Unless the thickness of a protein molecule is greater than 70 Å. (mol. wt. 150,000), it will not, if untreated, scatter a sufficient proportion of the incident electrons to render it visible against the usual background of carbon film. The first advance in technique was the use of metal shadowing (Williams and Wyckoff, 1945) which proved very successful with virus particles and was particularly useful for revealing detail of the surface and the height of the particle. The unavoidable granularity (20 Å.) of the evaporated metal film limited the effective magnification to about 50,000, which was not quite sufficient to reveal subunit structure in proteins. The method was, however, used to determine the lengths of some highly asymmetric molecules (Hall and Doty, 1958) and provided one of the earliest pictures of unattached antibody molecules (Hall *et al.*, 1959).

Two other general methods have been used for enhancing contrast. Positive staining, although satisfactory for sectioned material, is of little use at the molecular level because it is difficult to combine sufficient stain with the specimen (Valentine, 1961). Negative staining (more accurately, negative contrast), on the other hand, has proved to be both simple and effective for the study of viruses and of a variety of protein molecules. In its simplest form a droplet of the dilute (0.01%) protein solution, mixed with 2% sodium phosphotungstate or other suitable salt, is applied to a carbon (or nitrocellulose-supported carbon) film on the grid. After removing the excess fluid the film is allowed to dry. The molecules appear as low-density footprints in the thin layer of surrounding phosphotung-

state. Success depends on obtaining a faithful replica of the molecule in a uniform amorphous layer of a stain of high weight density. The properties and uses of various heavy metal salts have been discussed by Valentine and Horne (1962) and by Horne (1968).

Much of the work with antibodies has employed phosphotungstate, but recently silicotungstate, introduced by Wilcox *et al.* (1963) for use with viruses, has been found to give a slightly less granular background. It is more stable at neutral pH than phosphotungstate (Baker *et al.*, 1955) and the surface activity of its solutions causes it to spread more evenly at low protein concentration. A variation of the negative staining technique, described by Valentine *et al.* (1968) for use with enzymes, is worth repeating here with some additional details, in view of the excellent results it has given with antibodies. The molecules were picked up on carbon film deposited on freshly cleaved mica, by dipping the mica, film upward, into the protein solution (30–60 μg. antibody/ml.). The solution penetrated between the hydrophilic mica and the floating hydrophobic film and the molecules were adsorbed on the carbon in a few seconds. The film was transferred on the mica to a dish of 2% sodium silicotungstate, where it was left floating for a minute or two. A 400-mesh copper grid, coated with a thin layer of adhesive, was placed on the film, followed by a square of adsorbent paper (e.g., newsprint). The paper was removed together with the adhering grid and film and placed on filter paper to drain. The grid dries in a short time and is ready for examination. Uranyl salts have been used by Höglund (1967a,b) and give higher contrast and greater penetration than the tungstates, but this advantage is offset by the more granular background. Uranyl formate, in particular, penetrates further into molecules and between subunits (Leberman, 1965; Finch and Holmes, 1968), and this has been turned to advantage by Svehag *et al.* (1969) who were sometimes able to resolve H chains from each other and from L chains in fragments of IgM (see below). Objections that negative staining and drying may disrupt labile protein molecules are difficult to refute and cannot be disregarded. In some cases it has been shown that the biological activities of enzymes (Valentine *et al.*, 1968) and antibodies (Chesebro *et al.*, 1968) are not affected by drying down in the presence of the stain.

A further check for artifacts can be made by comparing the volume estimated from the linear dimensions with the molecular weight of the macromolecule (Rowe, 1966). The curves in Fig. 1 facilitate such estimations for spherical and cylindrical subunits. Unfortunately this can only be a very rough comparison since there are several possible sources of error in the measurement of overall linear dimensions. The blurred out-