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Phenomenon of Precipitation on Mixing Hemoglobin With Tissue Extract Antigen.*

REUBEN L. KAHN, ELIZABETH B. MCDERMOTT, ALBERT H. WHEELER, AND JACOB ADLER.

From the Clinical Laboratories, University Hospital, University of Michigan, Ann Arbor.

It was observed in this laboratory that precipitation occurs on mixing laked erythrocytes, referred to as hemoglobin solution, with tissue extract (Kahn) antigen. It was also observed that hemoglobin solutions derived from malarial (vivax) and nonmalarial erythrocytes showed some differences in their zones of precipitation. To our knowledge such observations have not heretofore been reported.

Zones of Precipitation with Malarial and Nonmalarial Hemoglobin Solutions: Hemoglobin solution is prepared as follows: Clots from whole blood specimens are broken up mechanically in about 5 cc of physiologic salt solution. Unbroken clumps remaining are removed by filtration through gauze. The resulting red cell suspension is washed free from serum as in the preparation of sheep cells for complement fixation tests. Three to four washings with saline are usually sufficient. The washed cells are packed in a calibrated centrifuge tube by centrifugation for 15 min at 1,800 rpm, the volume of the packed cells recorded, and the water-clear supernatant fluid discarded. The tubes are allowed to stand for 10 min and the residual supernatant fluid is removed as completely as possible. Distilled water is added to the packed cells to make a 1:5 dilution and the cells are hemolyzed as thoroughly as possible by stirring intermittently with a wooden applicator during a 10 min period. The resulting turbid solution is centrifuged for 15 min at 1,800 rpm. The supernatant hemoglobin solution is removed carefully from the sediment with a bulb pipette and is employed in preparing serial dilutions with distilled water ranging from 1:5 to 1:1,920.

Antigen suspension is prepared by mixing standard Kahn antigen with 0.3% NaCl solution instead of 0.9%. The method of mixing and the aging of the suspension before use are the same as employed in the Kahn test.

The serial dilutions of hemoglobin solution are added to the antigen suspension in the following ratios: 1:4, 1:2, 1:1, 3:1, 6:1, 12:1, 18:1, 24:1 and 30:1 (Table I.). The tubes are shaken by hand for a few seconds to assure proper mixing and the results are read.

Two main types of precipitation zones were obtained, the "limited zone" and the "wider zone," illustrated in Table I. It is apparent from this table that the two types differ mainly in the precipitation results obtained with 1:15 and 1:30 dilutions of hemoglobin in the 6:1 through the 24:1 ratios. In this "differential range," positive precipitation results are noted in the "wider zone" and negative results in the "limited zone."

Both types of precipitation zones were obtained with hemoglobin solutions of malarial and nonmalarial origin, but the hemoglobin solutions of malarial origin tended to give more frequently the "wider zone" of precipitation. The malarial specimens were provided us through the courtesy of the U. S. Naval Medical School, Bethesda, Maryland, and of the U. S. Naval Hospital, San Diego, California. The nonmalarial specimens were obtained from the Serological Laboratory of the University of Michigan Hospital.

Precipitation in the "Differential Range" with Malarial and Nonmalarial Hemoglobin Solutions: Table II. presents precipitation results of hemoglobin solutions from 10 malarial and 10 nonmalarial blood specimens.

These solutions in each instance were diluted 1:15 and 1:30 with distilled water and were tested with antigen suspension in ratios of 6:1 through 24:1. These ratios and hemoglobin dilutions represent the differential range of

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TABLE I.
Illustration of Zones of Precipitation Shown by Hemoglobin with Tissue Extract Antigen.

	Hemoglobin solution, cc								
	0.05	0.05	0.1	0.15	0.15	0.15	0.18	0.24	0.30
	Antigen suspension, cc								
	0.2	0.1	0.1	0.05	0.025	0.0125	0.01	0.01	0.01
	Ratios of hemoglobin dilution to antigen suspension								
	1:4	1:2	1:1	3:1	6:1	12:1	18:1	24:1	30:1
Dil. of hemoglobin with distilled water	Limited zone of precipitation								
1:5	4*	4	—	—	—	—	—	—	—
1:10	4	4	4	2	—	—	—	—	—
1:15	4	4	4	4	—	—	—	—	—
1:30	4	4	4	4	2	—	—	—	—
1:60	4	4	4	4	4	4	4	—	—
1:120	—	3	4	4	4	4	4	2	1
1:240	—	2	4	4	4	4	4	4	—
1:480	—	—	—	4	4	4	4	3	—
1:960	—	—	—	—	—	4	4	—	—
1:1920	—	—	—	—	—	—	—	—	—
	Wider zone of precipitation								
1:5	4	4	4	4	—	—	—	—	—
1:10	4	4	4	4	4	2	—	—	—
1:15	4	4	4	4	4	4	4	3	—
1:30	4	4	4	4	4	4	4	4	1
1:60	4	4	4	4	4	4	4	4	1
1:120	—	—	4	4	4	4	4	4	4
1:240	—	—	2	4	4	4	4	4	2
1:480	—	—	—	2	4	4	4	2	1
1:960	—	—	—	—	—	4	4	—	—
1:1920	—	—	—	—	—	—	—	—	—

* The numbers 4, 3, 2 and 1 represent precipitation in decreasing scale.

precipitation between hemoglobin solutions of malarial and nonmalarial origin. The blood specimens in this experiment were aged for 24 hr at room temperature. Ageing of blood specimens was found to be an important step in obtaining precipitation in the differential range. It is evident from the table that the hemoglobin solutions of malarial blood specimens gave more marked precipitation reactions than those of nonmalarial blood specimens.

A more extensive precipitation study was undertaken with 137 malarial blood specimens which had been received through the mails and had undergone approximately 48 hr ageing. About the same number of nonmalarial specimens, similarly aged, were employed as con-

TABLE II.
Precipitation with Malarial and Nonmalarial Hemoglobin Solutions.

	Malarial						Nonmalarial						
	Hemoglobin dilution with distilled water												
	1:15			1:30			1:15			1:30			
	Ratios of hemoglobin dilution to antigen suspension												
Spec. No.	6:1	12:1	18:1	12:1	18:1	24:1	Spec. No.	6:1	12:1	18:1	12:1	18:1	24:1
1	—	—	—	4	4	3	1	—	—	—	—	—	—
2	—	—	—	4	4	4	2	—	—	—	4	—	—
3	—	—	—	2	—	—	3	—	—	—	2	2	1
4	—	—	—	4	4	4	4	—	—	—	—	—	—
5	4	4	3	4	4	4	5	3	1	—	4	4	4
6	4	—	—	4	4	4	6	—	—	—	—	—	—
7	—	—	—	4	2	—	7	—	—	—	—	—	—
8	—	—	—	1	—	—	8	—	—	—	—	—	—
9	—	—	—	—	—	—	9	—	—	—	1	—	—
10	—	—	—	2	1	—	10	—	—	—	3	2	2

TABLE III.
Precipitation with Malarial and Nonmalarial Hemoglobin Solutions (Employing Aged Blood Specimens).

Malarial Spec. 137		Nonmalarial Spec. 138		Nonmalarial Spec. 306	
Aged about 48 hr at room temp.				Aged 4 hr at room temp.	
No.	%	No.	%	No.	%
Precipitation in the 1:15 and 1:30 hemoglobin dilutions*					
125	91	31	24	7	2
Precipitation only in the 1:30 hemoglobin dilution					
9	7	67	28	17	4
No precipitation in the 1:15 and 1:30 hemoglobin dilutions					
3	2	30	48	282	94

* The 1:15 hemoglobin dilution was examined in ratios of 6:1, 12:1 and 18:1 with antigen suspension. The 1:30 hemoglobin dilution was examined in ratios of 12:1, 18:1 and 24:1 with antigen suspension. If precipitation occurred in one or more ratios of either dilution, the result was recorded as positive.

trols. Table III. summarizes the results obtained.

As indicated in the table, 91% of malarial hemoglobin solutions in 1:15 and 1:30 dilutions with distilled water gave positive precipitation reactions. In addition, 7% of the hemoglobin solutions gave precipitation only in the 1:30 dilution. The remaining 2% showed no precipitation. Of the nonmalarial hemoglobin solutions, 24% gave precipitation both in the 1:15 and 1:30 dilutions and 28% only in the 1:30 dilution. The remaining 48% showed no precipitation. It is thus evident that under the ageing conditions specified, the malarial hemoglobin solutions showed a greater tendency toward precipitation than the nonmalarial hemoglobin solutions.

In Table III. is also presented an experiment in which 306 nonmalarial blood specimens were aged only 4 hr at room temperature. When hemoglobin dilutions from these specimens were examined with antigen suspension, only 6% showed precipitation. Preliminary findings indicate that hemoglobin dilutions from fresh malarial blood specimens give similar precipitation results.

Studies are in progress to determine the nature of the precipitation phenomenon described in this report and if the phenomenon is applicable to the development of a practical method for differentiation between hemoglobin solutions of malarial and nonmalarial origin.

Summary. It was observed that hemoglobin solutions (laked human erythrocytes in distilled water) when mixed with tissue extract antigen (Kahn antigen suspension) under proper conditions show immediate precipitation. This phenomenon of precipitation is manifested in a relatively wide range of hemoglobin-distilled water dilutions and also in various ratios of these dilutions to antigen suspension. Hemoglobin solutions derived from malarial blood specimens show a tendency toward a wider range of precipitation than hemoglobin solutions derived from nonmalarial blood specimens. Ageing of the blood specimens at a temperature above refrigeration, such as room temperature, is an important factor in increasing the tendency toward such precipitation.

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Release of Curare-Like Agent from Healthy Muscle and Its Bearing on Myasthenia Gravis.*

CLARA TORDA AND HAROLD G. WOLFF.

From the New York Hospital and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York City.

Several investigators, notably Nevins,[1, 2] have suggested the possibility of the presence in the blood or tissues of patients with myasthenia gravis of an abnormal substance or metabolite with a curare-like action which raises the threshold of skeletal muscle to the effects of acetylcholine. Walker[3] tested this

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