protective power against active infection with typhoid organisms is now under investigation.

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On the Blocking Antibody and the Zone Phenomenon in Human Anti-Rh Sera

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Recent findings by Race (*7*), Wiener (*8*), and Diamond (*2*) indicate the importance of the indirect test as evidence of active isoimmunization in Rh– mothers of erythroblastotic infants. According to Levine (*6*), only 50 per cent of Rh– mothers who recently delivered infants suffering from this hemolytic disease have active anti-Rh agglutinins in their sera. In the remaining sera which fail to agglutinate Rh– blood, antibodies are present which specifically coat the surface of Rh+ red cells, rendering them resistant to the action of potent anti-Rh agglutinins. Diamond and Abelson (*3*) found that these so-called "incomplete," "blocking," or "inhibiting" antibodies will agglutinate heavy suspensions of fresh blood in tests carried out on slides. Presumably, the determining factor is the use of serum instead of saline as the medium for the cell suspension (*9*).

These significant findings on the behavior of blocking antibodies were confirmed. However, Diamond's observation that blocking sera will agglutinate heavy suspensions of washed blood could not be repeated. In order to facilitate studies on the specificity of the agglutination reactions given by blocking antibodies, Diamond's method was modified by the use of a 2-per cent cell suspension in human serum and test tubes instead of slides.

In the course of these confirmatory tests additional observations were made on the properties of those anti-Rh sera which exhibit the so-called zone phenomenon. Obviously, these sera contain both anti-Rh agglutinins and blocking antibodies, but the concentration of the former is greater. By and large, such sera were not considered as useful diagnostic reagents unless the agglutinin in a particular serum was far more active than the blocking antibody. However, the experiments presented indicate that at least some of these sera can be absorbed with Rh+ blood, so that after the blocking antibody is specifically removed the treated serum becomes a useful diagnostic reagent.[1] A typical experiment, given in Table 1, was carried out with the same serum originally described as exhibiting the zone phenomenon.[2]

TABLE 1

	Serum Sil.* Diluted 1:									
	2	4	8	16	32	64	128	256	512	1,024
Untreated	tr	±	+	+	+±	++	+±	+	±	0
Absorbed at 37° C.	+++	+++	+++	++±	++±	+±	+	±	0	0
Absorbed at 0° C.	+++	+++	++±	++	+±	+±	+	+	±	0

* The serum of patient Sil. was drawn in July 1942, and tests indicated in the table were carried out in July 1945. The undiluted serum was absorbed with an equal volume of washed sediment of Rh+ blood at both 37° C. and 0° C., but the agglutinin activity of the absorbed and unabsorbed sera were tested at 37° C. with 2-per cent saline suspension of Rh+ blood.

The test shows a somewhat higher titer with the serum absorbed at the lower temperature. Presumably, a slight quantity of the agglutinating activity was absorbed at 37° C., along with all of the blocking antibody.

Similar results were obtained with two other anti-Rh sera, one of which gave only doubtful reactions in the course of the titration. On absorption at 0° the two sera had agglutination titers of 1:32 and 1:16, respectively. The corresponding titers, after absorption at 37° C., were 1:2 and 1:4.

As was to be expected, the absorption mixtures with undiluted serum exhibited no gross agglutination; this was a favorable circumstance, because unagglutinated cells present a much larger surface area for specific absorption than agglutinated cells. This view could be confirmed in parallel absorption tests of pure anti-Rh agglutinins which require several treatments for complete absorption, whereas blocking antibodies of identical degree of activity were almost completely removed by one absorption.

The result presented in Table 1 could be duplicated in tests with a mixture consisting of equal parts of an anti-Rh_0 serum with agglutinating activity and another serum containing only blocking antibodies. On absorption of this mixture, which simulates to a remarkable degree the so-called zone phenomenon, it was possible to recover a great deal of the agglutinating activity (Table 2).

[1] After these experiments were completed, reference was found to similar absorption tests carried out by Wiener (*8*), but there is no indication that he tested the activity of the residual agglutinins by titration.

[2] The serum used in this test did not exhibit the zone phenomenon when first studied in July 1942. On standing for several months in the ice chest the serum was no longer found to be useful for routine testing because it exhibited the zone phenomenon to a striking degree (*5*).

The results indicate a slight, but distinct, zone effect in the mixture absorbed at 0° C. To a more striking degree the same effect was observed in mixtures of the same blocking serum with each of two other potent anti-Rh_0 agglutinins. This effect is probably due to incomplete absorption of the blocking antibodies in the mixture at the lower temperature. On absorption at 37° C., the blocking antibody was removed completely, but at the same time, there was a slight, but definite, loss of agglutinin titer. Nevertheless, the treated serum is still useful as a diagnostic reagent.

TABLE 2*

Serum	Diluted 1: 2	4	8	16	32	64	128	256	512	1,024
1	+++	+++	+++	++±	++	++	+±	+	tr	0
2	0	0	0	0	±	+	++	++±	+++	+++
1 + 2, aa	0	0	0	ftr	±	+	+	±	ftr	0
1 + 2, absorbed at 37° C.	+++	+++	+++	+	±	tr	0	0	0	0
1 + 2, absorbed at 0° C.	+	+±	++	+++	++	+	±	0	0	0

* Absorption of mixture of Sera 1 and 2 with equal volume of washed sediment of Rh+ blood. The titrations were tested with a 2-per cent saline suspension of Rh+ blood. Serum 1 is a pure agglutinating serum (anti-Rh_0). Serum 2 is a "pure" blocking serum. The readings given for Serum 2 were made after the addition of a 1 : 10 dilution of a potent anti-Rh_0 serum. The result indicates a blocking antibody titer of 1 : 64–1 : 128.

Anti-Rh sera exhibiting the zone phenomenon behave somewhat differently in specific absorption at 37° and at 0° C. If the absorption of the blocking antibody is complete at the lower temperature, the treated serum is apt to give higher agglutinin titer than that shown by the serum absorbed at 37° C. On absorption at the higher temperature, the blocking antibody is removed first, and if this absorption is complete, some of the agglutinin activity may also be lost. Accordingly, one can expect to find for each serum a dosage of the absorbing blood which, under certain conditions, will remove all of the blocking antibody without influencing the agglutinin titer to any great extent.

Without carrying out absorption tests, it is difficult to state how often anti-Rh sera contain both blocking antibodies and agglutinins. The authors studied several sera with such concentration of the two antibodies that, even on titration, only traces of direct agglutination could be observed. Unless these tests are read microscopically, the weak degrees of agglutination in the higher dilutions could readily be overlooked. Furthermore, it seems probable that routine titration tests will entirely fail to detect those sera containing weak agglutinins and strong blocking antibodies. These views could be substantiated in experiments on mixtures of an agglutinating serum and an excess of a blocking serum. Although no zone effect could be obtained on titration, absorption of the undiluted mixture with Rh+ blood yielded a fluid which had strong agglutinating activity.

It is significant that in the observations reported, the tests were carried out with diluted cell suspensions in saline. Under these conditions, specific absorption of certain anti-Rh sera reveal the presence of agglutinins even in the presence of strong blocking antibodies.

These findings have some bearing on the nature of the agglutinating activity of blocking sera, and the available evidence indicates that this agglutination is a manifestation of the blocking antibody per se. This could be determined in a few instances by titration results of blocking sera diluted in normal human serum instead of saline. These tests were carried out not only on slides with heavy suspensions of unwashed blood, but also in test tubes with 2-per cent cell suspensions in serum. The results with the latter modification provide proof that the slide agglutination does not depend upon the use of a "large number of red cells to absorb the inhibiting substances (blocking antibody) and to combine with agglutinin in sufficient number to form easily visible clumps" (*3*). As first suggested by Wiener, the essential mechanism in the slide test as direct evidence of blocking activity is the use of serum as the medium for suspending the Rh+ red cells. In further support of this view is our failure to obtain a positive slide test with the use of washed blood.

In contrast to the observations on the slide test is the finding that the agglutinin in a particular anti-Rh serum masked by an excess of blocking antibody can be studied quantitatively by the usual procedures after specific absorption of the blocking antibody.

From a practical standpoint, the significance of these findings lies in the fact that the characteristics of the agglutinating antibody and the blocking antibody are sufficiently clear-cut so that, for example, there is no difficulty in the use of an anti-Rh serum which contains an anti-Rh′ agglutinin and also an anti-Rh_0 blocking antibody.

Addendum: After this paper was submitted (August 1945), several important studies on the characteristics of blocking antibodies have appeared (*1, 4, 10*). The use of the term "conglutination" suggested by Wiener for the test with serum-suspended cells was criticized by Coombs, Mourant, and Race. One of the objections is based upon the presence of a heat-labile "conglutinating" complement.

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Relapses Following Delayed Treatment of Naturally Induced *Vivax* Malaria of Pacific Origin

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A most difficult problem in the treatment of *vivax* malaria of South and Southwest Pacific origin is the stubborn tendency of the disease to relapse. The exact mechanism of relapse is not known (*9*), but the suggestion has been made that drug therapy which promptly and completely aborts the primary clinical attack may inhibit the development of acquired immunity (*8*). This, in turn, may be partially responsible for the relapse (*9*). Accordingly, it seemed reasonable to consider that prompt therapy of acute attacks such as soldiers obtain might be responsible for the large number of relapses, and to suggest the desirability of investigating the effect of withholding treatment if an opportunity arose (*6*). The establishment by the Surgeon General of a center for the treatment of neurosyphilis at an Army hospital, to which large numbers of soldiers with relapsing *vivax* malaria had been admitted, has given an opportunity to study various facets of the primary attack of malaria. This report presents data on the effect of delay of institution of quinacrine therapy on the tendency to relapse.

Ten white soldiers with malaria, acquired in the South or Southwest Pacific, volunteered as the original sources for the infection of the American anopheline mosquitoes (*10*) used for natural induction of the disease in 69 white soldiers who required malaria therapy because of neurosyphilis. The administration of quinacrine dihydrochloride (2.8 grams in 6 days) was delayed until the patients had had from 8 to 15 paroxysms with an average of approximately 40 hours of fever over 104° F. This level was reached on the average in approximately 20 days, during which blood smears were positive for malaria parasites. The patients were then followed either until a relapse occurred or for at least 60 days without a relapse. Thick smears of the blood were examined twice weekly for parasites during the period of observation, except during an interval of three weeks of furlough immediately after completion of quinacrine therapy. None of the patients received antiluetic therapy with heavy metals during the period of observation for relapse.

It is seen in Table 1 that 45 patients (65 per cent) have had a relapse. There were no significant differences between the mean hours of fever above 104° or between the mean days of parasitemia in those patients who relapsed and those who did not. Of 16 patients observed following quinacrine therapy of the first relapse, 11 (69 per cent) have had a second relapse. In a group of 124 patients who contracted *vivax* malaria in the same overseas areas and whose relapses at Harmon General Hospital were treated promptly with quinacrine, 92 (74 per cent) relapsed. Relapse rates of 70, 76, and 77 per cent following prompt treatment of initial attacks have been reported by Dieuaide (*3*) for three organizations serving overseas. The differences between the rates of relapse following prompt and delayed treatment can hardly be considered of practical significance. Such a result was to be expected from the findings of Boyd and Kitchen (*1*) that there was no relationship between the duration of illness prior to therapeutic termination of the primary attack and the rate of relapse.

TABLE 1

RELAPSES FOLLOWING QUINACRINE TREATMENT OF *Vivax* MALARIA OF SOUTH OR SOUTHWEST PACIFIC ORIGIN

	Delayed treatment of first attack of mosquito-induced therapeutic malaria		Prompt treatment of relapses of naturally acquired malaria	
No. of cases	69		124	
No. of relapses	45*		92†	
Per cent relapse	65		74	
Time to relapse in days‡	Relapses		Relapses	
	Number	Per cent (Cumulative)	Number	Per cent (Cumulative)
Less than 29	2	4	6	7
30– 59	32	76	45	55
60– 89	8	93	21	78
90–119	2	98	12	91
120–149	0	..	3	95
150–179	0	..	5	100
188	1	100	..	..
Total	45	100	92	100

* Average period of observation of nonrelapsers in days: 109 (range, 66–185).
† Average period of observation of nonrelapsers in days: 143 (range, 62–353).
‡ Calculated from time of completion of treatment.

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Seventy-six per cent of the relapses which developed within the period of observation following delayed treatment of the patients with mosquito-induced