

Melano flocculation (Henry) and Buffer Precipitation (Wolff) tests and their response to artificially produced alterations of the protein fractions of sera by some immunising agents

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The only experimental result suggesting that the Henry reaction (1929) is a specific antigen—antibody reaction for melanin is the report of Kritschewsky and Rubinstein (1935). These authors failed to produce a positive Henry reaction when injecting melanin intravenously into rabbits, but obtained positive results when suspending the melanin in pig serum. These results were explained by assuming that melanin was a haptene, which acquired full antigenic properties when combined with a protein, e.g. pig-serum.

Chorine (1937) obtained identical results with the Henry test when using distilled water only. The latter test, however, involved the use of a photometer. Melanin, therefore is to be considered as an intensifier only, which renders the non specific precipitation of euglobulins more easily read by the naked eye.

Wolff (1939, 1940) devised a method to avoid the need of a photometer by establishing the optimum flocculation of malarial sera at different pH values. This buffer precipitation test (B.P.T.) proved very useful in surveys and field trials (Bogen 1945, Robinson 1945).

Westphal (1950) reviewed the literature concerning the Henry test and filled the gap left by Kritschewsky and Rubinstein (1935) by investigating the effect of pig-serum injected without melanin into rabbits. He was able to show that pig-serum alone produced the same positive Henry reaction, as when given with melanin together. The occurrence of a positive Henry test after 3 to 7 injections of pig-serum supports the view that the Henry reaction is not a specific antigen—antibody reaction. His results suggest that the Henry reaction goes parallel with the appearance of antibodies specific for the immunising agent.

To prove this assumption and to investigate the behaviour of Wolff's B.P.T., 5 rabbits were injected intravenously with 1 ml. each of pig-, cattle-, horse-, human-serum, and T.A.B. vaccine. The rabbits were bled with 2 days interval for 2 weeks and finally at the end of the 3rd week. Henry (melano flocculation) test (Tyagaraja's

modification, 1938) was used along with the B.P.T., and antibody formation was followed up by precipitin and agglutination tests, respectively.

The results are shown in the following table.

				<i>Days after injection</i>						
		1	3	5	7	9	11	14	21	
Cattle-serum 1 ml i.v.	H.T.	..	—	—	+	++	++	++	++	
	B.P.T.	..	—	—	—	—	—	—	—	
	Prec.	..	—	—	—	+	++	++	++	
Pig-serum 1 ml i.v.	H.T.	..	—	—	—	++	++	++	++	
	B.P.T.	..	—	—	—	—	—	—	—	
	Prec.	..	—	—	—	+	++	++	++	
Horse-serum 1 ml i.v.	H.T.	..	—	—	+	+	++	+	+	
	B.P.T.	..	—	—	—	—	—	—	—	
	Prec.	..	—	—	—	+	++	++	++	
Human-serum 1 ml i.v.	H.T.	..	—	—	+	+	++	++	++	
	B.P.T.	..	—	—	—	—	—	—	—	
	Prec.	..	—	—	+	+	++	++	++	
T.A.B. vaccine 1 ml i.v.	H.T.	..	—	—	++	++	++	++	++	
	B.P.T.	..	—	—	—	—	—	—	—	
	Aggl.	..	—	+	++	++	++	++	++	

Key—H.T. = Henry Test. B.P.T. = Buffer Precipitation Test.

Prec. = Precipitin Test. Aggl. = Standard Agglutination Test.

The results show clearly that the melano flocculation (Henry) is a very sensitive lability reaction, which responds quickly to an increase of the euglobulins, whereas the B.P.T. remained negative throughout the experiment. Euglobulins are on the increase when antibody formation takes place. It becomes therefore quite understandable that the melano flocculation may yield positive results in other diseases as well. Kala-azar is known to be a potent source of error, but other diseases, such as typhoid, typhus, leprosy, trypanosomiasis, liver diseases, to enlist only a few, may also interfere with the melano flocculation, whereas the B.P.T. responds to kala-azar and disturbed liver functions in addition to its prompt reaction in malaria (Wolff 1939).

Both tests were performed on malarial sera and on sera sent for Standard Agglutination Test for typhoid and typhus (S.A.T.). As malaria is nearly eradicated in Ceylon, only a few (3) sera were available, all were positive with the melano flocculation, and one was positive with the B.P.T.

Sera positive in the S.A.T. (58) showed positivity with the melano flocculation in a rather high degree (48), whereas the B.P.T. was negative in all but one.

Sera negative in the S.A.T. (57) showed less positivity in the melano flocculation (39), and the B.P.T. was negative for all sera.

It is quite clear that none of these sero reactions can claim specificity for malaria, but from the above results it may be said that the B.P.T. reacts in a more exclusive way and should be given preference as the more 'specific' test.

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References

1. BOGEN, E., 1945, *Naval Med. Bull.*, 45, 1, 47.
2. CHORINE, V., 1937, *Ann. Inst. Pasteur*, 58, 78.
3. HENRY, F. A. X., 1929, *Abstr. Trop. Dis. Bull.*, 26, 941.
4. KRITSCHEWSKY, L. L., and RUBINSTEIN, P., 1935, *Zschr. Imm.forsch.*, 4, 397.
5. ROBINSON, A., 1945, *The serological Diagnosis of Malaria with special reference to the Buffer Precipitation Test of Wolff*. Thesis, Queen's University, Belfast.
6. TYAGARAJA, S., 1938, *J. of Ceyl. Br. B.M.A.*, XXXV, 4, 342.
7. WESTPHAL, A., 1950, *Zschr. f. Tropenmed und Paras.* II, 1, 68.
8. WOLFF, E. K., 1939, *Transact. Royal Soc. Trop. Med. and Hyg.*, XXXII, 6, 707.
9. WOLFF, E. K., 1940, *Ind. Med. Gaz.*, LXXV, 9, 517.