STUDIES OF SYPHILITIC ANTIBODIES*†

III. ANAMNESTIC REACTIONS AND 19S PREDOMINANCE OF THE ANTI-LIPOIDAL ANTIBODIES IN AGED PERSONS

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Syphilitic anti-lipoidal antibodies as detected by the standard tests for syphilis (STS) are usually recovered in both γ M (19S) and γ G (7S) globulin fractions (Davis, Moore, Kabat, and Harris, 1945; Laurell and Malmqvist, 1961; Aho, 1967), whereas substances responsible for biologic false positive (BFP) reactions are found in most instances solely in the γ M globulin fraction (Aho, 1968). Syphilis is a disease usually acquired in early adult life. Because of the prolonged immunogenic stimulation one might expect that the syphilitic antibodies at an advanced age would be mainly of γ G globulin type.

Aged persons may develop BFP reactions without any known cause except ageing *per se* (Tuffanelli, 1966). The present study was prompted by the accidental discovery of an elderly person with adequately treated old syphilis who exhibited rising titres in standard serum tests for syphilis. The frequency of such rising titres was studied and fractionation experiments were conducted to compare the distribution of anti-lipoidal and anti-treponemal antibodies.

Material and Methods

Records.—Since 1959, account has been kept of each patient with positive STS found at the State Serum Institute. In all new cases a special form has been sent to the doctor responsible for the patient's care, requesting information on the history, previous serological tests, and the present disease. Each patient has his own card on which the results of subsequent tests are then recorded. Up to the end of 1967, positive results were obtained from slightly over 11,000 patients. Serial specimens over a period of several years were recorded for about 15 per cent. of them.

Sera.—Those from patients with primary and secondary syphilis were from darkfield positive cases

with known dates of infection. Those from aged persons were found among specimens sent to the State Serum Institute for routine treponemal tests. They were positive in the TPI and FTA-ABS tests and had VDRL titres ranging from 4 to 128.

Methods.—The fractionations and the serological tests for syphilis as well as the measurements of immunoglobulins in the fractions were performed as described previously (Aho, 1967). In density gradient ultracentrifugations, nine successive 0.5 ml. fractions were collected by puncturing the tubes through the bottom. In tests for anti-lipoidal antibodies, all the fractions were titrated by the Kolmer complement-fixation test. Titrations were usually started from undiluted fractions. In tests for treponemal antibodies, only the fractions corresponding to the maximum of immunologically measured γ M and γ G globulin, respectively, were titrated.

In the FTA test an antiserum was used which had been raised in rabbits against the first protein peak of infectious mononucleosis serum eluted from Sephadex G-200 column. The antiserum was not specific for the immunoglobulins but reacted well with γ G, γ M, and γ A. Control experiments effected with labelled specific anti- γ G globulin serum indicated that there was fluorescence only in the fractions containing immunologically detectable γ G globulin.

Results

A search of the records from the State Serum Institute in 1959–67 revealed 27 cases (13 females and 14 males) fulfilling the following criteria:

(i) Positive STS had been detected in a person 60 years of age or older who on this occasion or some years previously had received penicillin in amounts considered to be sufficient for the treatment of syphilis;

(ii) After this there had been observed at least a 4-fold and usually slow increase in STS titres, and this titre increase had been confirmed in repeated specimens. In no instance was there a reversal to a lower titre level.

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The information concerning these 27 patients was supplemented, as far as possible, with data from hospital records. In two instances a note was found of previous completely negative tests. Twelve of the patients had evidence of old syphilis.

Treponemal tests were performed in only six cases. Two females and one male lacked treponemal antibodies and they apparently had BFP reactions. Some data concerning the twelve patients with evidence of syphilis are set out in Table I. In three cases treponemal tests were positive. Evidence of syphilis was obtained by other means in nine further cases. In the remaining twelve patients there was no valid information either for or against syphilis.

TABLE I
TWELVE PATIENTS SHOWING RISING TITRES WHO
HAD A HISTORY OF SYPHILIS

Patient No.	Sex	Age ¹ (yrs)	Titre Increase	Evidence of Syphilis
1	M	66	4-fold	Anamnestic ²
2	F	67	4-fold	Aortic insufficiency
3	F	68	4-fold	Anamnestic; aortic insufficiency
4	F	65	4-fold	Anamnestic; treponemal tests
5	F	69	4-fold	Syphilitic husband; treponemal tests
6	M	62	4-fold	Anamnestic
7	M	72	8-fold	Anamnestic; aortic insufficiency
8	M	71	8-fold	Anamnestic
9	F	68	8-fold	Anamnestic
10	M	74	8-fold	Anamnestic
11	F	70	8-fold	Tabes dorsalis
12	F	68	16-fold	Response to treatment ³

¹ Refers to the age at the time the highest titres were recorded.

² Information of early infectious syphilis.

³ Routine STS were found to be positive and they reverted to negative following penicillin treatment. Titre increase was noted many years later.

Table II illustrates the development of the reactions in one patient with syphilis. There was a gradual 8-fold increase in the VDRL titres from 1959 to 1963, and no change was observed during the following 3 years.

TABLE II
DEVELOPMENT OF STS IN PATIENT No. 8
(Born in 1892 early infectious syphilis treated in 1905)

Date	VDRL	Kahn
August 25, 1959	2 dils	1 dil
June 27, 1961	4 dils	2 dils
March 6, 1963	8 dils	8 dils
October 30, 1963	16 dils	16 dils
March 14, 1964	16 dils	16 dils
August 25, 1965	16 dils	8 dils
October 18, 1966	16 dils	8 dils

In one case the treponemal tests were performed on two serum samples with a 4-year interval. A 4-fold increase in VDRL titres had taken place while the Reiter protein complement-fixation (RPCF) test had reverted from positive to negative.

In two syphilitic cases, serum sent for the treponemal tests was available for ultracentrifugation studies. In one of them anti-lipoidal antibodies were found only in the 19S fraction, and in the other titres of the same level were found in both the 19S and 7S fractions.

Two of the three sera from patients with apparent BFP reactions were included in a previous study (Aho, 1968); their serological activity was confined to the γ M globulin fraction. In the third case serum was not available for fractionation studies.

To obtain more information about anti-lipoidal antibodies in aged persons, thirty additional sera from TPI-positive patients aged 65 years or more (mean 73) were fractionated by ultracentrifugation. In no instance were the fractions anticomplementary and therefore unsuitable for the tests. Table III shows that antibodies were detected only in the 19S fraction in nine sera. The sera with the highest titres in the series always contained some antibodies in the 7S fraction, although the titres in the 19S fraction were frequently 8- to 16-fold higher. In one serum antibodies were found only in the 7S fraction.

TABLE III
ANTI-LIPOIDAL ANTIBODIES IN TPI-POSITIVE
SERA OF 30 AGED PERSONS

Antibodies Present in	Number of Sera	Titre Range		Average Titre ¹	
		19S	7S	19S	7S
19S only	9	4-8	<1	4.7	<1
19S > 7S	16	2-64	1-16	12.3	2.5
19S = 7S	3	2-8	2-8	4	4
19S < 7S	1	2	4	2	4
7S only	1	<1	4	<1	4

¹ Calculated as the logarithmic mean of the titres.

Seven of the patients had symptomatic syphilis (aortic insufficiency (5) and benign tertiary disease (2)), and four of them had previously been treated with penicillin. The antibody distribution in the sera of these patients did not differ from that in the others.

The distribution of treponemal antibodies was studied in eight sera from patients with primary and four with secondary syphilis and in eight sera from the above TPI-positive series. Preliminary experiments had shown that antibodies active in the TPI and RPCF tests were present only in the 7S fraction even in the earliest phases of syphilis*. Therefore, fractions obtained by ultracentrifugation were titrated with the FTA and FTA-ABS systems.

* This may be due to the relative insensitivity of these tests; when the sera contained antibodies in high enough titres for fractionation to be performed, the shift from 19S to 7S had already taken place. It is also possible that γ M antibodies do not fix complement with certain antigens (Cunniff and Stollar, 1968).

The results of the tests without absorption are recorded in Table IV. The sera from patients with primary syphilis usually had slightly higher titres in the 7S fraction, and the difference was somewhat greater in secondary syphilis. In the sera from aged persons, by far the greatest proportion of the antibodies was in the 7S fraction, but usually some activity was still detected in the 19S fraction. In one serum the 19S and 7S fractions were separated by gel filtration on Sephadex G-200, and the presence of low titres in the 19S fraction was confirmed.

TABLE IV
DISTRIBUTION OF TREPONEMAL ANTIBODIES
IN 20 SERA

Syphilis	Number of Sera	Titre Range		Average Titre ¹		Difference 7S/19S
		19S	7S	19S	7S	
Primary	8	20-80	40-160	33	73	2.2
Secondary	4	40-80	160-320	56	226	4.0
Late	8	5-20	80-320	8.4	160	19

¹ Calculated as the logarithmic mean of the titres.

In some instances fractions were negative in the FTA-ABS system although the sera were positive, apparently because of the dilution that occurred in connexion with the ultracentrifugation. When the fractions were reactive, the titres paralleled those seen in the tests without absorption.

Discussion

The present series does not allow any firm conclusions to be drawn on the frequency of rising titres in STS in aged persons. They formed but a small proportion of the STS-positive cases in the older age groups. However, serial tests were performed in only a few of them and certainly not always during the period when the titre increase took place.

Several possible factors can be considered to explain such rising titres:

- BFP reaction;
- Relapse or asymptomatic re-infection;
- Anamnestic reaction.

Nearly half the patients exhibiting rising titres had evidence of old syphilis, and it is likely that at least some of those in whom the treponemal tests were not performed also had this disease. Penicillin treatment given to the patients before the titre increase is observed lessens the possibility of a relapse through lowered resistance that may occur when the patient is much older. The possibility of asymptomatic re-infection remains remote, because of the age of the patients and the fairly slow increase in the titres. One patient exhibiting rising titres in

STS had a concomitant reversal of the RPCF test from positive to negative.

Transient positivity in STS associated with conditions that only infrequently give rise to BFP reactions has been observed in patients with old seronegative syphilis (Lassus, Salo, and Aho, 1968). If the immunogenic stimulus leading to the production of syphilitic anti-lipoidal antibodies is given by treponemal lipids, BFP reactions are apparently caused by other mechanisms. By definition, such a transient positivity could then be regarded as an anamnestic reaction (Dorland, 1951).

It may be appropriate to consider ageing as a continuous stimulus capable of giving cause for persistent anamnestic responses, *i.e.* to redevelopment of positivity in STS or to titre increase. In the present series there was information of previous negative STS in only two patients, one of whom did have syphilis. However, reversal from seronegativity may have been present more frequently, but was not apparent from the records, since account was kept only of patients with positive STS.

Serum for fractionation studies was available from only two of the syphilitic patients with rising titres. A larger series was collected from TPI-positive aged patients for whom information about possible earlier tests was usually lacking. A marked 19S predominance was found in the anti-lipoidal antibodies. This differs from the situation in secondary and early latent syphilis, where the majority of these antibodies was found to be of 7S variety (Aho, 1967). In accordance with this work, the present complement-fixation experiments with Kolmer antigen were performed at 37°C. There is recent evidence indicating that γ G antibodies fix complement more effectively at 4°C. than at 37°C., whereas no such difference was found with γ M antibodies (Cunniff and Stollar, 1968). The use of an overnight incubation technique at 4°C. would probably have favoured 7S antibodies, but apparently in the same fashion at all stages of syphilis.

The 19S predominance in late syphilitic sera remains unexplained, and it is not known whether it is related to the rising titres. In any event, 19S predominance and rising titres are both peculiarities of anti-lipoidal antibodies, since most of the treponemal antibodies in the late syphilitic sera belonged to 7S globulins and no titre increase was found in them.

Summary

Records from the State Serum Institute in 1959-67 revealed 27 penicillin-treated patients over 60 years of age who exhibited a gradual 4- to 16-fold increase in STS titres. Three of the patients were

apparent BFP reactors, in twelve there was no firm evidence either for or against syphilis, and the remaining twelve had old syphilis. It was considered possible that the rising titres could be regarded as age-induced anamnestic reactions.

Sera from two of the last group of patients and from thirty TPI-positive patients over 65 years of age, with sufficiently high STS titres for fractionations to be performed, were subjected to sucrose gradient ultracentrifugation. In ten sera complement-fixing antibodies against Kolmer cardiolipin antigen were detected in the 19S fraction only, and the majority of the remaining sera had markedly higher titres in the 19S than in the 7S fraction. Titrations with the FTA and FTA-ABS systems indicated that most of the treponemal antibodies in the sera of aged persons with syphilis belonged to 7S globulins.

REFERENCES

- Aho, K. (1967). *Brit. J. vener. Dis.*, **43**, 259.
 — (1968). *Ibid.*, **44**, 49.
 Cunniff, R. V., and Stollar, B. D. (1968). *J. Immunol.*, **100**, 7.
 Davis, B. D., Moore, D. H., Kabat, E. A., and Harris, A. (1945). *Ibid.*, **50**, 1.
 Dorland, W. A. N. (1951). "The American Illustrated Medical Dictionary", 22nd ed., p. 1269. Saunders, Philadelphia.
 Lassus, A., Salo, O. P., and Aho, K. (1968). *Scand. J. clin. Lab. Invest.*, Suppl. 101, p. 41.
 Laurell, A.-B., and Malmqvist, J. (1961). *Acta path. microbiol. scand.*, **51**, 187.
 Tuffanelli, D. L. (1966). *Brit. J. vener. Dis.*, **42**, 40.

L'étude des anticorps syphilitiques. III

RÉSUMÉ

Les dossiers du State Serum Institute pendant les années 1959 à 1967 ont révélé 27 patients âgés de plus de soixante ans traités par la pénicilline qui ont montré une augmentation graduelle de 4 à 16 fois des titres STS. Trois des malades étaient apparemment des réacteurs BFP, et chez douze il n'y avait aucune preuve certaine pour ou contre la syphilis et les douze autres avaient une vieille syphilis. Il a été considéré comme possible que les titres qui augmentaient pouvaient être des réactions anamnestiques causées par l'âge.

Le sérum de deux malades du dernier groupe et celui le trente TPI positifs âgés de plus de soixante-cinq ans, ayant des titres STS suffisamment pour que des fractionnements soient faits, ont été soumis à l'ultracentrifugation (gradient sucrose), dans dix sérums des anticorps fixant le complément contre l'antigène du cardiolipine de Kolmer ont été découverts dans la fraction 19S seulement, et la majorité du reste des sérums avait des titres beaucoup plus élevés dans la fraction 19S que dans la fraction 7S. Les titrages d'après les systèmes FTA et FTA-ABS ont indiqué que la plupart des anticorps des tréponèmes du sérum des personnes âgées atteintes de syphilis appartenaient aux globulines 7S.