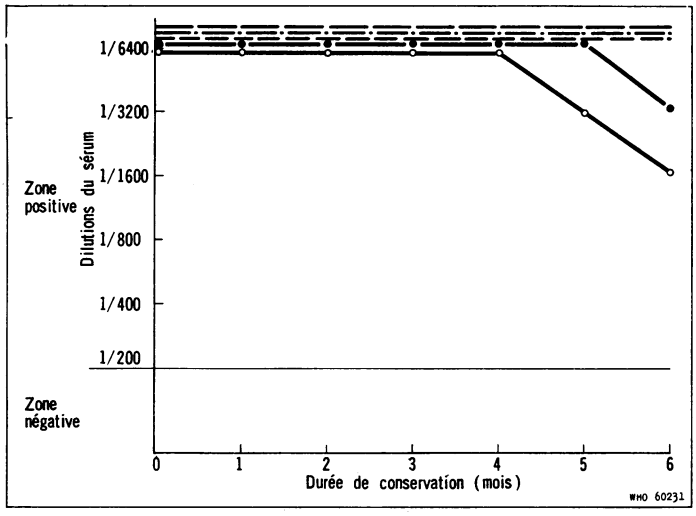

FIG. 6
ÉVOLUTION DES RÉACTIONS SÉROLOGIQUES (IF) EN FONCTION DE LA DURÉE ET DE LA TEMPÉRATURE DE CONSERVATION DES SÉRUMS

Sérum conservé à −180°C.
Sérum conservé à −20°C.
Sérum conservé à + 4°C.
Sérum conservé à + 22°C.
Sérum conservé à + 37°C.

Reiter, Kline, TIT, IF), à l'exception du Kahn qui accuse une légère baisse de sensibilité au 5e mois. A la température du laboratoire (20°C-24°C), on constate, sauf pour le Kline, une légère diminution de réactivité du sérum. A +37°C, les anticorps s'altèrent beaucoup plus rapidement; les titres diminuent très nettement puisque le sérum s'est même négativé au BW Kolmer au 6e mois.

Comparativement aux autres réactions, il semble que la réaction d'immuno-fluorescence soit la moins perturbée par la température. Elle a l'avantage d'être d'une très grande sensibilité (en plus de sa spécificité) et ce fait donne une marge de sécurité beaucoup plus grande pour le transport et la conservation des prélèvements.

Dans l'ensemble, les anticorps de la syphilis s'avèrent très stables aux basses températures et jusqu'à +4°C et, à condition que les sérums soient parfaitement stériles, aucune autre précaution ne semble nécessaire.

Studies on the Fluorescent Treponemal Antibody Test using Fluorescent Anti-γ, Anti-α, Anti-μ, Anti-λ, Anti-κ Chains and β1CA,E Globulin Reagents

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The heterogeneity of the γ-globulins has been discussed in connexion with almost every antigen-antibody system, including that of syphilis. Matuhasi & Mizuoka [a] have reported that the antibodies concerned in the fluorescent treponemal antibody (FTA) test and the cardiolipin test were found in both the γ-G and γ-M fractions eluted by diethylaminoethyl (DEAE) cellulose column chromatography, and that the activity of antibodies in the γ-G fraction was stable, but the activity of antibody in the γ-M fraction was labile after mercaptoethanol (ME) treatment. These authors also have confirmed the presence of the FTA antibodies by

[a] Matuhasi, T. & Mizuoka, K. (1963) *Jap. J. Bact.*, **18**, 243.

employing anti-γG and anti-γM globulin reagents conjugated with fluorescein isothiocyanate (FITC).[b]

The relationship between the character of the antibodies and the clinical stages in syphilis, as well as some interesting findings on the character of the antibodies produced during the course of this disease, also have been discussed by Mizuoka et al. (1964).[b]

Observations of FTA tests of syphilitic and non-syphilitic sera, using fluorescent anti-γ, -μ, -α, -κ chains and anti-β1CA,E globulins (human complement components) are reported below. The terminology used is that specified in " Nomenclature for Human Immunoglobulins." [c]

Preparation of antiglobulin reagents

Anti-γ and anti-μ chain reagents. Antiglobulin fractions 1 and 4 (An-1 and An-4) of syphilitic sera were prepared by Abelson's method, using DEAE cellulose column chromatography.[d] It was confirmed, by treatment with 0.2 M mercaptoethanol, that the An-1 fraction mainly contained γG reagin and that the An-4 fraction included γM reagin. Venereal Disease Reference Laboratory (VDRL) antigen suspension was mixed with each fraction in the optimal proportions. The precipitates obtained from each mixture were washed thoroughly and injected, with Freund's complete adjuvant, into the foot-pads of rabbits. Fortunately, nearly half of the antisera against the γG reagin precipitate contained only antibody corresponding to the γ-chain. Because most of the antisera against γM reagin precipitate contained anti-μ chain antibody and anti-γG antibodies, the antisera were absorbed with human γG to obtain only anti-μ chain antibody.[e, f]

Anti-α chain reagents. The γA-globulin was separated from the peritoneal fluid of a patient with A-myeloma by salting out with ammonium sulfate and by DEAE cellulose column chromatography. After the purity of the γA-globulin was confirmed by immunoelectrophoresis, it was injected, with Freund's complete adjuvant, into the foot-pads of rabbits.

In addition, anti-α chain antisera were prepared by the immunization of rabbits with the plasma of human milk.[f] The resultant antisera were adsorbed with cord serum to obtain anti-α chain antibody, since they contained not only anti-γA antibody but also antibodies against γG and some components of the β-globulins.

Anti-human complement reagents. Antisera against certain components of human complement were prepared by immunizing rabbits with antigen-antibody precipitate-fixing human complement, as reported elsewhere.[g] The antisera contained anti-β1CA,E antibodies.

Reagents to anti-κ and anti-λ chains. Type K and type L Bence-Jones proteins, with Freund's complete adjuvant, were injected into the foot-pads of rabbits. Reagents to the anti-κ and anti-λ chains were prepared by mutual absorption by the opposite types of Bence-Jones proteins.

Broad-spectrum antiglobulin reagents. Whole human sera, with Freund's complete adjuvant, were injected repeatedly into rabbits.

Only antisera with high titres against γG, γM, γA and β1CA,E globulins were used for the present experiments. The immuno-electrophoretic patterns and the findings by indirect anti-globulin titration are shown in Table 1.[f]

FITC labelling

One amount of crystalline FITC was used to label 100 amounts of γG globulin of the antisera according to the method recommended by the Japanese Committee on Fluorescent Treponemal Antibodies.[h]

Mercaptoethanol (ME) treatment

A volume of syphilitic serum was incubated with an equal amount of 0.2 M mercaptoethanol at 37°C for two hours following overnight dialysis against phosphate-buffered saline (PBS) at pH 7.2.[i]

FTA test

The test was performed according to the original FTA-200 method.[j]

Results

Detection of γG and γM syphilitic antibodies by DEAE cellulose column chromatography and mercaptoethanol treatment. The An-1, An-2, An-3 and An-4 fractions of nine sera of several stages of human

[b] Mizuoka, K., Usui, M., Matuhasi, T. & Suzuki, K. (1964) *Jap. J. Dermatol. (Ser. A)*, **74**, 650.

[c] *Bull. Wld Hlth Org.*, 1964, **30**, 447-450.

[d] Abelson, N. & Rawson, A. J. (1959) *J. Immunol.*, **82**, 435.

[e] Matuhasi, T. (1964) *Jap. J. Bact.*, **19**, 382.

[f] Matuhasi, T. & Usui, M. (1964) *Jap. J. clin. Med.*, **22**, 2624.

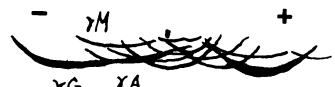
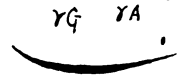
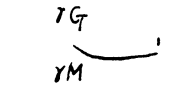
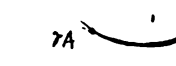
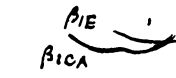
[g] Matuhasi, T. & Usui, M. (1954) *Med. of Biol.*, **44**, 123.

[h] Kawamura, A. (1964) *Jap. J. Bact.*, **19**, 381.

[i] Mollison, P. L. (1962) *Blood transfusion in clinical medicine*, Oxford, Blackwell, p. 218.

[j] Deacon, W. E., Freeman, E. N. & Harris, A. (1960) *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 827.

TABLE 1
SPECIFICITY OF VARIOUS ANTI-GLOBULIN REAGENTS

Sera (Rabbit No.)	Reagent character: anti-	Schema of immuno-electrophoresis	Anti-globulin titre against RBC coated with anti-				
			D [a]	E [a]	Le λ EDTA [c]	Le λ C'	Incom. cold [a]
1360	Broad spectrum		1024	256	256	512	128
7613	γ-chain (7Sγ)		2048	2048	—	—	—
7616	μ-chain (19Sγ)		—	—	512	32	—
7623	α-chain (A myeloma)		—	—	—	64	4
7630	α-chain (IgA milk plasma)		—	—	8	32	16
7619	β1CA,E (human complement)		—	—	—	256	128

[a] Anti-D, anti-E = eluate from red cells of HDN by anti-D, anti-E.
[b] Incom. cold = incomplete cold antibody.
[c] Ethylenediaminetetra-acetic acid.

syphilis were eluted by DEAE chromatography, using Abelson's method.[4] The An-1 fraction, which contained, principally, γG globulin, was positive in the FTA, VDRL, Reiter protein complement-fixation (RCPF), cardiolipin, and Wassermann (Ogata) tests in all cases, and these results were not affected by ME treatment. There were, however, some very puzzling cases in which FTA-negative An-2 fractions, which usually are without reactivity to the tests, became positive after treatment with ME.

In three of nine cases, the FTA test of An-3 fractions was positive; one of the six FTA-negative An-3 fractions became positive after ME treatment. Only one of nine Ogata tests, one of nine VDRL tests, and none of three RCPF tests yielded positive results, and both of these positives became negative after ME treatment. On the other hand, all samples in the An-4 fraction were positive by both the VDRL and Ogata tests and became negative after ME treatment.

As shown in Table 2, these experiments confirm that γG and γM antibodies are produced in the course of syphilitic infection. Observations made at various stages of these experiments yield the impression that γM antibodies diminish during the later stages of the disease, as in late or congenital syphilis.

Detection of γG and γM syphilitic antibodies by means of anti-γ and anti-μ reagents. The FTA test, using fluorescent anti-γ and anti-μ reagents, was carried out with sera from both syphilitic and non-syphilitic individuals. Most of the sera from syphilitics showed positive reactions to both reagents. Non-syphilitic sera with positive reactions to the FTA test in 1/5-1/20 dilutions also reacted to both of these reagents. Representative results from these trials are shown in Table 3. After ME treatment, a small decrease in the reaction by fluorescent anti-γ antibodies occurred, but all non-syphilitic sera and the serum from one syphilitic individual decreased in antibody titre by fluorescent anti-μ antibodies. Surprisingly, however, a small decrease in titre after ME treatment was observed in the fluorescent anti-μ antibody test in the later stages of syphilis. The reason for this lack of influence of ME treatment is at present being sought.

Complement-binding property of syphilitic antibodies detected by anti-β1CA,E reagents. Several experiments were carried out to investigate the complement-binding property of syphilitic antibodies

TABLE 2
FTA ANTIBODIES IN DEAEC FRACTIONS BY BROAD-SPECTRUM FLUORESCENT ANTI-GLOBULIN REAGENT

Sera (Patient No.)	Type of syphilis	DEAE cellulose column fractions			
		An-1 (γG)		An-4 (γM, etc.)	
		Saline	ME	Saline	ME
420	Primary	++++	+++	++	±
475	Primary	++++	++++	++	—
496	Primary	++++	++++	+++	—
383	Secondary	++++	++++	+++	—
450	Secondary	++++	++++	++++	—
455	Late	++++	++++	++	—
513	Late	++++	++++	+	—
405	Congenital	++++	++++	—	—

in the FTA test. In tests using fluorescent anti-β1CA,E on syphilitic sera inactivated at 56°C for 30 minutes, results were negative for all cases. Some of the sera became reactive, but others did not, after they had been mixed, in 1/50 dilutions, with fresh human sera that were negative by the FTA test. The findings in one such experiment are shown in Table 4.

It would appear that, among the antibodies concerned in the FTA test, there are some with very limited ability to fix complement. Nevertheless, there is no need to inactivate sera for testing by the FTA.

Different classes of antibody in the various stages of syphilis, as detected by fluorescent anti-γ, anti-μ, anti-α and anti-Fab-fragment reagents. The FTA tests of patient sera from individuals in different clinical stages of syphilis were performed with fluorescent anti-γ, anti-μ, anti-α and anti-Fab-fragment reagents, with the results shown in Table 5.

TABLE 3
FTA TESTS BY FLUORESCENT ANTI-γ AND ANTI-μ REAGENTS

Sera (Patient No.)	Diagnosis [a]	Treatment	FTA AB titre against	
			anti-γ	anti-μ
474	STS negative	Saline [b]	—	5
		ME	—	—
512	STS negative	Saline	5	5
		ME	5	—
481	STS negative	Saline	20	10
		ME	10	—
496	Primar syphilis	Saline	256	128
		ME	128	8
450	Secondary syphilis	Saline	2048	128
		ME	1024	128
455	Late syphilis	Saline	2048	128
		ME	1024	128

[a] STS = serological tests for syphilis.
[b] ME = mercaptoethanol.

TABLE 4

FTA TESTS BY FLUORESCENT ANTI-HUMAN COMPLEMENT REAGENT

Sera (Patient No.)	Anti-human globulins inactivate (control)	Anti-human complement	
		Inactivated	Add fresh human serum
370	++±	−	+
383	+++	−	++
439	+++	−	++
440	+++	−	±
443	+++	−	±
444	++++	−	±
448	++++	−	++
452	++±	−	+±
456	+++	−	+
475	++	−	+±
459	−	−	−

Serum from one patient with an initial chancre reacted positively with fluorescent anti-μ reagent. Reactivity against anti-γ reagent, only, was found in five syphilitic sera: three congenital, one late, and one early secondary; the sera of the remaining cases showed positive reactions with both anti-γ and anti-μ reagents. Positive test results with anti-α reagents were found in some cases of secondary syphilis. The fluorescent anti-Fab-fragment reagents always showed positive results.

Generally speaking, the positive anti-μ and anti-α FTA test results were found with sera from the early stages of syphilis; FTA tests that were positive only with anti-γ reagents were found in later stages of the disease, such as congenital or late syphilis. It might be considered that γM antibodies are produced in the early stages of syphilis and that the γG antibodies gradually become dominant during the course of the infection.

Light chain type of FTA antibodies detected by fluorescent anti-κ and anti-λ reagents. Syphilitic and

TABLE 5

FTA TESTS BY FLUORESCENT ANTI-γ, ANTI-μ, ANTI-α AND ANTI-FAB-FRAGMENT REAGENTS

Fluorescent antibody against					Sera	
γ (γG)	μ (γM)	α (γA)[b]	α (γA)[c]	Fab (S-frag.)	Diagnosis [a]	Case number
−	±	−	−	+	Primary syphilis	1
+	+	+	−	+	Secondary syphilis	2
					STS positive	1
+	+	+	+	+	Secondary syphilis	3
+	+	−	−	+	Primary syphilis (treated)	1
					Secondary syphilis	3
					Secondary syphilis (latent)	1
					Congenital syphilis	1
					STS positive	1
+	−	−	−	+	Primary syphilis (treated)	1
					Late syphilis (latent)	1
					Congenital syphilis	3
					STS positive	1
−	−	−	−	−	Normal	5

[a] STS = serological tests for syphilis.
[b] Immunized with A-myeloma protein.
[c] Immunized with human milk plasma.

TABLE 6

FTA BY FLUORESCENT ANTI-γ, ANTI-μ, ANTI-κ AND ANTI-λ REAGENTS [a]

Sera (Patient No.)	Stage of syphilis	Fluorescent antibodies			
		Anti-γ	Anti-μ	Anti-k	Anti-λ
492-1	Primary	−	±	+	−
663	Primary	++±	+	++±	+±
492-2 [b]	Primary	+	±	+	+
581	Primary	++	±	+±	±
557	Secondary	++++	++	++++	+++
549	Secondary	++++	++	++++	++++
538	Secondary	+++±	++	++++	+±
527	Secondary	++++	++	++++	+++±
583	Secondary	+++±	+±	+++±	+++
598	Late	+++	±	++	++
628	Late	+±	±	+	+
650	Congenital	+++	+	++	+±
594	Congenital	+++	±	++	+±

[a] Syphilitic sera in 1/200 dilution.
[b] Serum 492-2 was taken from the same patient as serum 492-1, but at a later stage.

non-syphilitic sera were checked, without titration, by fluorescent anti-κ and anti-λ reagents to determine whether the antibodies were produced monoclonally, as is known to be the situation with multiple myeloma [k]. Representative results of these investigations are shown in Tables 6 and 7. No great differences in reactivity, using these two reagents, has been demonstrated in sera from any stage of syphilis or sera from non-syphilitics. When sera were titrated by fluorescent anti-κ and anti-λ reagents, no significant differences between titres using either of these reagents was observed, either in syphilitic or in non-syphilitic sera.

DISCUSSION AND CONCLUSIONS

The immunological character of syphilitic antibodies active in the FTA test was investigated by the use of various fluorescent anti-globulin reagents. The results reported above are, in some points, similar to the observations on blood-group antibody production; [l] that is, in the early stages of syphilitic infection, only γM antibody may be detected, but later, γG antibody appears and gradually increases during the course of immunization until, finally, only γG antibody is produced. There may be a γA FTA antibody in certain stages of syphilis, since

TABLE 7

FTA TESTS BY FLUORESCENT ANTI-γ, ANTI-μ, ANTI-κ AND ANTI-λ REAGENTS [a]

Sera (Patient No)	Fluorescent antibodies			
	Anti-γ	Anti-μ	Anti-κ	Anti-λ
651	−	±	±	−
637	+	+	++	++
636	+++	+±	+++	++±
621	++	−	+	±
610	+++	+	++	+
603	+±	+	++	++
601	++	+	++	+±
594	++±	+±	++	+±
596	+±	+	+±	+±
572	++	−	++	+++

[a] Non-syphilitic sera in 1/5 dilution.

[k] Bernier, G. M. & Putnam, F. W. (1964) *Progr. Hemat.*, 4, 160.
[l] Mollison, P. L. (1962) *Blood transfusion in clinical medicine*, Oxford, Blackwell, chapter 7.

sera from six of 20 cases of this disease reacted fairly strongly to the fluorescent anti-α reagents. However, the antibody belonging to the γA immunoglobulins may appear rather early in the infection, since most of the positive test results were found with sera from patients with secondary syphilis. From the point of the character of the FTA antibody, the clinical significance for therapy was investigated, but no close correlation between the class of antibody present and stage of the disease has been observed.

The finding that light chains of both Type K and Type L are usually found in FTA antibodies suggests that the antibodies against *Treponema pallidum* evolved in syphilitic infection are produced, not in a single clone, but in two or more clones. However, in the course of the present investigation, one case of early syphilis was encountered in which the FTA antibody could be detected only with anti-Type K light chain reagent, suggesting that, in the early stage of syphilis, FTA antibody may be produced monoclonally.

La sériciculture et l'éradication du paludisme

par D. ATHANASSOV, *Conseiller paludologue, Organisation mondiale de la Santé, Genève, Suisse*

L'introduction des insecticides à action rémanente dans la lutte contre le paludisme a eu pour effet une régression spectaculaire de cette maladie et a permis d'envisager son éradication. Toutefois, dans certains pays à cultures séricicoles, des pulvérisations imprudentes d'insecticides à action rémanente ont causé une mortalité en masse des vers à soie amenant l'interdiction de ces mesures dans ces zones et entravant de ce fait la lutte contre le paludisme.

L'importance des facteurs sanitaires et économiques liés au paludisme et à la sériciculture devait déterminer de nombreuses recherches pour éclaircir les causes de cet inconvénient attribué aux insecticides et pour y apporter les remèdes de circonstance. Notre exposé présente les résultats des travaux que nous avons entrepris en Bulgarie, les confronte avec ceux d'autres auteurs et aboutit à un certain nombre de conclusions d'ordre pratique.

Dès avant 1952, nous avions démontré que, dans certaines conditions, le ver à soie peut vivre et se développer normalement dans des locaux traités au DDT et que ce dernier n'agit pas à distance. Nous entreprîmes alors des expériences dans deux régions de la Bulgarie.[a]

Notre première expérience a été menée dans un village de la région de Mikaïlovgrad. Nous y avons utilisé la race locale (jaune) de vers à soie et appliqué le DDT à la concentration de 6%, soit en solution de pétrole, soit en suspension suivant différentes variantes présentées dans le tableau 1. Comme matière adhésive pour fixer les particules de DDT, nous avons utilisé l'amidon, la colle-gomme arabique ou la chaux.

L'élevage des vers à soie a été assuré par une ouvrière dûment formée et instruite des règles d'hygiène requises. Nous avons utilisé comme témoins les vers à soie de la même éclosion élevés dans la maison de l'ouvrière ainsi que les vers à soie des coopérateurs.

Tout le matériel (tables, planches, perches, paniers, sacs, etc.) était neuf et les tables étaient placées à 50 cm des murs, sauf dans la variante chambre II où elles touchaient les murs. Le balayage à sec était interdit, à l'exception de la chambre I, et il était également interdit de poser les feuilles de mûrier sur le plancher. Au début de notre expérience et en raison de l'abondance des fourmis dans toutes les pièces, nous avons par précaution badigeonné les pieds des tables avec une colle spéciale « anti-chenille ».

Les pulvérisations de DDT ont commencé le 15 avril 1952 à raison de 2 à 2,5 g/m² et pour les chambres IV et VI à la dose de 3 g/m². Les chambres ont été aérées jour et nuit pour éviter l'accumulation de vapeurs du solvant auxquelles le ver à soie est très sensible. Le 8 et le 13 mai, les larves ont été distribuées dans les chambres. Elles étaient du 1er et du 3e âge (ces dernières dès leur éclosion étaient entre-

[a] Bogdanov, V. T. & Athanassov, D. M. (1955) *Rev. Inst. Rech. scient. Min. Agr. (Sofia)*, **4**, 11.