EXPERIENCE WITH BRITISH LATEX SUSPENSION FOR AGGLUTINATION TESTS IN RHEUMATOID ARTHRITIS

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Since the initial utilization of polystyrene particles by Plotz and Singer (1956) as a macroscopic indicator basis replacing sheep erythrocytes in the methods originated by Waaler (1940) and Rose, Ragan, Pearce, and Lipman (1948) for the detection of the enhancement of agglutination by a macroglobulin in sera of rheumatoid arthritic cases, many variations in practical techniques have been published. Burby and Behr (1958) recorded a method employing Bovine Cohn Fraction II gamma-globulins as the sensitizing agent. Shortly after this, a British-produced polystyrene (Latex) particle suspension became available (Coke, 1958), and a comparison of the sensitivity of this method with that of the standard Rose-Waaler or sheep cell agglutination test (S.C.A.T.) would seem to be of interest.

Ziff (1957) demonstrated that an element of normal serum protein had a capacity to inhibit agglutination, which was lost or blocked in rheumatoid arthritic serum. He claimed that his method was much more accurate so that 98 per cent. of rheumatoid patients gave positive results, but the technique involved was difficult and time consuming.

The method of Burby and Behr, used in conjunction with the original British polystyrene suspension, was also found to depend on the inhibitory capacity of normal sera and the failure of inhibitory capacity in rheumatoid arthritic sera. Modifications of the British suspension have since been made, and these require the changes in technical procedure described under "Methods".

Any numerical assessment of specific serological tests of the clinical condition in rheumatoid arthritis must rely upon the physician's definition or interpretation of the clinical condition. Until a factual aetiology for this disease process is established, the unknown variations of clinical computation must play a part in the relative value of figures derived from any series of such tests. The clinical estimation becomes more variable in the mild, early, and doubtful ambulant case than in the severe, active, hospitalized case; the latter provides intensely

positive serological results which do not assist the definition of differentiation between positive and negative. The comparison, however, of two serological techniques such as the latex inhibition test of Burby and Behr, and the modified S.C.A.T. of Rose and Waaler, affords a factual numerical assessment, unaffected by clinical doubts and definitions.

While the purport of these results is the factual numerical correlation between the two technical methods, each test must be related to the clinical condition. The severity of the rheumatoid arthritis in the present series varied from the active advanced case with much functional distress to the relatively mild early case. The code of the revised criteria of the committee of the American Rheumatism Association (Ropes, 1959), together with their specified exclusions has provided the guiding principles. The clinical establishment of rheumatoid arthritis required the presence of observed joint swellings with tenderness (dissociated from any evidence of osteo-arthritis either clinically or radiologically), measured muscle weakness, perspiration of the palms, morning stiffness, and possibly a history of cyclical variations of exacerbations and remissions. Additional evidence was given by the raised erythrocyte sedimentation rate and at times by confirmatory radiological appearances. Differentiation from the degenerative endocrine polyarthritis of the climacteric in females is a more difficult and extensive proposition, to be dealt with in a separate communication.

Methods

S.C.A.T.—The original method of Rose and others (1948) was employed with two modifications. Preliminary absorption of heterophil agglutinins (Ball, 1950) was used, and the sheep erythrocytes were sensitized by the addition of 5 minimum haemolytic doses of rabbit anti-sheep cell serum (Difco). Agglutination at a dilution of 1 : 32 or more was considered as positive.

Latex Inhibition Test.—Latex suspension was prepared by diluting the commercial preparation in glycine buffer, so that it had a transmission of 35–41 per cent. or an

optical density $E_{1\text{ cm.}} = 0.46-0.39$ with an Ilford filter 608, with a maximum of $690\text{ m}\mu$. This necessitated a dilution of 1:1,000 with the original Polystyrene Company's product, and of 1:300 with the present-day product distributed by B.D.H. All the results recorded here were made with the first original product.

0.05 ml. patient's serum is added to a very clear unscratched test tube $3 \times \frac{3}{8}$ in. containing 0.95 ml. glycine buffer. Mix and remove 0.5 ml. to a second tube containing 0.5 ml. glycine buffer, and so on to make four dilutions 1:20 to 1:160. 0.1 ml. Armour's Bovine Fraction II gamma-globulin in a strength of 0.5 per cent. in glycine buffer is added to each tube. 0.5 ml. Polystyrene suspension, diluted as above to correct transmission value, is added to each tube. The control tube contains Latex suspension and Bovine globulin only, without any added serum. All tubes are treated for 2 hours in a water bath at 56°C . The tubes are removed and allowed to stand in rack overnight at room temperature. Results are read after 18 hrs, by viewing by transmitted daylight, preferably not in sunlight. Three stages of agglutination are noted:

- (i) A change in colour of opacity from a transparent blue tint to less transparent yellowish tint, which if undisturbed by agitation, can be seen as sedimenting with a line of demarcation below the top level of the fluid.
- (ii) A greater degree of agglutination, with particles visible through a hand lens and some small sediment at the bottom of the tube.
- (iii) A complete agglutination and sedimentation.

Any one of these three types occurring in the 1:20 and 1:40 dilutions is read as a positive reaction. Complete agglutination usually occurs when the S.C.A.T. reaches a titre of 1:256 or higher. The control tube should always show a complete agglutination.

The modern B.D.H. product does not agglutinate in the same way, but, as suggested by Burby and Behr (1959), gives a quite satisfactory reagent, if it is diluted to the correct transmission value and the Bovine gamma-globulin is increased to 0.2 ml. per tube. The same inhibitory phenomenon can then be utilized.

Glycine buffer is used as described by Burby and Behr (1958).

More recent experience suggests that these Latex colloidal suspensions may suffer with "ageing", as a result of which they become quite useless for these purposes. A control tube of Latex suspension, diluted first to the correct transmission value and later as in the standard test, heated, and cooled overnight, should show a pale bluish opalescence only. If it is "aged", then opacity and agglutination may occur.

Results

Bovine Fraction II globulin by itself causes agglutination of the Latex particles. Table I shows a typical experiment and indicates the quantitative nature of the reaction. From such results, the employment of 0.1 ml. 0.5 per cent. solution of Bovine globulin as the optimal amount was derived for a standard test, being the most sensitive position giving no appreciable agglutination with normal serum to the dilution of 1:80. 300 consecutive specimens of sera from ambulant patients (149 with rheumatoid arthritis and 151 with other types of arthritic disease) were determined for their agglutination capacity by both tests, each of the two tests being carried out simultaneously by different sections of the laboratory staff. The results were compared after a clinical assessment of the diagnosis had been obtained. Table II (opposite) gives the general results.

The non-rheumatoid arthritic group included cases of ankylosing spondylitis, rheumatic fever, gout, osteo-arthritis, non-articular manifestations and physical or traumatic deformities. The definition of a polyarthritis in the female of the climacteric period and during the degenerative period in the later decades, is becoming recognized as not neces-

TABLE I
LATEX AGGLUTINATION RELATED TO BOVINE F.II AS SENSITIZING AGENT
Results with Latex Suspension (Original Polystyrene) 0.5 ml. with Different Amounts of Bovine F.II 0.5 per cent.

Dilution of Normal Serum	..	..	..	..	1/20	1/40	1/80	1/160	1/320	1/640	1/1,280
Bovine F.II 0.5 per cent. (ml. per tube)	0.2	0	0	0	0	0	0	0	0	0	0
	0.1	0	0	0	0	0	0	0	0	0	0
	0.05	0	0	0	0	0	0	0	0	0	0
	0.025	0	0	0	0	0	0	0	0	0	0

Results with Different Types of Latex Suspension (0.5 ml.) with Different Proportions of Glycine Buffer and Bovine F.II

Glycine Buffer (ml.)	..	..	..	..	0.5	0.45	0.4	0.35	0.3	0.25	0.2
Bovine F.II 0.5 per cent. (ml.)	..	..	..	..	0	0.05	0.1	0.15	0.2	0.25	0.3
Latex Suspension	Original Polystyrene	..	..	..	0	+	++	++	++	++	++
	B.D.H. Suspension	..	..	..	0	0	+	++	++	++	++

0 = No agglutination

+ = Partial agglutination

++ = Complete agglutination

TABLE II
SIMULTANEOUS COMPARISON OF 300 CASES WITH THE SHEEP-CELL AGGLUTINATION TEST
AND THE LATEX PARTICLE INHIBITION TEST

Condition	No. of Cases	Result of Test	Test			
			S.C.A.T.		Latex	
			No.	Per cent.	No.	Per cent.
Rheumatoid Arthritis	149	+	109	73·15	105	70·5
		—	40	26·85	44	29·5
Non-rheumatoid Arthritis	151	+	4	2·7	16	10·6
		—	147	97·3	135	89·4

sarily homogeneous. This has been suggested by Olsen and Rantz (1958) and by Miall, Ball, and Kellgren (1958). Sex differences of this type were reported earlier by Ball (1952), Jacobson, Kammerer, Wolf, Epstein, and Heller (1956), and Alexander and de Forest (1954). Climacteric degenerative endocrine polyarthritis has been included as non-rheumatoid arthritis where the clinical evidence was sufficient to establish it.

Table III enumerates divergencies in greater detail; the last column shows the figure reported by Steinberg (1959) for 504 cases, in a similar comparison between the S.C.A.T. and the Plotz and Singer technique, using the Dow Latex suspension. His figures have been recalculated to the same total numbers.

Discussion

There is an apparent close relationship between the results of the two test techniques (83·3 per cent.). The correlation is closer with the Dow latex sus-

pension although the British polystyrene latex suspension does not fall far behind. For clinical assessment the S.C.A.T. is slightly more sensitive and accurate, especially in its lower rate of false positives. The nature of this group of clinical cases provides a series of the most difficult types to differentiate both serologically as well as clinically. The latex technique allows a considerable saving in time and labour, and it is possible to get the result in a routine laboratory within 24 hours of the venepuncture. Only the established diagnosis has been taken into account, without considering the effect of treatment or the severity or duration of symptoms. The stability of positive reactions irrespective of rheumatic activity and other features has been recognized, but recent reports suggest that changes may occur as a result of treatment (Michotte, Vanslype, Tritsmans, and Verstraete, 1959; De Forest, Mucci, and Boisvert, 1958) and many of the patients in this series are in fact undergoing active therapy.

TABLE III
DETAILED COMPARISON OF CONVERGENCE AND DIVERGENCE IN SHEEP-CELL AGGLUTINATION TEST
AND THE LATEX PARTICLE INHIBITION TEST

Condition	No. of Cases	Results of Tests		Present Series		Steinberg	
		S.C.A.T.	Latex	Convergence	Divergence	Convergence	Divergence
Rheumatoid Arthritis	149	+	+	92		88	
		+	—		17		9
		—	+	27	13	47	6
Total				119	30	135	15
Non-rheumatoid Arthritis	151	+	+	0		2	
		+	—		4		3
		—	+	131	16	142	4
Total				131	20	144	7
Grand Totals		No.		250	50	279	22
		Per cent.		83·3	16·7	92·7	7·3

It must be emphasized that these results, in so far as the latex technique is concerned, are derived entirely from the application of the Burby and Behr technique which, with the original British preparation, was found to involve the inhibition by normal sera, and they are not necessarily applicable to other techniques. Greenbury (1958) reported that, in his experience, this form of Latex suspension was inferior to the American (Dow) preparation, but his observations related to the Plotz and Singer technique, which is probably fundamentally different in its chemical reactions and does not necessarily depend upon the inhibitory functions of the normal serum. Burby and Behr (1959) report satisfactory correlative findings. The employment of these relatively simple mixtures affords a means of determining the nature of these reactions in greater detail, and it is hoped to report upon them in the near future.

Summary

Technical details of a method utilizing British Polystyrene Latex particles in the agglutination test for rheumatoid arthritis described by Burby and Behr (1958, 1959) are set out. The results of this test are compared with those of the standard sheep cell agglutination test in 300 simultaneous examinations. The two tests agreed in 83.3 per cent. of results. In assessment with the clinical diagnosis, the sheep cell agglutination test was found to be more sensitive and accurate, mainly because of the smaller number of false positive reactions.

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Essai de la suspension britannique de Latex pour les réactions d'agglutination dans l'arthrite rhumatismale

RÉSUMÉ

On présente les détails techniques d'un procédé employant des particules de Latex, un polystyrène britannique, dans la réaction d'agglutination pour l'arthrite rhumatismale, selon Burby et Behr (1958, 1959). On compare les résultats de cette réaction à ceux obtenus avec la réaction d'agglutination de globules de mouton standard en 300 examens simultanés.

Il y eut un accord entre les deux réactions dans 83,3% des résultats. Par rapport aux procédés chimiques, la réaction d'agglutination de globules de mouton s'avéra plus sensible et plus précise, surtout en raison d'un nombre plus petit de réactions positives fausses.

Experiencia con suspensión británica de Latex para reacciones de aglutinación en la artritis reumatoide

SUMARIO

Se presentan los detalles técnicos de un método usando partículas de Latex, un polistireno británico, en la reacción de aglutinación para la artritis reumatoide, según Burby y Behr (1958, 1959). Se comparan los resultados de esta reacción a los obtenidos con la reacción de aglutinación de eritrocitos de carnero standard en 300 investigaciones simultáneas.

Ambas reacciones se acordaron en un 83% de los resultados. En comparación con los métodos químicos, la reacción de aglutinación de eritrocitos de cordero se mostró más sensible y más precisa, particularmente por dar menos falsas reacciones positivas.