AN UNUSUAL CASE OF SEROLOGICAL DISCORD*

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" Serological discord " or " serological dissociation " are expressive terms used by French workers to describe the finding that different serological tests for syphilis give variable results on the same specimen of serum. When a battery consisting of a number of tests is used, an occasional discord among the results is inevitable and may render interpretation of the results difficult. It is most often due to differences in the sensitivity of the tests employed, so that when a serum contains only a small amount of reagin the least sensitive tests of the battery fail to detect it. This state of affairs exists in primary syphilis which is just entering the sero-positive stage, and in treated syphilis which is approaching sero-negativity and in which the reactions may fluctuate from positive to negative and back again before becoming permanently negative. When discord is found in strongly positive sera, it is most often due to a prozone phenomenon, to which all flocculation tests are liable ; the serum contains so much reagin that it swamps the lipoidal antigen when tested in the serum-antigen ratio used in the test and fails to give a visible precipitate. When suitably diluted such serum gives a strongly positive reaction. Complement-fixation tests seem much less liable to this drawback.

The patient whose case history is reported below provided an example of serological discord and presented serological features which are sufficiently unusual to merit recording.

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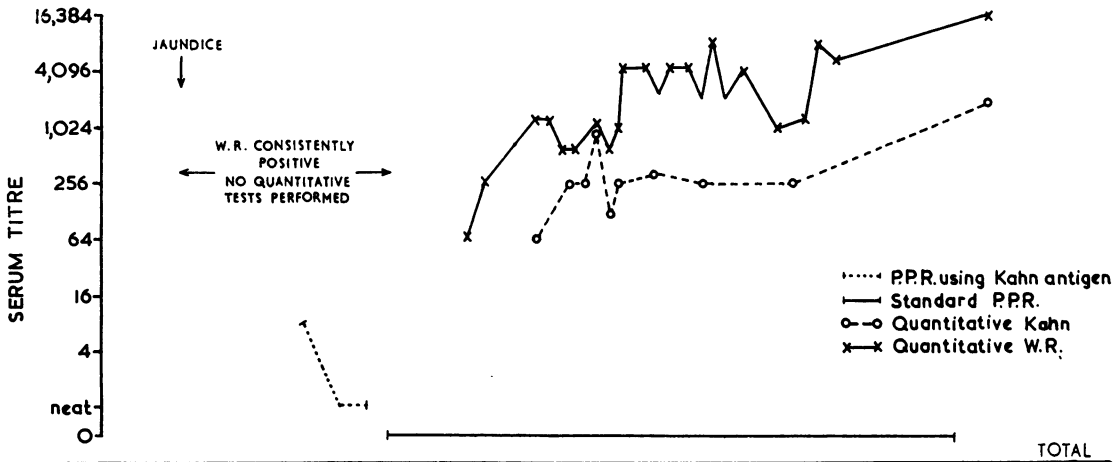

	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	TOTAL
N.A.B. (g.)	3 COURSES 9·9		2½ COURSES 9·95								19·85
BISMUTH (g.)	4 COURSES 11·6	2 COURSES	1½ COURSES 3·8			1 COURSE 2·4	1 COURSE 1·6				27·00
PENICILLIN (mu.)						6	7				13·00

FIG. 1.—Serological progress from 1944–1953.

Case Report

A 46-year-old male first attended the Whitechapel Clinic on December 11, 1943, complaining of a transient urethral discharge following an exposure 2 weeks previously; he had treated himself by painting his penis with tincture of iodine. He had had gonorrhoea when aged 20.

Examination.—There was a small, rather hard ulcer of the meatus, which was reddened. There was no inguinal adenitis and no urethral discharge, and the urine was clear. Dark ground examination was negative but the Wassermann reaction (Harrison-Wyler technique) and Kahn test were reported as positive.

Diagnosis.—Sero-positive primary syphilis.

Treatment.—N.A.B. and bismuth. It was noted that, before this was started, the sore had healed.

Progress.—On January 21, 1944, he was noticed to have a left facial palsy. Jaundice developed in July, 1944, during his third course of N.A.B., and arsenicals were withheld until February, 1946, but treatment was continued with bismuth. The Wassermann reaction remained obstinately positive, while the Kahn fluctuated between positive and negative during 1945 and 1946. Price's precipitation reaction (P.P.R.), using Kahn antigen (Price, 1946), fell from a titre of 8 in December, 1945, to 1 in August, 1946. At this time cardioscopy showed no evidence of aortitis and the cerebro-spinal fluid was normal, as it was when he was re-examined in August, 1948.

When the P.P.R. using uncholesterolized antigen (Price, 1948) was adopted for routine use in the laboratory in place of the standard Kahn test, it was noticed that although the Wassermann reaction remained strongly positive, the P.P.R. was consistently negative, and repeated tests with a quantitative technique showed that this was not due to a prozone phenomenon. Quantitative W.R. tests using the Whitechapel technique showed very high titres and this serological pattern has persisted ever since. The patient's serological progress is shown in Fig. 1. The general trend has been towards a progressive rise in W.R. and Kahn titres, while the P.P.R. remains obstinately negative.

In July, 1949, a patch of dermatitis was noted on the buttocks; this persisted, despite local treatment with Lassar's paste, and in February, 1950, the patient still complained of irritation of the buttocks and natal cleft. The lesion was described as dusky red with a serpiginous outline and slightly elevated margin with no scaling (Fig. 2). This was considered to be a tertiary syphilide and further treatment, consisting of eight weekly injections of 0·2 g. bismuth, followed by 7 mega units aqueous penicillin, was given, but 5 months later it was noted that the lesion was still present and unchanged in appearance.

Because of the patient's persistently anomalous serological reactions, a Treponemal Immobilization Test (TPI) was carried out in January, 1952, and was reported as negative. A similar result was obtained on a second specimen of serum.* In order to exclude a possible zone effect in the TPI, a third test was performed at the V.D. Reference Laboratory in July, 1953, with serum dilutions ranging from 1/10 to 1/1000, and negative results were obtained at all dilutions. Thus, after having received treatment totalling 19·85 g. N.A.B., 27·5 g. bismuth (as metal), and 13 mega units penicillin, the patient's peculiar serological pattern of reaction remained apparently unchanged.

Serological Investigations

The patient shows a serological pattern of behaviour which has not been previously seen in this laboratory, an extraordinarily high degree of reactivity in complement-fixation tests, using both crude heart extract and cardiolipin antigens,† with

*These two TPI tests were kindly carried out by Dr. G. W. Chacko at St. Mary's Hospital.

†The W.R. titres using cardiolipin antigen ran parallel to those with crude heart extract antigen but have been omitted from Fig. 1 for the sake of simplicity.

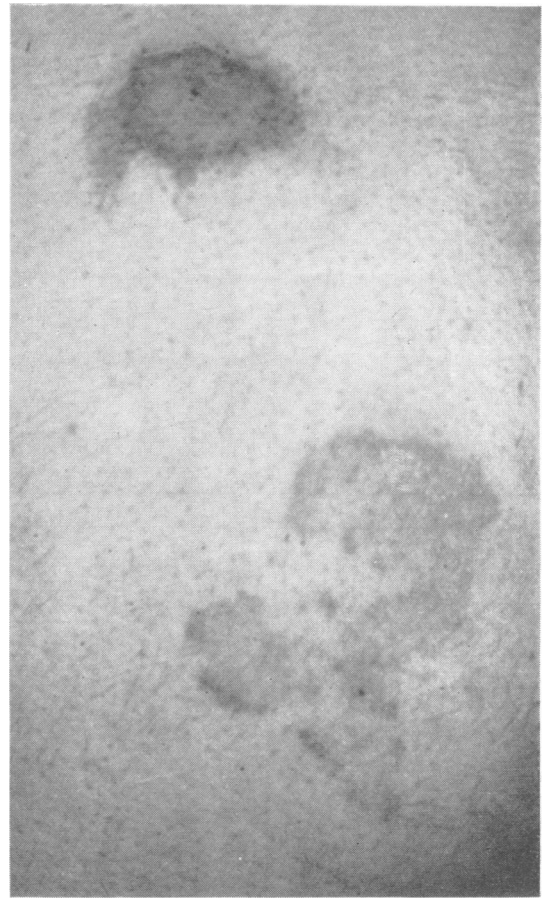

FIG. 2.—Skin lesion on buttock.

the Kahn test, and with V.D.R.L. cardiolipin antigens, and yet an apparently complete absence of reactivity with P.P.R. antigen. The titres with the complement-fixation tests have on some occasions reached the highest values recorded in the laboratory. A specimen of serum obtained on July 27, 1953, was therefore studied in some detail.

P.P.R. antigen differs in one important respect from the other antigens used, in that it is a simple alcoholic extract of beef heart which is not fortified with cholesterol. When cholesterol was added to the alcoholic extract in concentrations from 0·01–0·5 per cent. and the serum examined with these cholesterolized antigens at their respective optimum titres the results shown in Table I were obtained. Optimal reactivity was given by the antigen containing 0·25 per cent. cholesterol; above this value there was a slight but definite decrease in the density of the precipitates and in the titre reached, and with antigens with cholesterol contents below 0·25 per cent. the density of the precipitates became less and the tendency to zoning progressively more marked. It was also noted that with the uncholesterolized antigen there was a very slight precipitate at the serum dilutions 1/40 and 1/80. Price (1948) has shown that cholesterolization of the P.P.R. antigen does not enhance its reactivity with syphilitic sera. For comparison with Table I the behaviour of a pooled positive serum with the cholesterolized P.P.R. antigens is shown in Table II. It will be seen that cholesterolization in amounts up to 0·1 per cent. gave no significant increase in reactivity, whereas concentrations in excess of 0·1 per cent. caused a definite decrease in reactivity.

Since these experiments showed that the serum

TABLE I

EFFECT OF CHOLESTEROLIZATION OF P.P.R. ANTIGEN ON REACTIVITY WITH PATIENT'S SERUM

Serum Dilution	Cholesterol Content of Antigens (per cent.)					
	0·5	0·25	0·1	0·05	0·01	0*
10	++++	++++	+	±	0	0
20	++++	++++	+	+	0	0
40	++++	++++	++	+	±	±
80	++++	++++	++	+	±	±
160	++	+++	+	+	±	0
320	+	++	+	±	0	0
640	±	+	+	0	0	0
1,280	0	±	0	0	0	0
Negative Serum	0	0	0	0	0	0

*Antigen with no added cholesterol.

++++ complete precipitation.
0 no precipitation.

TABLE II

EFFECT OF CHOLESTEROLIZATION OF P.P.R. ANTIGEN ON REACTIVITY WITH POOLED POSITIVE SERUM

Serum Dilution	Cholesterol Content of Antigens (per cent.)					
	0·5	0·25	0·1	0·05	0·01	0*
Neat	++	++	++	++	++	++
2	±	+	++	++	++	++
4	0	0	+	+	+	+
8	0	0	±	±	±	±
16	0	0	±	0	0	0
Negative Serum	0	0	0	0	0	0

*Antigen with no added cholesterol.

did in fact react to a slight degree with standard P.P.R. antigen over a relatively narrow range of serum dilutions, the effect of centrifugalization was examined. Doubling dilutions of the serum were examined by the routine method of shaking the serum-antigen mixtures for the standard time of 5 minutes on a Kahn shaker and by centrifuging the mixtures at 2000 r.p.m. for 10 minutes. The results are shown in Table III together with the results of tests made at the same time with P.P.R. antigen made from Kahn antigen and with the V.D.R.L. tube test using a cardiolipin antigen. The latter two tests gave complete precipitation to a high titre with no evidence of a zone of inhibition. The two tests with P.P.R. antigen* on the other hand showed

TABLE III

PATTERN OF PRECIPITATION OF PATIENT'S SERUM WITH DIFFERENT ANTIGENS

Serum Dilution	P.P.R.			V.D.R.L. Tube Test
	Shaking	Centrifuging	Kahn Antigen	
Neat	0	0	++++	++++
2	0	0	++++	++++
4	0	0	++++	++++
8	0	0	++++	++++
16	±	±	++++	++++
32	+	+	++++	++++
64	+	+	++++	++++
128	+	+	++++	++++
256	±	+	++++	++++
512	0	±	+++	++++
1,024	0	0	+	++
2,048	0	0	0	0
Negative Serum	0	0	0	0

*This was a different batch from that used in the experiment shown in Table I.

an extremely atypical zone reaction in which precipitation was minimal over several serum dilutions. In the usual zone reaction given by the P.P.R. with a strongly reacting serum, after the prozone of inhibition has been passed, the flocculation rapidly becomes maximal and then progressively diminishes as the limiting titre is reached. In this case, the degree of precipitation was very slight and was unchanged throughout the range of precipitation. Centrifugalization produced essentially the same picture, but the observed titre was a little higher ; there was no difference in the degree of precipitation as compared with that produced by shaking. It should be emphasized that quantitative P.P.R. tests had been repeatedly carried out on earlier specimens of this patient's serum but no evidence of a zone phenomenon had previously been obtained.

Discussion

This patient poses questions both clinical and serological. The original diagnosis of syphilis was based primarily on serological grounds ; the fact that no treponemes were found in the lesion and that healing occurred before treatment was instituted may cast some doubt on the validity of the original diagnosis. Moreover, the subsequent clinical behaviour shown by the patient presents curious features. He had received a considerable amount of treatment, regularly administered (although treatment with arsenic had to be temporarily interrupted because of jaundice). Despite this, he developed a lesion which appeared to be a tertiary syphilide and was diagnosed as such by a venereologist of great experience. Five months after treatment with penicillin and bismuth the lesion was unchanged, whereas a syphilide would usually be expected to heal fairly rapidly. The negative immobilization test, thrice repeated, also suggests strongly that the patient was not then suffering from late syphilis since at this stage of the disease the test is almost invariably positive. While these criticisms are certainly not conclusive, their cumulative effect does raise some doubt as to whether the patient was in fact suffering from syphilis.

There are three possibilities to consider :

(*a*) The treponemal immobilization test was at fault and the patient did have primary syphilis and did develop a tertiary lesion. The negative result given by the immobilization test is certainly valid since it was performed on three specimens of serum in two laboratories and a possible prozone effect was excluded. The immobilization test is usually positive in cases of treated late syphilis ; Moore and Mohr (1952b) found 97·8 per cent. positive results among 262 cases of this type. Thus it is possible that the patient belongs to this small proportion of patients with late syphilis who give negative immobilization tests.

(*b*) The original diagnosis of primary syphilis was correct, but the lesion on the buttock was not syphilitic. This assumes that non-specific reagin tests may have been superimposed on an old treated syphilis. The fluctuating Kahn tests in 1945 and 1946 could be explained by the approach of sero-reversal in a case of primary syphilis, although the W.R. was consistently positive. In such cases the Kahn usually remains positive longer than the W.R. Had quantitative tests been available when the patient first came under observation they might well have given a useful indication of their specificity. The immobilization test would probably have been negative at the times it was performed in a patient with treated primary syphilis.

(*c*) The patient did not have syphilis in the first place and the reagin tests have given non-specific reactions throughout the period of observation. In general, such non-specific reactions are low titred. If, as the negative immobilization test suggests, the reactions given by this patient's serum are non-specific, they are the strongest reactions of this type which have been found in this laboratory.

Investigation of patients who give persistent non-specific reactions with reagin tests as judged by negative immobilization tests has shown that some may be suffering from unsuspected disease. Moore and Mohr (1952a) examined 51 patients showing this serological pattern : five were found to have " definite " and 21 " probable " diseases of the collagen group ; fourteen were clinically normal but laboratory tests gave abnormal results (raised sedimentation rate, abnormal liver function tests or serum globulin values) ; six others showed no abnormality. Haserick and Long (1952) have also reported five cases in which the appearance of presumed non-specific serum tests for syphilis preceded the onset of disseminated lupus erythematosus by periods of 1 to 7 years.

In the light of these findings further investigations of the patient were carried out.

November 26, 1953.—Serum bilirubin 0·2 mg./100 ml.
Serum alkaline phosphatase 7·5 King-Armstrong units.
Thymol turbidity 9·8 units.
Total plasma proteins 7·9 g./100 ml. (albumin 5·4 g./100 ml., globulin 2·5 g./100 ml.).

December, 12, 1953.—Total protein 8·1 g./100 ml. (albumin 4·7 g./100 ml., globulin 3·4 g./100 ml.).
Sedimentation rate 30 mm./hr. (Westergren),
Total white cell count 6,700 per cu. mm.
Differential count normal,
Haemoglobin 15·5 g./100 ml.,
Total red cell count 5,200,000 per cu. mm.
No L.E. cells seen in peripheral blood.

These laboratory findings showed definite abnormalities : a raised thymol turbidity value, an increase

in serum globulin, and a raised sedimentation rate. These all form part of the pattern found by Moore and Mohr (1952a) in their studies of patients with persistent non-specific serum reactions. In the present case they may possibly be explained by the development of liver damage following the attack of jaundice suffered by the patient early in the course of his treatment.

It is not possible to decide finally which of the three explanations of the patient's clinical and serological behaviour advanced above is correct. The writer favours the second (*b*) as the most likely, but, on the evidence available, this can only be a matter of opinion.

The patient is also of great serological interest. The long-continued failure to obtain a positive reaction with the P.P.R. despite the extremely high titres with complement-fixation tests and other flocculation reactions is striking. Schoch and Alexander (1944) have reported a case which shows some similarities to the present one; their patient had been treated for secondary syphilis and, when sero-reversal had almost been achieved, suddenly showed a very high titre with one brand of Kolmer Wassermann antigen while another brand of the same antigen gave negative or feebly positive results. This phenomenon lasted for 7 months and was associated with the development of multiple lung abscesses. They considered it to be a non-specific reaction due to the lung infection superimposed on a true syphilitic reaction. The anomalous serum reactions in the present case have lasted for some 6 years and our patient appears to be in good health.

The peculiar zone reaction with the P.P.R. on the last specimen of serum, coupled with the very strong flocculation to high titre with cholesterolized P.P.R. antigen, Kahn, and V.D.R.L. antigens without any suspicion of a prozone, is unique in the writer's experience. Brown (1943) has reported the occurrence of an inhibition phenomenon in precipitation tests due to the colloidal state of some sera, which allows union of antibody and antigen to occur but prevents the formation of precipitates. This is distinct from a prozone, in that dilution does not cause the inhibition to disappear while centrifugation overcomes the effect of the inhibitory factor and large flaky precipitates are formed. In the present case, centrifugation did not alter the character of the scanty precipitate produced, although it did produce a slight rise in titre. An attempt was also made to demonstrate an inhibitory agent by mixing equal amounts of the patient's serum and a pooled serum giving a strongly positive reaction with the P.P.R. The mixture of sera gave a reaction of comparable strength to that of a mixture of the positive serum with an equal volume of a P.P.R.-negative serum.

P.P.R. antigen differs from the other antigens used in that it is not fortified by the addition of cholesterol, though it can be shown to contain a small amount of this substance, which presumably comes from the heart muscle during the process of extraction. Attempts to increase the sensitivity of the antigen by the addition of cholesterol have been unsuccessful. In the case of this patient's serum, however, cholesterolization of the antigen is necessary for full reactivity, and in this respect he appears to be a serological curiosity.

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