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Some Aspects of the Fluorescent Antibody Test for Bilharziasis

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Introduction

During the second half of 1963 and early in 1964 much interest was aroused by what appeared to be an excellent new screening test for detecting patients possibly suffering from bilharziasis, using the fluorescent antibody technique (Cookson, 1963). The test is within the scope of a routine laboratory, provided that experienced staff and a regular supply of cercariae can be obtained. In response to many enquiries this laboratory started to use the test, the Bilharzia Research Laboratory kindly supplying the cercariae. The technique followed was that of Cookson, as in use at that time at the Bilharzia Research Laboratory.

With any new test it takes time to evaluate results, but it became apparent in the first few months of using the Fluorescent Antibody Test (thereafter referred to as the F.A. test) that more positive results were being obtained than statistics would indicate; and, secondly, a small number of negative results were obtained in persons known to be excreting viable schistosoma ova. The Bilharzia Research Laboratory was finding similar results.

It seemed a pity to discredit and discard this test after such a short time, especially since the test was also in use in South Africa and the U.S.A.

Much basic research had been done at the Walter Reed hospital in America by Sadun, Anderson and co-workers (1961), and their technique differed in several aspects from that currently used in Salisbury. Most of their work was published in American scientific journals, but these were obtained through the co-operation of the Bilharzia Research Laboratory and the University College. It was decided to compare their technique with Cookson's method and attempt to reach some definite conclusions regarding their respective accuracy.

Sources of Material

Cercariae.—*S. mansoni* were initially supplied to us stained and ready for use, but during this work we requested live cercariae for staining and fixing by Sadun's method.

Rhodamine.—Lissamine Rhodamine R.B.200 conjugated to bovine albumen was obtained from Difco and Micro-Biological Associates.

Serum.—Serum from patients sent to the laboratory for possible bilharziasis, from known positive volunteers and from blood donors of different blood groups was used in the tests.

Fluorescent Coombs Reagent.—Fluorescent anti-human globulin was obtained from Difco, Stayne, Progressive Laboratories Inc., Sylvana Co. and Burroughs Wellcome, plus a small quantity prepared in the Bilharzia Research Laboratory. For reasons unknown, Difco and Stayne have found difficulty in providing a satisfactory fluorescent A.H.G., while we obtained poor results with the products of two other suppliers.

Technique

We do not intend going into the details of the Coombs technique in general or of fluorescent microscopy; firms producing U.V. microscopes will guide laboratories in their use and difficulties, while there are a number of excellent books on fluorescent methods, e.g., *Nairn-Fluorescent Protein Tracing* (E. & S. Livingstone Ltd.). However, a brief outline follows for those unacquainted with the nature of this type of test.

The patient's serum is added to a buffered suspension of dead cercariae. If antibodies to bilharziasis are present in the serum, these become attached to the surface of the cercariae.

After washing off the unadsorbed serum the cercariae are treated with anti-human globulin reagent (prepared in rabbits, horses or goats) which has been tagged with a fluorescent dye.

This reagent will, in turn, adhere to any previously adsorbed human antibody on the surface of the cercariae.

The excess reagent is washed off and the cercariae viewed under the ultra-violet microscope. In positive tests the cercariae are outlined by a rim of brilliant yellow-green fluorescence. Negative tests show simply a dull

red colour resulting from preliminary counter-staining with Rhodamine (*vide* infra). Doubtful results show either a very narrow rim of fluorescence or uneven, broken fluorescence.

The Two Techniques

The techniques of Cookson (1963) and of Sadun *et al.* (1960) differ in the following main essentials:

(1) The need to inactivate the serum at 56° C.—recommended by Sadun, but not mentioned by Cookson.

(2) The vital-staining of cercariae with Rhodamine to block non-specific internal fluorescence in cercariae used after 24 hours storage—recommended by Sadun *et al.* (1960). Cookson used Rhodamine simply to counter-stain dead cercariae. (It is convenient to prepare large numbers of cercariae at one time and store them for future use.)

(3) Dilution of the serum 1 in 4 with buffer to avoid interference from non-specific antibodies—recommended by Sadun, but not mentioned by Cookson.

Discussion on Differences

(1) Heat inactivation of serum is routine in serological tests of this nature and had been employed in this laboratory from the beginning. It destroys many non-specific antibodies, leaving intact the important immune antibodies.

(2) The effect of vital-staining of the cercariae with Rhodamine is apparent in the final stage of the test when viewing the cercariae under the U.V. microscope. Hence it is largely subjective and dependent on the microscopist, but we are unanimous that vital-staining gives the following three advantages:

(a) It does eliminate non-specific internal fluorescence in stored cercariae, as claimed by Sadun *et al.* (1961).

(b) It preserves the cercariae in good condition, preventing their heads and tails from parting company (an event which leads to false positive staining of the tails).

(c) The adsorption of the antibodies on to the surface of the cercariae is more even, both on the individual cercaria and in the group of cercariae used in each test. This greatly facilitates the final "reading" under the U.V. microscope.

(3) This difference, alone of the three, permits of objective comparison. Our results follow, comparing the use of undiluted serum and of serum diluted 1 in 4 with buffer.

(a) *Negative Controls*

Twenty-two European blood donors were examined in an attempt to obtain satisfactory negative controls. (It will be appreciated that finding persons locally with no possible contact with bilharziasis is somewhat of a task, and this group of healthy donors was felt to be the best approximation.)

Undiluted sera gave seven positive results, seven doubtful results and eight negative results. Sera diluted 1 in 4 yielded two positive results and 20 negative results. These two positive donors were positive by both methods and both donors pursue sports which expose them frequently to possible infestation. Table I compares the results in more detail.

No association was found between these results and either the ABO or Rhesus blood groups.

(b) *Positive Controls*

Thirty-one patients (24 Africans and seven Europeans) known to be excreting ova or to have viable ova in surgical specimens (appendices or rectal snips) were examined. Neat sera gave 20 positive results, nine doubtful

Table I

Comparison of Results with Negative Controls Using Undiluted Sera and Sera Diluted One in Four with Buffer

		Totals	Positive	Doubtful	Negative
			2	0	20
Undiluted sera	Positive	7	2	0	5
	Doubtful	7	0	0	7
	Negative	8	0	0	8

results and two negative results. Diluted sera yielded only one doubtful result, the remaining 30 being positive. Table II compares the results in more detail.

(c) *In Practice*

In patients referred for testing in view of possible previous contact, a positive test rate of 35 per cent. has been found using Sadun's method.

The Effect of Diluting the Serum

Two possibilities present themselves: Are the false positive results found with undiluted serum caused by weak non-specific antibodies not removed by the preliminary heat inactivation? Or are the false negative results with undiluted sera demonstrating a pro-zone phenomenon with the true anti-bilharziasis antibody?

Our results suggest that both these factors are concerned. The nature of the antibody rendered undetectable by a 1 in 4 dilution has evaded all workers. We have been unable to show any relationship to the Forsmann group or to the Paul-Bunnell type of antibody. Other workers have suggested Properdin as this weak, inherent, anti-helminthic substance (Kagan, 1958).

The pro-zone phenomenon is common in viral and bacterial serology and is, therefore, possible with worms. But helminthic serology is, comparatively, in its infancy.

Conclusion

Our results indicate that the technique developed by Sadun, Anderson and Williams (1961) is the more reliable with regard to avoiding both false positive and false negative results. Our number of patients is too small to assess the frequency of false results, but Sadun *et al.* assess these at 5 per cent. of infested persons (Sadun *et al.*, 1961). Using their technique, we found one doubtful result among 31 patients who were undoubtedly infested.

General Discussion

Antibodies are produced by the body to prevent or abort the entry of foreign organisms, proteins or lower forms of animal life. The F.A. test for bilharziasis demonstrates the production of an antibody by the human body against the cercariae, presumably with the aim of limiting the disease they initiate.

The use of the test is still under evaluation, but the current consensus of opinion is that a negative result leaves little likelihood of active infestation. However, it takes some two to three months following exposure for the F.A. test to become positive, so it is of no use in diagnosing the early stage of the disease or the acute form of bilharziasis.

Positive results are less specific for a number of reasons.

(1) The test remains positive in most people after effective treatment or natural conquest of the disease. In some persons the antibodies appear to persist for life, so the test gives no indication of the current activity of the disease.

(2) Abortive attacks by non-pathogenic schistosomes (e.g., bird schistosomes and *S. mattheei* of cattle) are known to occur and will stimulate antibody production (Olivier, 1963).

(3) Unisexual bilharziasis infestations without deposition of ova are possible, if not common (personal communication from Mr. V. de V. Clarke of the Bilharzia Research Laboratory).

(4) Cross-reactions occur with trichinosis and fasciola infestation (both uncommon in Central Africa) (Cookson, 1963; Sadun *et al.*, 1960).

Summary

Our findings indicate that the Fluorescent Antibody Test for bilharziasis evolved by Sadun, Anderson *et al.* is preferable to that of Cookson.

Table II

Comparison of Results with Positive Controls, Using Undiluted Sera and Sera Diluted One in Four with Buffer

		Totals	Positive	Doubtful	Negative
			30	1	0
Undiluted sera	Positive	20	19	1	0
	Doubtful	9	9	0	0
	Negative	2	2	0	0

The significance of the results is discussed briefly.

It should be stressed that a positive result is no measure of the activity of the disease.

REFERENCES

1. Cookson, L. O. C. (1963). *C. Afr. J. Med.*, 9, Nov. and Dec., 429, 469.
2. Kagan, I. G. (1958). *Rice Inst. Pamphlet*, 45, 151.
3. Olivier, L. (1953). *J. parasitol.*, 39, 237.
4. Sadun, E. H., Williams, J. S. & Anderson, R. L. (1960). *Proc. Soc. exper. Biol. & Med.*, 105, 289.
5. Sadun, E. H., Williams, J. S. & Anderson, R. L. (1961). *Exper. parasitol.*, 11, 220.
6. Sadun, E. H., Anderson, R. L. & Williams, J. S. (1961). *Exper. parasitol.*, 11, 117.

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