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MITOCHONDRIAL AND OTHER TISSUE AUTOANTIBODIES IN PATIENTS WITH BIOLOGICAL FALSE POSITIVE REACTIONS FOR SYPHILIS

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SUMMARY

Mitochondrial antibodies ('M' fluorescence) similar to those seen in patients with primary biliary cirrhosis but of low titre and predominantly of IgM class were found in 51% of forty-one patients showing chronic false positive reactions for syphilis, in the absence of detectable liver abnormalities. The pathogenic significance of this association is not understood but the antigen of Wasserman reaction and Venereal Disease Research Laboratory tests, cardiolipin, is situated in close proximity to the distinct lipoprotein reactive in 'M' fluorescence, both being components of the mitochondrial inner membranes. Since these two antibodies have a low incidence in the population, it is likely that their association in a highly selected group of patients represents a particular immunological abnormality in the context of the collagenoses. Thus four of the cases presented with unusual neurological features.

The presence of 'M' fluorescence in biological false positive reactors was strongly correlated with systemic disease. This test, together with those for antinuclear and other tissue antibodies may prove of help in differentiating unusual variants of connective tissue disorders and in the follow-up of symptomless patients who are liable to develop these diseases in later life.

INTRODUCTION

Some of the serological tests used in the diagnosis of syphilis, for example the Wassermann reaction (WR) and the Venereal Diseases Research Laboratory test (VDRL), detect antibodies to cardiolipin which is a phospholipid specific to the mitochondrial inner membranes. Although infections with *treponema pallidum* and related organisms regularly provoke the appearance of these antibodies in the human and in normal animals, and a proportion of the lipids extracted from these organisms is closely related to mammalian tissue cardiolipins (Faure & Coulon-Morelec, 1963), it is difficult at present to decide whether to consider the WR reagins as cross-reacting treponemal antibodies (Eagle & Fleishman, 1948) or as auto-

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antibodies stimulated by the adjuvant effect of the treponeme (Kahn, 1950). In favour of the second viewpoint is the high incidence of false positive reactions in malaria, leprosy and several viral infections, and their appearance in non-infectious conditions such as systemic lupus erythematosus (SLE) and related collagenoses. In these disorders, biological false positive reactions for syphilis (BFP) may persist for many years and conversely, prolonged follow-up of apparently healthy BFP reactors has shown that a proportion of them develop signs of collagen disease many years later (Moore & Mohr, 1952; Harvey & Shulman, 1966).

Autoantibodies reacting in the immunofluorescence test with a lipoprotein component of the mitochondrial inner membranes but not with cardiolipin (Berg *et al.*, 1969) were first described in patients suffering from primary biliary cirrhosis (Walker *et al.*, 1965) where they occur in about 90% of cases, often in high titre. These antibodies are also found in about 25–30% of patients with 'lupoid' hepatitis and 'cryptogenic' cirrhosis, the only other two liver disorders associated with persistent autoimmune phenomena (Doniach & Walker, 1969). Since cardiolipin antibodies are also in a sense mitochondrial, the antibody reacting in liver diseases will be referred to as 'M' fluorescence. 'M' fluorescence is uncommon in other diseases, being detected in 0·8% of mixed hospital patients and of healthy middle-aged controls. Even in SLE, Sjögren's syndrome and rheumatoid arthritis the incidence of the reaction does not exceed 8%. It was therefore of interest to find 'M' fluorescence in some BFP reactors (Walker, Doniach & Doniach, 1970) and this prompted a systematic search for these and other tissue antibodies in forty-one patients with chronic false positive standard tests for syphilis (STS) and four additional cases showing only false positive Reiter complement fixation, as well as in seventy-five proven syphilitics.

TABLE 1. Mode of presentation in forty-one patients with BFP reactions

Standard tests for syphilis done for:	Number of patients
Pregnancy	13
VD clinic	9
Investigation of systemic disease	18
Healthy relatives of cirrhosis patient	1

MATERIALS AND METHODS

Patients with biological false positive (BFP) reactions

The forty-one cases included twenty-eight females and thirteen males varying in age from 21 to 77 years, and Table 1 shows their mode of presentation. All but six of these patients have been followed up clinically by one of us (RDC) for periods up to 5 years and auto-antibody tests were carried out on at least two and sometimes as many as five separate specimens of serum taken at intervals. The four patients with positive Reiter complement-fixation test (CFT) had no evidence of syphilis and are included for comparison with the BFP reactors.

Syphilitic patients

These included thirty-eight males varying in age between 12 and 61 years: eleven had

primary syphilis, fourteen had secondary syphilis of whom only four were untreated. Thirteen patients were in the late stages of the disease. Autoantibody tests were also performed on the sera of thirty-seven females aged 21–88, all with positive treponemal immobilization (TPI) and fluorescent treponemal antibodies (FTA) tests, only two having untreated secondary syphilitic rashes.

Serological methods

The sera used for testing were either fresh or stored at −20°C for up to 1 year. The immunofluorescence tests were performed using thyrotoxic thyroid, human stomach and kidney, and rat liver as tissue substrates. Fluorescein conjugates of anti-human IgG and IgM were used in separate tests and the fluorescence titre was established by repeating the tests on serial dilutions of serum. A few sera were also tested with anti-β_{1C} conjugate after addition of normal fresh serum as a source of complement. Thyroglobulin antibodies were detected by the tanned red cell (TRC) test. Latex FII tests were titrated on serial dilutions of sera showing a positive result in the Hyland slide test at 1:20. Complement fixation was carried out on serum dilutions of 1:2 to 1:2048 with the Takatsy microtitration apparatus using lyophilized rat liver mitochondria and freshly prepared saline homogenates of rat kidney with 2MHD of complement. Thyroid cytoplasmic antibodies were titrated by CFT with thyrotoxic thyroid homogenate. All these methods are described in the World Health Organization manual for autoimmune serology (Roitt & Doniach, 1969). STS tests were performed in the venereology laboratory of the Middlesex Hospital and sera were sent to the Veneral Diseases reference laboratory at the London Hospital for TPI. Individual immunoglobulin assays for IgG, IgA and IgM were performed by the method of Mancini, Carbonara & Heremans (1965) using Hyland plates and standards.

Absorption of sera with VDRL antigen

0·5 ml of VDRL antigen (Dade Reagents Inc. Miami, Florida, 33152, Lot VDA 38) was reconstituted as for VDRL testing and centrifuged at 1300 *g* for 5 min. 0·15 ml of serum was added to the sediment incubated at 37°C for 30 min, then left overnight at 4°C and centrifuged again. The supernatant was used for the fluorescence test and for CFT.

Liver function tests

Serum bilirubin, serum glutamic pyruvic transaminase, alkaline phosphatase and albumin-globulin ratios were carried out on fifteen patients showing mitochondrial fluorescence, while bromsulphthalein retention tests were done in six cases. The biochemical tests were performed in the laboratory of the Courtauld Institute.

RESULTS

BFP reactors

STS

Results are shown on Table 2. In thirty-one cases both the cardiolipin WR and VDRL tests were positive, seven patients reacted only in WR and four only in VDRL. By definition BFP reactors must have negative TPI and FTA tests but one patient was included in spite of a positive FTA on one occasion. She was a hypertensive elderly woman with no past or present evidence of syphilitic infection, admitted to hospital with a left-sided hemiplegia

following a cerebrovascular accident, in whom an anti-nuclear titre of 1:100 suggested a possible collagen disease.

TABLE 2. Serological tests for syphilis in forty-one patients with biological false positive reactions

	Number positive
Cardiolipin Wassermann reaction	37 (90%)
Venereal Disease Research Laboratory test	34 (83%)
Reiter protein complement fixation test	0
Fluorescent treponemal antibody test	1
Treponemal immobilization test	0

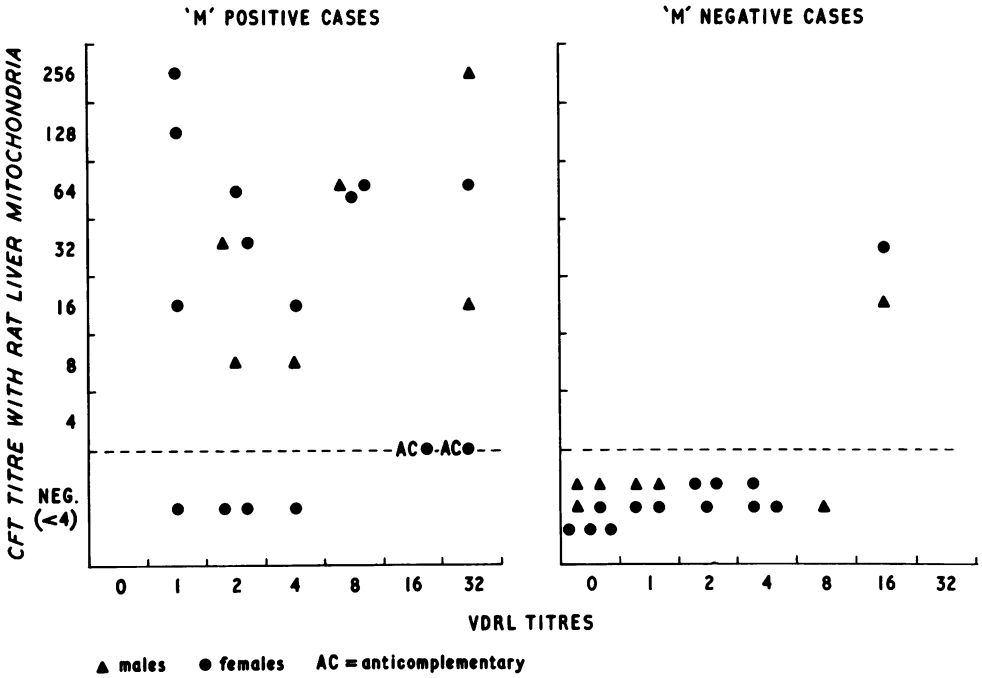

FIG. 1. Relationship of Mitochondrial CFT and VDRL titres in forty-one patients with chronic BFP reactions. In cases without 'M' fluorescence the CFT could be inhibited by absorption with VDRL antigen.

Autoantibodies

Table 3 shows the overall results in the forty-one BFP reactors, thirty-nine of whom had one or more positive tests. 'M' fluorescence patterns similar to those seen in primary biliary cirrhosis were obtained in twenty-one of these patients (51%) in titres of 1:5–1:50. The antibodies were mainly of IgM class in ten cases, a further eight patients reacted with both

anti-IgG and -IgM conjugates (seven of them in low titre) and only three had 'M' fluorescence mainly of IgG type. Unlike primary biliary cirrhosis, in BFP reactors the CFT titres obtained with rat liver mitochondria were invariably higher than those of the fluorescence

TABLE 3. Autoantibodies in forty-one patients with chronic BFP reactions

Antibody	Number positive	Percentage positive
Mitochondrial immunofluorescence	21	51
CFT rat liver mitochondria	16	39
Smooth muscle fluorescence	10	24
Antinuclear antibodies	19	46
CFT rat kidney homogenate	32	80
Rheumatoid factors (Latex FII)	6	14
Thyroid specific antibodies	10	24
Gastric parietal cell antibodies	3	7
Total patients with antibodies	39	95

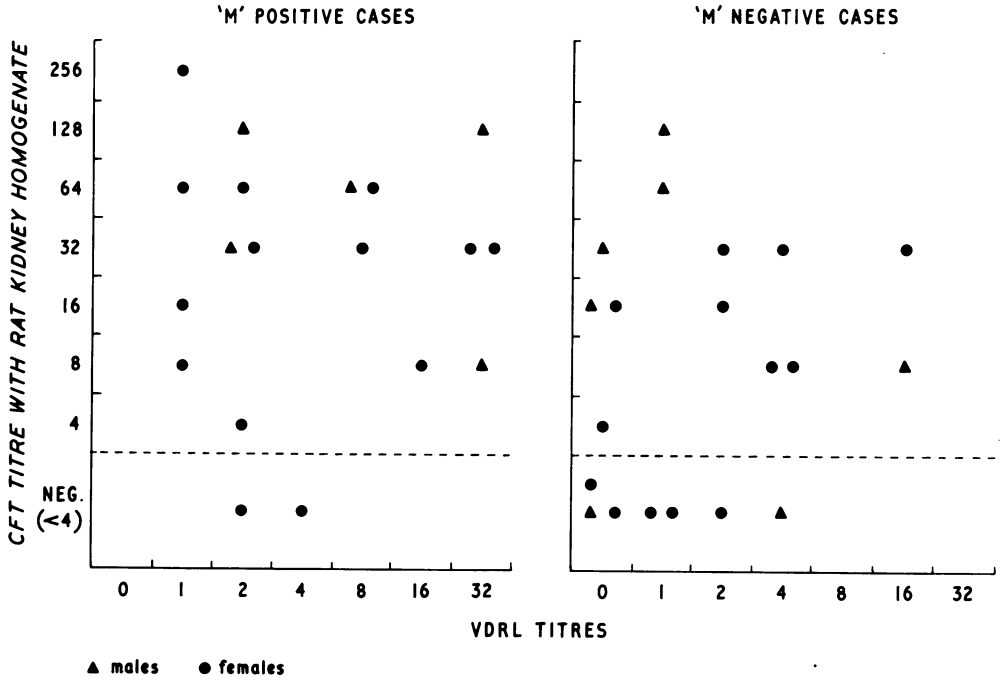

FIG. 2. Lack of correlation between 'M' fluorescence, CFT with crude tissue homogenates and VDRL titres in forty-one BFP reactors. Results indicate presence of further unidentified tissue antibodies.

test, possibly due to better complement fixing ability of IgM antibodies and to the co-existence of cardiolipin antibodies in these patients.

Fig. 1 shows the correlation between mitochondrial CFT and VDRL titres in BFP reactors. Experience with syphilitic sera devoid of 'M' antibodies showed that sera with VDRL titres greater than 1:8 gave positive CFT with liver mitochondria by virtue of the cardiolipin content of this preparation, and that this reaction could be inhibited by prior absorption of the sera with VDRL antigen. In BFP reactors, VDRL titres tended to be low, and in cases with positive 'M' fluorescence there was no correlation with the mitochondrial CFT titre. Absorption with VDRL antigen reversed the CFT in the two patients having no 'M' fluorescence and lowered the titre from 1:256 to 1:32 in a patient having both antibodies. The patient's fluorescence titre (anti-IgM) remained at 1:30 before and after VDRL absorption. Similar absorptions were carried out in eighteen of the twenty-one positive BFP reactors: the tissue fluorescence remained unaltered in seventeen instances and became negative in one case.

Non-organ-specific CFT reactions were obtained with rat kidney homogenates in thirty-two cases and Fig. 2 shows that the titres are independent both of the presence or absence of 'M' fluorescence. The complement-fixing 'M' antibody could not have contributed to the reaction with rat kidney homogenate in the twenty patients having negative fluorescence, yet CFT titres of up to 1:128 were obtained in patients with low or negative VDRL reactions.

Antinuclear antibodies (ANA) were found in 46% of the BFP patients, eleven (27%) having titres of 1:20–1:320, i.e. outside those usually seen in healthy controls. The ANA were predominantly of IgG class and of the 'diffuse' variety, as seen in SLE and 'lupoid' hepatitis and unlike primary biliary and cryptogenic cirrhosis, where these reactions are frequently of the 'speckled' type. Another antibody marker, namely smooth muscle fluorescence, which is rarely seen outside the liver disorders mentioned, was present in a surprising number of BFP reactors (24%). On the other hand rheumatoid antiglobulins and thyroid specific antibodies had an incidence only slightly above that expected in control groups, while organ-specific gastric parietal cell fluorescence was present in three cases (7%).

False positive Reiter CFT

Table 4 shows the diagnosis and serological results obtained in four patients. None had ANA, 'M' or smooth muscle fluorescence. One patient gave gastric parietal cell fluorescence and one man suffering from non-specific urethritis gave kidney CFT titres of 1:16–1:32 on repeated testing.

Syphilitic patients

Results of autoantibody tests in seventy-five cases are shown in Table 5. True 'M' fluorescence was not present in any of these patients who were all tested with both anti-IgG and anti-IgM conjugates. However during the course of this investigation it was found in collaboration with another team, that certain syphilitic sera produced a tissue fluorescence resembling that obtained with 'M' antibodies but which could be absorbed out with VDRL antigen and with purified cardiolipin. Work on this new antibody is reported separately (Wright *et al.*, 1970). CFT with rat liver mitochondria was positive in thirty cases (twenty-five male, five female) and the correlation with VDRL titres is shown in Fig. 3. Patients with VDRL titres of 1:8 upwards reacted roughly in proportion with the strength of the flocculation reactions and the CFT could be absorbed out with VDRL antigen. A proportion of the female patients had a low VDRL titre and were selected on the basis of

FTA and TPI reactions. Three of the five patients giving a positive CFT with kidney homogenate also reacted with mitochondria and had high VDRL titres but in two cases titres of 1:128 and 1:8 were observed with kidney associated with VDRL titres of only 1:2 and 1:1 respectively, suggesting that the CFT with kidney reflected the presence of antibodies distinct from those to cardiolipin or the 'M' antigen. The antinuclear and smooth muscle reactions were all of low titre (less than 1:10) and thyroid and gastric fluorescence was found mostly in middle-aged patients and was of low titre.

TABLE 4. Four patients free of syphilis with persistently positive Reiter CFT

Sex	Age	Diagnosis	WR, VDRL, FTA and TPI	Reiter CFT	ANA, 'M' and smooth muscle fluorescence	Other autoantibodies
M	30	Cured Gono.	−	+	−	Gastric parietal cell +
M	26	Arthritis	−	+	−	—
M	30	Non-specific urethritis	−	+	−	Kidney CFT 16–32
F	27	Pregnancy	−	+	−	—

TABLE 5. Incidence of tissue autoantibodies in seventy-five syphilitic patients

Antibody	Females	Males
Number tested	37	38
Mean age and range (years)	42 (21–88)	32 (12–61)
'M' fluorescence*	0	0
CFT rat liver mitochondria	5	25 (absorbed out with VDRL Ag)
Smooth muscle fluorescence	1	2
Antinuclear factors (ANF)	4 (titres <1:10)	0
CFT rat kidney homogenate	7	6/6
Thyroid cytoplasmic fluorescence (organ-specific)	4	1
Gastric parietal cell fluorescence (organ-specific)	4	0

* Cardiolipin fluorescent (F) antibodies were observed in 3 females and 5 males.

Evaluation of clinical significance of positive immunofluorescence tests in patients with chronic BFP reactions

Comparison of clinical and serological features in twenty-one patients giving positive 'M' fluorescence and twenty negative cases is shown in Table 6. The sex ratio was nearly three females to one male and in the positive group 67% suffered from systemic disease. There was a higher incidence of all autoantibodies and the titres tended to be higher in 'M'

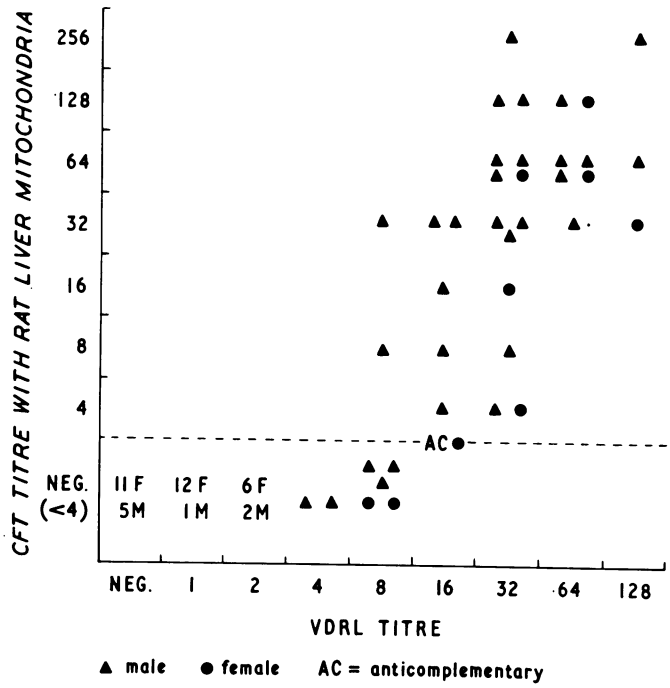

FIG. 3. Correlation of mitochondrial CFT with VDRL titres in seventy-five syphilitic patients. The CFT could be inhibited by absorption with VDRL antigen.

TABLE 6. Comparison of 'M' positive and negative cases

	Mitochondrial immunofluorescence	
	Positive	Negative
Total number of patients	21 (15F; 6M)	20 (13F; 7M)
Age (Mean)	41	34
(Range)	(22–77)	(21–74)
Systemic disease present	14 (67%)	5
Apparently healthy	7	15 (75%)
ANF positive	13	6
Smooth muscle antibodies	8	2
Thyroid antibodies*	9	1
Gastric parietal cell*	2	1
Latex FII	4	2
WR positive	19	18
VDRL positive	21	13
CFT rat kidney	19	13

* Organ-specific thyroid and gastric cytoplasmic fluorescence could only be assessed accurately when either 'M' fluorescence negative or in different Ig class.

positive than in negative cases. Biochemical tests of liver function proved entirely normal in fifteen patients tested. One patient had had idiopathic aortitis of the Takayashu type in 1964 when her STS were found positive. Mitochondrial antibodies were first looked for in 1968 when she developed severe liver necrosis probably due to virus hepatitis. However,

TABLE 7. Systemic disease in forty-one patients with chronic BFP reactions

Disease	Twenty-seven patients with positive immunofluorescence (including 'M' and ANA)		Fourteen patients with negative fluorescence tests	
	Number	Sex	Number	Sex
Systemic lupus erythematosus	1	F		
Neurological syndromes ?collagenoses with vasculitis	3	F	1	M
Sjögren's syndrome	2	F		
Rheumatoid arthritis and Peyronie's disease	1	M		
Undefined collagenoses	2	F		
Discoid LE and AIHD	1	M		
Auto-immune haemolytic disease (AIHD)	1	F	12F, 5M	
Idiopathic aortitis and subacute liver necrosis	1	F		
Chronic superficial dermatitis	1	F		
Senile dementia	1	F		
Aortic stenosis unknown cause	1	M		
Rheumatic mitral stenosis	1	M		
Lymphadenopathy and heroin addiction	1	M		
Total percentage with systemic disease	63%		7%	
Apparently healthy	10	(6F, 4M)	13	10F, 3M

TABLE 8. Comparison of reactions obtained with Wassermann and 'M' antibodies

Test	Cardiolipin 'WR' antibodies	Mitochondrial 'M' antibodies
WR	+	−
VDRL	+	−
Reiter CF, FTA and TPI	−	−
CFT purified liver mitochondria	+	+
„ after absorption with VDRL	−	+
CFT rat kidney homogenate	+	+
Immunofluorescence on human thyroid, stomach, kidney and rat heart	−	+
„ after absorption with VDRL	−	+
„ Alcohol or acetone fixed sections	−	−
„ Deoxycholate 0·02%, 1 sec	−	+

one year later her liver function tests returned to normal, yet she still had BFP and 'M' positive reactions, so that these are probably connected with the aortitis rather than with the transient liver disorder.

A still better separation between apparently healthy BFP reactors and those with systemic disease was obtained when both mitochondrial and ANA fluorescence were taken into account and Table 7 shows that of fourteen patients negative in all fluorescence tests only one suffered from possible collagen disease, whereas of the twenty-seven patients with positive tests seventeen had systemic illnesses. One of the 'healthy' subjects was a man aged seventy-seven who had two sisters with primary biliary cirrhosis.

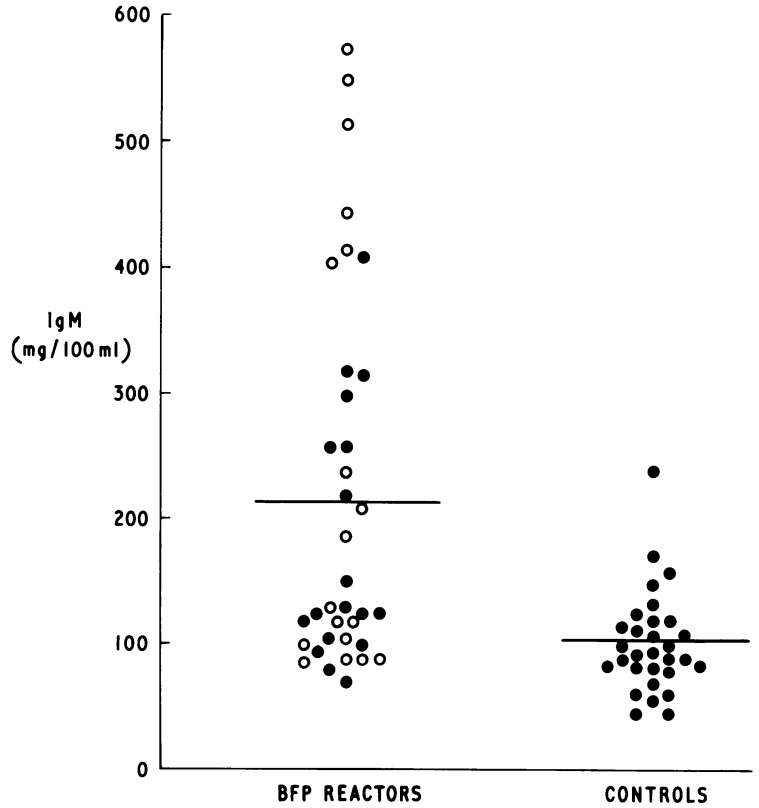

FIG. 4. Serum IgM levels in thirty-six BFP reactors and thirty healthy male controls. The horizontal lines represent mean values. ○ Patients with systemic disease. ● 'Healthy' patients and controls.

Experiments to demonstrate the separate nature of Wassermann and 'M' antibodies

Table 8 compares the behaviour of the two antibody systems in the various tests used. Patients with primary biliary cirrhosis having high titre 'M' fluorescence do not give positive STS as a rule, although BFP reactions have been reported in 2%–3% of patients with active cirrhosis (Dubois, 1966). Since crude tissues homogenates and purified mitochondria contain cardiolipin, syphilitics and BFP reactors gave positive CFT with both reagents. Absorption experiments showed that the mitochondrial CFT titre of PBC patients is not altered by incubation with VDRL antigen whilst this reaction is abolished in syphilitic sera. Some BFP reactors clearly have both antibodies simultaneously. This was demonstrated on absorption with VDRL antigen, which failed to alter the 'M' fluorescence titre while cardiolipin

flocculation became negative and the mitochondrial CFT titre dropped to a level comparable with that of the fluorescent test. Sera from treated syphilitics did not give rise to 'M' fluorescence on tissue sections including heart muscle, using conjugates of anti-IgG, -IgM and -IgA and anti-β_{1C}. Fixation of the sections in alcohol, acetone, and treatment with varying concentrations of deoxycholate failed to reveal the cardiolipin antigenic sites. These sera also failed to block the 'M' fluorescence produced by a direct conjugate of PBC serum.

Immunoglobulin levels in BFP reactors

Individual assays of IgG, IgM and IgA levels were carried out in thirty-six of the forty-one BFP reactors. The only striking abnormality was an evaluation of IgM values in sixteen patients (Fig. 4), thirteen of whom also had mitochondrial and/or ANA fluorescence. An attempt was made to correlate serum IgM levels with the presence of systemic disease: 50% of 'ill' patients had a raised IgM as compared with 39% of apparently healthy BFP reactors ($P>0{\cdot}05$). IgG was raised in only two patients with Sjögren's syndrome and IgA levels greater than 500 mg/100 ml were found in four cases but bore no relation to the titre of antibodies or the state of health of the patients.

DISCUSSION

The high incidence of tissue antibodies in our series of BFP reactors is related to the fact that about half the cases were general hospital patients investigated for systemic disease, especially neurological and collagen disorders. There was a diversity of clinical conditions; nevertheless thirteen of the eighteen patients with systemic involvement had some form of 'collagenosis' or related immunopathological disease, though only one was considered to suffer from SLE. Four BFP reactors presented with a curious neurological picture resembling multiple sclerosis and a paretic type of Lange curve. The detailed clinical features will be reported separately (Catterall, Kremer & Fullerton, to be published). The combination of this neurological presentation with positive STS and 'M' antibodies, appears to constitute a syndrome.

The mitrochondrial fluorescence found in the BFP reactors was unrelated to VDRL titre and could not be absorbed out with this antigen except in one case. In appearance and serological behaviour it was similar to that found in primary biliary cirrhosis and allied autoimmune liver diseases. However whereas PBC patients usually reacted to high titres (fluorescence up to 1 : 6000; CFT being always less sensitive) and the antibodies could be regularly detected in the three main immunoglobulin classes, in BFP reactors 'M' fluorescence was of low titre usually less than 1 : 40, and in the majority of cases the antibodies were present only in one immunoglobulin class, mainly IgM. In this context it is of interest that WR reagins also tend to be mostly in the IgM fraction in this type of patient (Aho, 1968) in contrast with cases of syphilis where they are mainly IgG. The BFP reactors also differed from other groups of patients with collagen disorders having 'M' antibodies (Walker *et al.*, 1970) in that none could be shown to have any abnormalities of liver function.

When it is considered that 'M' fluorescence is detected in only 5%–8% of patients with collagenoses and that false positive tests for syphilis occur in 15% of cases in this group of diseases (Dubois, 1966), the simultaneous presence of these two distinct antibodies suggests some special immunological abnormality. The association is the more remarkable considering the low incidence of each of these reactions in healthy subjects since chronic BFP

tests are found in less than 1 : 1000 (Carr, Becker & Carpenter, 1966) and 'M' fluorescence in less than 1 : 100 in the normal population.

The failure of classical WR reagins to react in the fluorescence test is perhaps unexpected. It must be presumed that the antigenic site is situated in a hydrophobic part of the cardiolipin molecule inaccessible to antibody in tissue sections when the phospholipid is combined with proteins in the mitochondrial inner membranes. As mentioned above, some syphilitics and occasional BFP reactors have now been found to possess a second cardiolipin antibody (Wright *et al.*, 1970) detectable by an immunofluorescence which can be fully absorbed out with cardiolipin and where the antibody is presumably directed against a hydrophilic site on the molecule.

Not unexpectedly, the patients with systemic disorders had a higher incidence of other tissue antibodies, particularly those known to occur in the collagen disorders. Antinuclear antibodies were found in two thirds of 'ill' patients but ANA was also positive in six of twenty-three apparently healthy individuals, two men having titres of 1 : 160 and 1 : 320 respectively. The significance of CFT reactions obtained with crude tissue homogenates is difficult to assess in these patients. The test detects a whole range of poorly defined tissue antibodies; neither the cardiolipin reagin, nor the 'M' antibodies could entirely account for the positive results obtained in some of our cases. About 3% of healthy subjects give these reactions while BFP reactors, even when symptomless, had a far higher incidence, though the titres were low.

It is interesting to observe that almost half of BFP reactors had a raised serum IgM level, while the other two main immunoglobulin classes showed no significant abnormalities. The same changes have been reported in primary biliary cirrhosis (Feizi, 1968) but in neither group of patients was there any correlation of serum IgM values with 'M' or ANA fluorescence titres.

Isolated positive Reiter CFT reactions are unusual and are not thought to be related to collagen disorders. It has been suggested that these antibodies may occasionally arise from saprophytic spirochetes. Our four cases showed none of the antibody markers specifically associated with connective tissue disorders.

Previous studies in BFP reactors have stressed the occurrence of collagen diseases and of antinuclear antibodies (Harvey & Shulman, 1966; Catterall, 1961; Putkonen *et al.*, 1967; Berglund & Carlsson, 1966; Tuffanelli *et al.*, 1967). It was also shown that false positive tests for syphilis are more frequent in patients with acute SLE and positive LE cell tests than in chronic or mild cases (Strejcek, Malina & Bielicky, 1968). Mixed cryoglobulins (Mustakallio *et al.*, 1967) and rheumatoid factors (Tuffanelli *et al.*, 1967; Mustakallio *et al.*, 1967) were found in over 25% of BFP reactors of whom a high proportion suffered from SLE. In our series the incidence of positive Latex FII tests was not high and LE cells were only found in one patient with definite SLE. The pathological significance of 'M' antibodies in association with BFP reactions is not yet clear but is proving of great interest in the study of certain unexplained neurological cases. In more general terms it seems that BFP reactors having positive immunofluorescence of significant titre show a greater tendency to systemic diseases. These tests could therefore prove of help in the selection of cases requiring prolonged medical follow-up.

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REFERENCES

AHO, K. (1968) Studies of syphilitic antibodies. II. Substances responsible for biological false positive sero reactions. *Brit. J. vener. Dis.* **44,** 49.

BERG, P.A., ROITT, I.M., DONIACH, D. & COOPER, H.M. (1969) Mitochondrial antibodies in primary biliary cirrhosis. IV. Significance of membrane structure for the complement-fixing antigen. *Immunology*, **17,** 281.

BERGLUND, S. & CARLSSON, M. (1966) Clinical significance of biologic false positive reactions and antinuclear factors. *Acta med. scand.* **180,** 407.

CARR, R.D., BECKER, S.W. & CARPENTER, C.M. (1966) The biologic false positive (BFP) phenomenon in elderly men. *Arch. Derm.* **93**, 393.

CATTERALL, R.D. (1961) Collagen disease and the chronic biological false positive (BFP) phenomenon. *Quart. J. Med.* **30,** 41.

CATTERALL, R.D., KREMER, M. & FULLERTON, P. (In preparation).

DONIACH, D. & WALKER, J.G. (1969) A unified concept of autoimmune hepatitis. *Lancet*, **i,** 813.

DUBOIS, E.L. (1966) *Lupus Erythematosus*. McGraw-Hill, New York.

EAGLE, H. & FLEISHMAN, R. (1948) The antibody response in rabbits to killed suspensions of pathogenic *treponema pallidum*. *J. exp. Med.* **87,** 369.

FAURE, M., COULON-MORELEC, M.J. (1963) Rapports entre la structure du cardiolipine et son activité sérologique. *Ann. Inst. Pasteur* **104,** 246.

FEIZI, T. (1968) Immunoglobulins in chronic liver disease. *Gut*, **9,** 193.

HARVEY, A.M. & SHULMAN, L.E. (1966) Connective tissue diseases and the chronic biologic false positive test for syphilis (BFP reaction). *Med. Clin. N. Amer.* **50,** 1271.

KAHN, R.L. (1950) *Serology with Lipid Antigen*. Baillère, Tindall & Cox, London.

MANCINI, G., CARBONARA, O. & HEREMANS, J.F. (1965) Immunochemical quantification of antigens by single radial immunodiffusion. *Int. J. Immunochem.* **2,** 235.

MOORE, J.E. & MOHR, C.F. (1952) Biologic false positive serological tests for syphilis; incidence and cause. *J. Amer. med. Ass.* **150,** 467.

MUSTAKALLIO, K.K., LASSUS, A., PUTKONEN, T. & WAGER, O. (1967) Cryoglobulins and rheumatoid factor in sera from chronic false positive sero reactors for syphilis. *Acta derm.-vereneol. (Stockh.)*, **47,** 249.

PUTKONEN, T., JOKINEN, E.F., LASSUS, A. & MUSTAKALLIO, K.K. (1967) Chronic biologic false positive seroreactions for syphilis as a harbinger of SLE. *Acta derm.-venereol. (Stockh.)*, **47,** 83.

ROITT, I.M. & DONIACH, D. (1969) *WHO Manual for Autoimmune Serology*. Geneva.

STREJCEK, J., MALINA, L. & BIELICKY, T. (1968) Antinuclear factors, rheumatoid factors, and Bordet-Wassermann reaction in chronic and systemic lupus erythematosus. *Acta derm.-venereol. (Stockh.)*, **48,** 198.

TUFFANELLI, D.L., WUEPPER, K.D., BRADFORD, L.L., WOOD, R.M. (1967) Fluorescent treponemal-antibody absorption tests; studies of false positive reactions to tests for syphilis. *New Engl. J. Med.* **276,** 258.

WALKER, J.G., DONIACH, D. & DONIACH, I. (1970) Mitochondrial antibodies in subclinical liver disease. *Quart. J. Med.* **39,** 31.

WALKER, J.G., DONIACH, D. ROITT, I.M. & SHERLOCK, S. (1965) Serological tests in the diagnosis of primary biliary cirrhosis. *Lancet*, **i,** 827.

WRIGHT, D.J.H., DONIACH, D., TURK, J.L., LESSOF, M.H., GRIMBLE, A.S. & CATTERALL, R.D. (1970) New antibody in early syphilis. *Lancet*, **i,** 740.

ABBREVIATIONS

ANA, anti-nuclear antibody
BFP, biological false positive reactions for syphilis
FTA, fluorescent treponemal antibodies
'M', mitochondrial fluorescent antibodies found in cirrhosis
Reiter CFT, CFT with extract of Reiter treponemes
STS, standard tests for syphilis
TPI, treponemol immobilization