AUTO-IMMUNITY IN UVEITIS*

BY

E. S. PERKINS AND R. M. WOOD†

From the Institute of Ophthalmology, University of London, and the Wilmer Institute, Johns Hopkins Hospital, Baltimore, Maryland, U.S.A.

Since the discovery that auto-antibodies to thyroglobulin were present in cases of Hashimoto's disease (Doniach and Roitt, 1957), a search for auto-antibodies has been made in various diseases of otherwise obscure origin. Ophthalmology has already provided the first confirmed example of an auto-antibody causing disease in the condition endophthalmitis phako-anaphylactica; a similar explanation has been advanced in the case of sympathetic ophthalmitis (Woods, 1917).

Although there are many known causes of endogenous uveitis, there remains a large group of cases in which no direct infective cause has been demonstrated and in which the aetiology is obscure. This group of cases is typified by the acute anterior uveitis in adults which is usually considered as a manifestation of uveal hypersensitivity. Auto-immunity to uveal tissue, perhaps following alteration of its antigenic status by circulating toxins of bacterial origin, would provide a satisfactory explanation of the clinical findings. It must be emphasized, however, that the demonstration of circulating antibodies to a tissue is not a proof of auto-immune disease as the cause of the condition. It is quite likely that, in any disease in which breakdown of tissue occurs, some antibodies to this tissue might be produced as a secondary process following the original insult—traumatic, infective, etc.

Disturbances in serum gamma-globulin have been reported in uveitis (Woods and Stone, 1958), although no consistent pattern could be recognized—this suggests an immunological basis for some types of uveitis; but the only direct evidence of auto-antibodies to uveal tissue apart from cases of sympathetic ophthalmitis has been the report of Hallett, Wolkowicz, Leopold, Canamucio, and Wijewski (1962). They performed complement-fixation tests, using uveal tissue, kidney, and liver as antigen, on the sera from normal individuals, patients with uveitis, and patients suffering from other ocular conditions. Positive results were obtained in less than 10 per cent. of the normal sera, in about 52 per cent. of the uveitis group, and in about 32 per cent. of the group with other ocular diseases. There was no predominance of positive results in any one type of uveitis and the occurrence of 32 per cent. positive results in other eye diseases raises doubt concerning the specificity of the test. The authors suggest that there may be other hitherto unrecognized instances of auto-immune ocular disease accounting for the results in ocular diseases other than uveitis.

We have attempted to find evidence of auto-immune antibodies in the sera of uveitis patients, using the complement-fixation test and fluorescent antibody method.

* Received for publication May 27, 1963.

† This work was supported in part by United States Public Health Service grants NB 00043-15 and NB 03692-02.

Methods

Complement-Fixation Test

The procedure used for the complement-fixation method was that described by Kolmer, Spaulding, and Robinson (1951). Two antigen preparations were used: one antigen consisted of a suspension of uvea (choroid, ciliary body, and iris) and the other antigen was lens. Both antigens were prepared from freshly enucleated bovine eyes.

To the uveal tissue of each eye 5 ml. buffered physiological saline (pH 7.3) was added. The tissues were then homogenized in the Waring blender for 5 minutes. The suspension was centrifuged at 1500 G for 15 minutes and the supernate removed and used as antigen.

The lens antigen was prepared by homogenizing twelve bovine lenses in 120 ml. buffered physiological saline for 5 minutes in the Waring blender. This suspension was then centrifuged at 1500 G for 30 minutes and the supernate used as antigen.

Fluorescent Antibody Test

The indirect reaction was used in most instances. In this method the serum to be tested is applied to the antigen (ocular tissues) and the presence of any globulin fixed to the antigen detected by applying a fluorescent-labelled anti-globulin.

Frozen sections of human and guinea-pig eyes 4 μ in thickness were mounted on clean glass slides, dried in air at room temperature, and fixed in cold acetone for 3 minutes. The sera to be tested were diluted with saline and placed on the sections. The slides were kept in a moist box and gently agitated in a mechanical shaker for 30 minutes; they were then washed in three changes of saline for 3 minutes each time. Fluorescein-conjugated anti-human gamma-globulin (Progressive Laboratories, Baltimore 28, Md., U.S.A.), diluted in saline 1:20, was added to the sections, and these were again agitated for 30 minutes and washed as before in saline. After the excess saline had been shaken off, a drop of buffered glycerine and a cover slip were applied and the slides were examined under the ultra-violet microscope.

Results

Complement-Fixation Test

(1) *Using Uveal Extract as Antigen.*—Preliminary screening of sera from 36 patients with uveitis gave five (13.8 per cent.) positive results, as shown in Table I.

TABLE I

DETAILS OF FIVE POSITIVE COMPLEMENT-FIXATION TESTS USING BOVINE UVEAL TISSUE AS ANTIGEN

(29 TESTS ON OTHER SERA WERE NEGATIVE)

Serum No.	Diagnosis of Uveitis	Titre
C. 1918	Heterochromic	1:80
C. 2068		1:80
C. 827	Acute anterior	1:80
C. 1266	Acute posterior	1:40
C. 2000		1:20

Tests using lens as antigen were then found to give more satisfactory results and complement-fixation tests using uvea as antigen were discontinued.

(2) *Using Bovine Lens as Antigen*

(a) CONTROL SERA.—175 sera were obtained from the blood bank. Of these 170 were negative, and three were positive at a titre of less than 1:5, one at 1:5, and one at 1:10.

(b) UVEITIS SERA.—Initial tests on stored sera showed that a high proportion of the sera were anti-complementary and, although it seemed likely that this was due to the fact that they had been stored for a long period, further investigations of this phenomenon are in progress by one of us (R.M.W.).

In the results quoted below (Tables II and III), anti-complementary sera have been excluded, excepting those which were positive in dilutions beyond the anti-complementary end-point. The total number of positives shown in Table III is lower than that in Table II,

TABLE II
RESULTS OF COMPLEMENT-FIXATION TESTS USING BOVINE LENS AS ANTIGEN

Diagnosis of Uveitis		Total Number of Sera	Positive Sera	
			Number	Per cent.
Anterior	Acute	55	32	58
	Chronic	21	15	71.4
Posterior	Acute	53	34	64
	Chronic	25	16	64
Generalized	Acute	14	6	43
	Chronic	27	15	55
Total		195	118	60

TABLE III
HEIGHT OF TITRE OF POSITIVE COMPLEMENT-FIXATION TESTS SHOWN IN TABLE II

Type of Uveitis		Titre					
		< 1:5	1:5	1:10	1:20	1:40	1:80
Anterior	Acute	3	5	3	12	4	2
	Chronic	3	1	4	3	2	0
Posterior	Acute	5	7	4	10	5	0
	Chronic	2	1	4	8	1	0
Generalized	Acute	0	1	2	3	0	0
	Chronic	3	3	7	0	2	0
Total		16	18	24	36	14	2

as more than one sample of serum was available in some of the cases reported in Table II. The results of repeated tests are shown in Table IV.

TABLE IV
RESULTS OF COMPLEMENT-FIXATION TESTS ON MORE THAN ONE SAMPLE OF SERUM

Results of Tests	No. of Cases	Maximum Time Interval (yrs)
First positive and repeat positive	13	5
First positive and repeat negative	4	1½
First negative and repeat negative	6	1

The interval between testing in this series was very variable, and it is difficult to draw firm conclusions. It can be said, however, that in nineteen out of 23 cases the test gave the same result on two separate occasions.

Discussion of Complement-Fixation Results.—As no control sera were tested with uveal antigen, this discussion will be confined to the results obtained with lens as antigen. These results show a remarkably high incidence of positive complement-fixation tests in the uveitis cases compared with the control (blood bank) sera. However, as Hallett and others (1962) found, there is little difference in the percentage of positive tests between the different clinical categories of uveitis. If the complement-fixation test indicates the presence or absence of antibodies to the antigen used, then these results must mean that antibodies to lens were present in the sera of over half the cases of uveitis studied. This is a very surprising finding, as only rarely is there any clinical evidence of reaction to the lens in cases of uveitis, *e.g.* in endophthalmitis phako-anaphylactica. Cataract may ensue in any long-standing case of uveitis, but this is usually assumed to be due to disease of the ciliary body interfering with the nutrition of the lens.

We know that other tissues contain components which behave antigenically like some constituent of lens (Perkins and Wood, 1963), and it is possible that the antibodies demonstrated in these cases of uveitis are really auto-antibodies to some other tissue—uvea, for example—in which a similar antigen to lens is present.

In view of the low incidence of positive results in the control sera, the results can hardly be dismissed as “non-specific”. As yet we do not know whether the test is specific for uveitis, and it is possible that other diseases may be associated with positive complement-fixation tests using lens as antigen. The analogy with the Wassermann reaction comes to mind in this context. This reaction is essentially a complement-fixation test using a tissue extract as antigen, and yet it is highly specific not for a disease affecting one particular tissue but for an infection with a particular organism. The wide variety of clinical conditions shown here to give a positive complement-fixation test suggests that a single infective agent is unlikely to be responsible for all the positive results. If it is there are at the moment no clues as to its nature.

For the moment, the simplest explanation is to accept that in many cases of uveitis the serum contains antibodies to one or more antigenic components of bovine lens.

Fluorescent Antibody Method

(1) Before the complement-fixation tests were done, a series of 115 sera from uveitis cases was tested undiluted. It was hoped to find some sera which gave strongly positive results so that these could be studied in serial dilution. Unfortunately none of the sections studied was unequivocally positive, although seven out of 115 sera seemed to give a more marked fluorescence in the anterior uveal tissues, particularly in the ciliary body. In some cases the lens also fluoresced more strongly than with sera from cases of uveitis and with normal sera. These seven sera were then tested in a dilution of 1:5 and compared with normal sera in the same dilution. Again, there appeared to be more fluorescence in the uveal tissues of the sections treated with the uveitis sera.

(2) Selected sera from cases of active anterior uveitis, in some associated with Reiter's syndrome, were compared with a series of normal sera at dilutions of 1:10 and 1:20. Again, the impression was that the stroma of the ciliary body fluoresced more strongly with the uveitis sera than with the normal. However, the differences were small, and no specific structures could be seen which took up fluorescein in the sections treated with uveitis serum which did not show with the normal serum.

(3) Five sera from patients with sympathetic ophthalmitis showed a definite staining with fluorescein in the choroid as compared with normal sera. These sera were also run against extracts of uveal tissue dried on slides, and two sera which showed up as definitely positive using this method were the two which showed the most clear-cut increase in fluorescence on the sections.

(4) The sera from seven patients with Fuchs's heterochromia were compared with normal sera. No differences in fluorescent staining between the patients' sera and the normal could be detected.

(5) Sections of normal human prostate were prepared and treated with sera from eight cases of Reiter's syndrome. No specific differences in fluorescence could be observed between sections treated with the patients' sera and the normal controls.

(6) When positive results were obtained with the complement-fixation test using uveal antigen, these sera were tested using the fluorescent antibody method on sections of human and guinea-pig eyes.

Human Sections.—Human sections were treated with the first two sera giving positive complement-fixation tests and with one normal serum, in dilutions of 1:5 and 1:10. In both dilutions the sections treated with the positive sera showed increased fluorescence in the lens, iris, and ciliary body after application of fluorescein-conjugated anti-gamma-globulin. In the iris and ciliary body the increased fluorescence was mainly due to a bright line on the surface of the epithelial cells, with little change in the stroma.

Guinea-pig Sections.—The same sera were also tested on freshly sectioned guinea-pig eyes. After processing there appeared to be a specific line of staining along the margin of the pigmented epithelium of the iris on sections treated with the positive sera. The lens fibres also fluoresced more strongly with these than with the normal sera, and there was some increased fluorescence in the supporting tissue of the retina. With one positive serum there was also increased fluorescence in the corneal stroma and interstitial tissue of the extra-ocular muscle fibres—the whole picture being reminiscent of sections treated with anti-serum to guinea-pig uveal tissue.

In view of these results on the guinea-pig sections, a further series of sera from normal subjects and uveitis patients was tested on guinea-pig sections, paying particular attention to the variations in fluorescence in the lens. Four normal sera and ten uveitis sera were used in a dilution of 1:10. Four of the uveitis sera showed a definite increase in fluorescence of the lens. Photographs (Ektachrome transparencies) were taken of the lens on the sections, choosing areas of approximately equal thickness. Measurements of the transmission of white (tungsten) light were made on the transparencies by using a photo-electric cell and galvanometer. Table V shows the results on these sera.

The correlation between the complement-fixation results, the subjective evaluation of the slides, and the photo-electric determinations of the photographs is good in one (L.R.) of the three sera on which the complement-fixation test was done, but not in the other two. The photographs and photo-electric measurements are probably more reliable than the subjective results of examining the slides, although three of the four thought to be positive on inspection were confirmed positive by the photo-electric measurements of the transparencies. It must be remembered that uveal antigen was used for the complement-fixation tests and the fluorescent results apply only to lens.

The differences between some of the uveitis sera and normal sera were definitely easier to detect on sections of guinea-pig eye than on human sections. The reason for this is probably concerned with the freshness of the material. No human eyes were obtained less than 12 hours after death, and, although they were subsequently kept at $-20^{\circ}\text{C}.$, it was noticeable that there was a steady deterioration in the staining-properties of the sections during storage. The guinea-pig eyes on the other hand were removed from freshly killed animals and sectioned immediately.

No conclusions regarding the type of uveitis most likely to be associated with auto-immune antibodies can be drawn from this small series of cases. There is a suggestion that a higher proportion of positives occurs in sympathetic ophthalmitis. The most dramatic result shown in Table V (opposite) is the serum from L.R. This patient was a girl aged 8 years who had a chronic posterior uveitis with fundus lesions very suggestive of infestation with *Toxocara canis*. However, a rather similar case also thought to be due to *Toxocara canis* gave negative results with both the complement-fixation test and the fluorescent antibody test.

Summary of Results

The findings of Hallett and others (1962) that auto-immune antibodies, as demonstrated by complement-fixation tests using ocular tissue as antigen, are present in the sera from some cases of uveitis have been confirmed.

Antibodies to human uveal tissue in some sera from uveitis cases were also demonstrated by the fluorescent antibody technique. Sera giving positive results with human tissue were also found to give positive results with guinea-pig tissues.

Discussion

Perhaps the most disappointing aspect of this work and that of Hallett and others (1962) is the apparent lack of specificity in the results. With the possible exception

TABLE V

CORRELATION BETWEEN COMPLEMENT-FIXATION TESTS USING BOVINE UVEA AS ANTIGEN, FLUORESCENT RESULTS USING SECTIONS OF GUINEA-PIG EYES, AND PHOTO-ELECTRIC MEASUREMENT OF FLUORESCENCE IN LENS OF GUINEA-PIG SECTIONS

Sera	Complement-fixation Result	Fluorescent Result	Photo-electric Measurement (Galvanometer Scale Reading)
Uveitis	2272	Positive 1:40	Negative
	S.R.		Negative
	2170		Negative
	L.R.	Positive 1:80	Positive
	1874		Negative
	643		Negative
	935		Positive
	2615		Positive
	1262		Positive
	2114	Positive 1:10	Negative
Mean			9.08
Normal	309	Negative	5.5
	308	Negative	6.2
	130	Negative	7.2
	258	Negative	6.0
Mean			6.2

of sympathetic ophthalmitis, no particular type of uveitis seems to be associated with the presence of auto-antibodies. Hallett and others found auto-antibodies in cases of uveitis in which an infective basis was probable. They suggest that auto-immune disease and infection are not mutually exclusive but that uveal inflammation initiated by infection could be perpetuated by an auto-immune response. If such a sequence of events takes place in uveitis, it would seem likely that a similar sequence occurs in all infective conditions and does not imply that uveitis should be included among the auto-immune diseases.

According to Burnet (1962), the diseases which either have been or may eventually be shown to have an auto-immune aetiology may be listed as in Table VI.

TABLE VI

CLASSIFICATION OF DISEASE CONDITIONS KNOWN OR SUSPECTED TO BE OF AUTO-IMMUNE NATURE (BURNET, 1962)

I	II	III	IV
Hashimoto's disease	Systemic lupus erythematosus	Rheumatic fever	Macroglobulinaemia
Myasthenia gravis	Acquired haemolytic anaemia	Glomerulonephritis	Hypergammaglobulinaemia
Idiopathic Addison's disease	Rheumatoid arthritis	Secondary acquired haemolytic anaemia	
Sjögren's disease	Dermatomyositis, etc.		
Acute encephalopathy			
Polyneuritis			
Multiple sclerosis			

Group I (typified by Hashimoto's disease) consists of conditions which result from immunological attack on constituents which are specific for a single organ or group of organs and appear to be directed against antigenic determinants which rarely come into the extracellular environment, including in this the blood and lymph circulation.

Group II contains the classical auto-immune diseases where the attack is on common, widely distributed components of the tissues (typified by systemic lupus erythematosus).

Group III contains conditions which are apparently initiated by some bacterial (usually streptococcal) or virus infection and undergo more or less rapid remission when the infection is eliminated.

Group IV includes conditions on the borderline between auto-immune diseases and neoplasia.

If uveitis is an auto-immune disease, where would it fit in Burnet's classification? Group I demands a tissue isolated from the bloodstream—a condition possibly fulfilled by the uveal tract, although the undoubted barrier between the aqueous and the bloodstream does not imply a barrier between the uveal tissue and the bloodstream. The lens, however, is an ideal tissue in this context and endophthalmitis phako-anaphylactica clearly comes into this group of auto-immune diseases. The possibility of antibodies to lens being of importance in other types of uveitis is suggested by the demonstration of such antibodies in the sera from cases of uveitis not obviously resulting from the liberation of lens protein into the eye.

It has been shown (Maisel and Langman, 1961; Perkins and Wood, 1963) that the uveal tissue contains an antigen very similar to one of the antigenic components of the lens, and if this antigen was involved in the auto-immune process it could account for the positive results with lens reported here. The conditions in Group I can be considered as "disturbed antigen" diseases, compared with Group II in which "disturbed tolerance" is the main factor (Hijmans, Doniach, Roitt, and Holborow 1961). Some of the factors ascribed to "disturbed antigen" diseases by these authors hold good for uveitis, and if uveitis is an auto-immune disease it would seem to fit best into this category. Burnet's Group III does bring the infective aspect to the fore, and it may be that the auto-antibodies which have been demonstrated in cases of uveitis in which infection is thought to play a dominant part belong to this group.

There are some general characteristics of auto-immune diseases (Burnet, 1962), and it is interesting to consider uveitis in this context:

(1) A constitutional background is involved and there is evidence that genetic and familial factors are of importance in Hashimoto's disease and systemic lupus. There is no evidence of such factors in uveitis.

(2) In virtually all the accepted auto-immune diseases women are more frequently affected than men. There are differences in the sex distribution of different types of uveitis (Perkins, 1961), but in the cases in which an infective aetiology seems likely (toxoplasmosis, for example), the incidence is equal in the two sexes, whereas in the non-specific inflammations in which an immunological disease process seems more likely more cases occur in men than in women.

(3) The diseases in Groups I and II are often progressive and fatal conditions. Although there are some types of uveitis (that associated with Behçet's disease, for example) which run a progressive course and end in destruction of the eye, the vast majority of cases are episodic and tend to recover.

(4) Many patients show some symptoms or signs of some auto-immune condition other than that of his or her primary diagnosis. With the possible exception of rheumatoid arthritis, uveitis does not occur in association with any of the known or suspected auto-immune diseases.

Uveitis does not fit easily into these general characteristics of auto-immune disease, but in the present state of knowledge it would be rash to exclude the possibility now that auto-antibodies to uveal tissue have been demonstrated in some cases.

Summary

Sera from cases of uveitis were examined for the presence of antibodies to ocular tissues using the complement-fixation and fluorescent antibody techniques.

175 sera from blood donors gave 2·8 per cent. positive complement-fixation tests using bovine lens as antigen, whereas the test was positive in 60 per cent. of sera from cases of uveitis. 13·8 per cent. of positive complement-fixation tests were obtained in a series of 36 sera from uveitis patients when bovine uvea was used as the antigen.

The presence of antibodies to uveal and lens tissue in some sera from uveitis patients was also shown by the fluorescent antibody method on frozen sections of human and guinea-pig eyes.

There did not seem to be any correlation between the type of uveitis and the results of the tests.

The findings are discussed in the light of our knowledge of other auto-immune diseases.

Table VI is included by kind permission of the author and by courtesy of the honorary editors of the *Proceedings of the Royal Society of Medicine*.

REFERENCES

- BURNET, MACFARLANE (1962). *Proc. roy. Soc. Med.*, **55**, 619.
 DONIACH, D., and ROITT, I. M. (1957). *J. clin. Endocr.*, **17**, 1293.
 HALLETT, J. W., WOLKOWICZ, M. I., LEOPOLD, I. H., CANAMUCIO, C., and WIJEWSKI, E. (1962). *Arch. Ophthalm.*, **68**, 168.
 HIJMANS, W., DONIACH, D., ROITT, I. M., and HOLBOROW, E. J. (1961). *Brit. med. J.*, **2**, 909.
 KOLMER, J. A., SPAULDING, E. H., and ROBINSON, H. W. (1951) "Approved Laboratory Technic", 5th ed., p. 797. Appleton Century-Crofts, New York.
 MAISEL, H., and LANGMAN, J. (1961). *Anat. Rec.*, **140**, 183.
 PERKINS, E. S. (1961). "Uveitis and Toxoplasmosis". Churchill, London.
 ——— and WOOD, R. M. (1963). *Exp. Eye Res.*, **2**, 255.
 WOODS, A. C. (1917). *Arch. Ophthalm.*, **46**, 283.
 ——— and STONE, H. H. (1958). *Amer. J. Ophthalm.*, **45**, 11.