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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

THE ETIOLOGIC DIAGNOSIS OF UVEITIS BY THE METHOD
OF FLUORESCENT ANTIBODIES

Bulletin des societes d'ophtalmologie
(Bulletin of the Ophthalmologic
Societies) Vol. 65:268-70, 1965

M. R. HEITZ

The biochemist, to whom we have entrusted the aqueous humor of the eye in the case of uveitis, can only rarely satisfy our curiosity and provide us with a useful conclusion. His analysis too often consists of a simple estimation of proteins and research on streptococcic or toxoplasmic antibodies.

Then our studies suffer from lack of data! As a matter of fact, research on the aqueous humor runs into a great obstacle that is said to be insurmountable: the low volume of aqueous humor. Although the ophthalmologist will be proud to have studied two or three tenths of a cubic centimeter, the chemist will explain the impossibility of making precise analyses with such a low volume.

One might think that in the age of transistors, it would be possible to miniaturize traditional biochemical methods. I do not intend to cast aspersions on all micromethods - some, if not all, of which give us good results. But experience has shown us that the more the initial volume is reduced, the more errors occur. We can only subscribe to the overly well known sally of Marc Amsler: "Micromethods give micro-results".

Now then, a few years ago there was increasing talk of immunofluorescence. This technique, which emanates from America and which offers such sensational applications in all clinical areas, has only just scratched the surface in ophthalmology and it is quite a pity. The reason is that there is a method, the indirect immunofluorescence test, which is currently being used, especially for the diagnosis of syphilis and toxoplasmosis, and is enormously interesting in that it requires only a drop, or one-half of a drop, of liquid. This method offers a sensitivity, a specificity, and a reproductability equal to or greater than traditional methods.

What is the essence of this miracle method? Its principle is relatively simple. Let us take the example of toxoplasmic antibody research. In the first place, we prepare a smear of toxoplasmes, serving as an antigen, on a glass slide for the microscope. Then we deposit our drop or half-drop of aqueous humor on a small area of the preparation. The toxoplasmic antibodies, possibly present in our liquid, attach themselves to the toxoplasmic antigens. Finally, it will be enough to have these antibodies appear. This is what we do in making them fluorescent by the action of human antigamma-globulin conjugated by fluorescein isothiocyanate. Successive dilutions will be read by ultraviolet microscopy. The inverse ratio of the last solution still showing a net fluorescence will be called positive. To recapitulate, it is a matter of incorporating the antibodies from the two layers made up of the antigen, which here is the toxoplasm, and the fluorescent globulin. Hence the name two-layer method, from the indirect immunofluorescence method, or the sandwich method.

We repeat that this technique offers guarantees of specificity and sensitivity comparable to those of other methods. Moreover, it is relatively simple and is of satisfactory reproductability. Finally, let us recall a matter that is essential for research on the aqueous humor: handling requires only the volume necessary to cover a small portion of the microscope slide, or about five hundredths of a cubic centimeter. Because of all these qualities, this technique will probably spread very quickly and will replace micro-techniques currently in use that are rather artisanal.

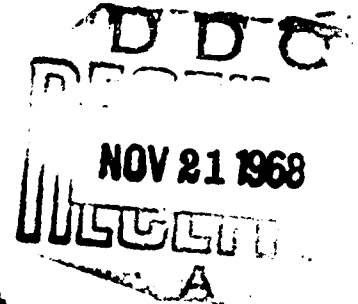
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applications. As has already been done in the field of general pathology, through numerous assays of bacterial, viral and tissue antibodies -- it is not impossible that these benefits will spread to the field of ophthalmology.