Comments and Communications

The Structure of Antigen Films and Long-Range Forces

Recently, A. Rothen (*Science*, November 2, 1945, p. 446; *J. biol. Chem.*, 1947, 168, 75) has reported experiments involving the interaction between films of spread antigens and homologous antisera. The effects of the deposition of screens of barium stearate, octadecylamine, and Formvar on the antigenic film prior to treatment with antibody were studied. He found that these inert films did not completely prevent the specific immobilization of antibody. After considering and rejecting interpretations of these results in terms of holes in the screens and diffusion of the protein molecules, Rothen has proposed the conception of specific long-range forces, extending over 200 A, to account for his observations.

A basic assumption in Rothen's argument is the claim "that the antigenic films consisted of completely unfolded molecules, since the thickness per monolayer was found consistently between 8 and 9 A after transfer under 8 dynes of pressure" (*J. biol. Chem.*, 1947, 168, 79). It has appeared to us that this claim is unjustified and that, in fact, the antigenic film is not of constant thickness but may contain projections as high as 200 A. On this basis it is possible to account for Rothen's results without the need to assume specific long-range forces. We have carried out electron microscope examination of antigenic films, and the evidence obtained does indeed indicate an irregular structure.

The fallacy in the above quotation, as to the smoothness of the antigenic film, arises from the fact that the method employed by Rothen (*Rev. sci. Instr.*, 1945, 16, 26) determines only an average optical thickness and will not detect irregularities whose dimensions are small compared to the wave length of light. This is clear from the derivation of Drude's equations upon which this method is based (*Theory of Optics*. New York: Longmans' Green, 1913. P. 287).[1] Thus, since visible light was used, it is not excluded that there may have been peaks as high as 200 A in the antigen films.

In attempting to understand how an irregular film can arise from the original compressed monolayer of protein on water, it must be remembered that the protein layers are dried after transfer to the slide and before the adsorption experiment is conducted. The stability of a protein monolayer on water depends upon the interaction between water molecules and the polar side chains of the protein (H. B. Bull. *Adv. prot. Chem.*, 1947, III, 95). On drying, this stabilization effect is lost or greatly reduced. It is therefore necessary to consider the possibility that a refolding of the extended molecules occurs, followed by molecular aggregation or, perhaps, even the formation of microcrystals. The fact that reversible extension of protein molecules in solution occurs is well known, as, for example, in the case of urea-denatured serum albumin (H. Neurath, G. R. Cooper, and J. O. Erickson. *J. phys. Chem.*, 1942, 46, 203).

On the basis of such a picture, it is only necessary to assume that when the barrier films are laid down over the antigen film, the protein peaks project through and can thus interact directly with the antibody. It is to be expected that there will be a distribution in height of these peaks. As a result, the number of peaks exposed per unit area (or, preferably, the area of protein exposed per unit area of slide) would decrease with increasing thickness of blanket. This result is consistent with the experimental relation Rothen found between the amount of adsorbed antibody and the thickness of screen.

Other observations reported by Rothen can readily be accounted for in terms of the interpretation suggested above. For example, conditioning of a slide covered by an optical gage of barium stearate with uranyl acetate increases the number of layers of bovine albumin which can subsequently be deposited. Since such conditioning results in an increase of optical thickness of about 8 A, it would appear likely that there has been a deposition of uranyl salts, probably in the form of small crystals. It might be expected, then, that these crystals would act as polar sites on which the protein would be held and on which folding and aggregation might occur. Further, Rothen found that bovine serum albumin showed much more striking effects than did egg albumin. It appears to us as significant in this connection that bovine albumin can, after denaturation, much more readily revert to a soluble form resembling the native protein than can egg albumin (H. Neurath, J. P. Greenstein, F. W. Putnam, and J. O. Erickson. *Chem. Rev.*, 1944, 34, 243).

As mentioned above, we have sought confirmation of our views of the structure of the dried antigenic film by electron microscope examination. Monolayers of bovine serum albumin were prepared on twice-distilled water in accordance with Rothen's procedure.[2] The protein was then transferred to carefully cleaned glass slides by repeated immersion. The slides were rigidly held in a vertical position and were moved by rack and pinion. The transfer could be followed by the reduction in surface pressure resulting from the removal of protein from the water surface onto the slide. It was found that the transfer occurred only during the withdrawal of the slide from the water, since no change was observed during immersion. It was possible to transfer as many as 6 mono-

[1] Drude's method for measuring film thickness has been subject to question and, recently, refinements in the method have been reported (Lucy. *J. Chem. Phys.*, 1948, **16**, 167). It is reasonable to expect that a film with irregularities which are small compared to the wave length of the light used will cause plane polarized light to become elliptically polarized upon reflection through it, but the effective index of refraction and the relation between the actual mean thickness and the optical thickness given by the method requires further investigation.

[2] We are indebted to Dr. Hayashi, of Columbia University, for assistance in the preparation of the protein monolayers.

layers, a fact which in itself suggests the importance of the polarity of the surface in the retention of the protein. It should be pointed out that our antigen films differ from Rothen's in that his were deposited on an optical gage of barium stearate or octadecylamine.

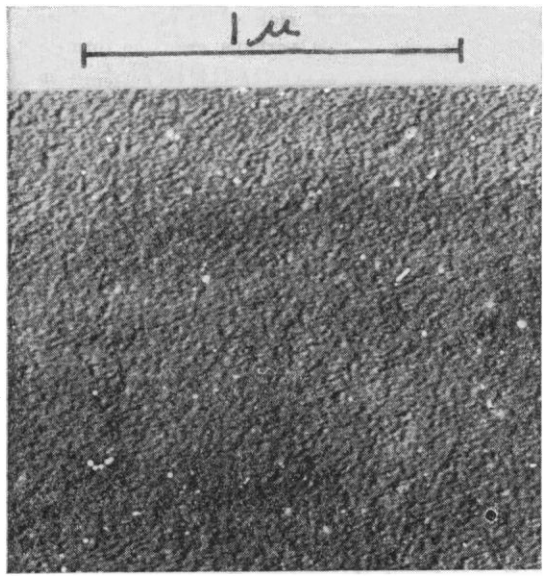

FIG. 1

A direct collodion replica of the protein film was prepared by casting in the usual manner from an amyl acetate solution. The collodion film was stripped and the negative replica obtained was shadowed with chromium at an angle of 1: 5. Fig. 1, representing a magnification of 50,000 ×, shows the appearance of the protein film resulting from the transfer of 6 monolayers of bovine serum albumin. What appear as depressions or pits correspond to ridges or peaks in the original protein film. The heights of these peaks can be calculated from the electron micrograph of the replica by assuming that their slopes are the same on both sides. Such measurements reveal that the projections in this case range generally between 50 and 85 A, with a few greater than 100 A.

In addition, the preshadow replica technique (R. C. Williams and R. W. G. Wyckoff. *J. appl. Phys.*, 1946, 17, 23) was used to prepare a direct positive representation of a slide with one monolayer of protein. Here, too, projections of significant proportions were evident. Control replicas of clean glass slides produced practically structureless micrographs, as expected from collodion cast on glass.

In conclusion, it appears to us that, in view of the considerations and evidence presented above, the claim that the experiments of Rothen establish the existence of *specific* long-range forces is unjustified at this time. It is perhaps unnecessary to remark that because of the fundamental significance of such a conception the most critical appraisal, both theoretical and experimental, of the hypothesis of specific long-range forces should be made before it is regarded as validated.

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Research and the Geographic Factor

In the February 6 issue of *Science* (pp. 127–130) Clarence Mills brought forward a strong indictment of existing methods and institutions concerned with the distribution of funds for research on the basis of discrimination against certain geographic areas by favoritism to others. In the April 16 issue of *Science* (p. 391), Thomas Turner has argued contrariwise to the effect that disbursing bodies are under obligation to place research funds where they believe the most productive immediate results will be achieved and that the results of research benefit not merely a community but the country as a whole. The points which Mills and Turner make are interesting, but both writers imply a certain deliberation in the distribution of funds according to an artificially simplified plan which may be questioned. In the past two years the American Neurological Association has been concerned with the collection of certain information which may be considered to cast some light upon the question of the geographic distribution of research funds. It was the specific intention of the American Neurological Association to determine what agencies had in the past allotted funds for research dealing with the nervous system, or for training in one of the disciplines concerned with that system. The Association was further interested in determining what proportion such aid bore to other aid made by the same agencies, whether financial assistance of this type had proportionally increased or declined, and what institutions and geographic areas had been so aided.

The over-all implications of the survey were clear and in conformity with expectation. The principal contributors to such research and training were governmental agencies, over-all aid had increased absolutely and had remained relatively unchanged (though certain basic fields dealing with the neural system were all but ignored), and most of the aid had gone to the larger and better-known institutions north of the Mason-Dixon line and east of the Mississippi.

The survey disclosed certain factors which have a direct bearing upon the last consideration. The agencies, foundations, and funds able and willing to give an account of their present expenditures were few. Still fewer were able and willing to compare their present with past disbursements. Very few indeed were able and willing to make any kind of a breakdown in terms of the exact disciplines aided. There are several obvious reasons why a comprehensive picture could not be obtained from all the agencies, funds, and foundations contacted. In the first place, the mortality among such organizations is high, and only a few of the better known have been in existence or have held to a consistent policy long enough

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