17166. Nature of the Plasma Factor Responsible for *In vitro* Lysis of Leucocytes by Tuberculoprotein.*

JOSEPH M. MILLER,† CUTTING B. FAVOUR, BARBARA A. WILSON, AND MERLE A. UMBARGER. (Introduced by J. H. Mueller.)

From the Medical Clinics, Peter Bent Brigham Hospital and the Department of Medicine, Harvard Medical School.

In vitro tissue culture studies on the tuberculin reaction,[1] using washed buffy coats of sensitized animals, have stressed the role of cells rather than serum antibodies in the tuberculin type of hypersensitivity. Since the tuberculin reaction may be passively transferred to a normal host with white cells and not with the serum from sensitized animals,[2] and since white cell suspensions from tuberculous animals[3] and humans[4] are lysed by tuberculin within one hour, it would seem that the delayed type of hypersensitivity is in some way mediated via the white cells of the animal body.

In an earlier report[5] using the one hour observation technic on white cell suspensions it was noted that white cells of normal tuberculin-negative and tuberculous humans have an equal affinity for tuberculin antigen. Subsequent experiments[6] with the same technic, using thoroughly washed white cells of both normal tuberculin-negative humans and tuberculous patients, indicate that the one hour *in vitro* cytotoxic effect of tuberculin is dependent on the presence of some factor in tuberculous plasma. The present report extends these observations on the tuberculous plasma factor.

Experimental. Because normal white blood cells were shown to undergo the same lysis as do sensitized cells when incubated with tuberculin antigen and tuberculous plasma[6] and in order to eliminate any property of the tuberculous cell as a factor in cytolysis, thoroughly washed white cells from healthy tuberculin-negative human subjects were used in all experiments. Tuberculous plasma (or serum) was obtained from tuberculous patients hospitalized for acute tuberculosis. Normal white cell concentrates were diluted with this plasma in amounts sufficient to bring the final cell concentration to the range of 4,000-15,000 cells per cu mm. The tuberculous plasma was used in several ways:

a) freshly drawn;

b) after storage at 10°C for 7 days;

c) after dialysis for 2 days against isotonic saline;

d) after heating at 56°C for 15 minutes; and

e) as two fractions obtained as follows:

1. the globulin precipitated by dialysis of the plasma for 2 days against distilled water; and

2. the supernatant plasma freed of precipitate by centrifugation and redialized against isotonic saline. The supernatant plasma was added to one tube containing normal cells; the precipitate, after being suspended in 1 ml of isotonic saline, was added to a similar tube of normal cells.

In all cases where tuberculous plasma was subjected to heat or was used at any time subsequent to the day of collection from the patients, fresh normal guinea pig serum, containing approximately 400 units of active complement per ml, was added to the medium. To 0.4 ml of such white cell suspensions was

* Work done under an U.S.P.H.S. Research Grant.

† Research Fellow, National Institute of Health.

1 Rich, A. R., and Lewis, M. R., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 115.

2 Chase, M., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

3 Favour, C. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 269.

4 Fremont-Smith, P., and Favour, C. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 502.

5 Favour, C. B., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 369.

6 Miller, J. M., Favour, C. B., Wilson, B. A., and Umbarger, M. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 738.

TABLE I.
In vitro Effect of Tuberculin on Normal Leucocytes in Presence of Normal and Tuberculous Plasma Before and After Saline Dialysis at 10°C.

Normal cells	X	X	X	X	X	X	X	X
Tbc. Plasma (freshly drawn)	X	X						
Normal Plasma (freshly drawn)			X	X				
Tbc. Plasma (dialysis 48 hr against isotonic saline)					X	X		
Normal Plasma (dialysis 48 hr against isotonic saline)							X	X
Tuberculin antigen	0.1		0.1		0.1		0.1	
Saline		0.1		0.1		0.1		0.1
Total WBC								
5 min.	8,770	9,420	8,530	9,450	7,380	8,290	9,270	11,260
60 min.	6,510	9,390	8,500	9,360	5,330	8,320	9,210	11,080
% Decrement	—25.8	—0.3	—0.5	—0.8	—27.6	+0.2	—0.7	—1.5

added 0.1 cc of tuberculin antigen (prepared as described earlier[3]). Cell system controls with isotonic saline replacing the tuberculin antigen were studied in similar manner. In every instance, normal plasma from a tuberculin-negative donor was subjected to the same treatment as was tuberculous plasma (heat, dialysis, etc.) and added to normal white cell concentrates in the presence of "Old Tuberculin" and saline. Cytolysis was demonstrated by doing total white counts before and after a 60 minute period of incubation at 37°C. The accompanying protocols (Tables I, II, III) illustrate the method of experimentation. Essentially similar results have been obtained many times on different tuberculous patients.

Results. 1. Washed white cells from normal tuberculin-negative humans undergo significant cytolysis when suspended in the plasma of tuberculous patients in the presence of "Old Tuberculin" under conditions of these experiments (Table I).

2. The ability of tuberculous plasma to produce cell lysis is retained after dialyzing such plasma against isotonic saline for 48 hours at 10°C (Table I).

3. Heating tuberculous plasma to 56°C for 15 minutes destroys its ability to lyse white cells, even when fresh guinea pig complement is added to the system (Table II).

TABLE II.
In vitro Effect of Tuberculin on Normal Leucocytes in Presence of Tuberculous Plasma Before and After Heating Plasma to 56°C for 15 Minutes.

Normal cells	X	X	X	X
Tbc. plasma	X	X		
Tbc plasma* (heated 15 min. at 56°C)			X	X
Tuberculin antigen	0.1		0.1	
Saline		0.1		0.1
Total WBC				
5 min.	7,790	7,470	7,310	6,730
60 min.	5,840	7,540	7,270	6,720
% decrement	—24.8	+0.8	—0.8	—0.3

* Fresh guinea pig complement added.

4. If tuberculous plasma is dialyzed at 10°C for 48 hours against distilled water, a white precipitate appears. The supernatant plasma, after redialysis against isotonic saline, has no effect on white cells in the presence of "Old Tuberculin." The precipitate, when resuspended in isotonic saline, is capable of causing white cell cytolysis (Table III).

TABLE III.
In vitro Effect of Tuberculin on Normal Leucocytes in Presence of Normal and Tuberculous Plasmas Before and After Dialysis Against Distilled Water.

Normal cells	X	X	X	X	X	X	X	X	X	X	X	X
Tbc. plasma*	X	X										
Normal plasma*			X	X								
Supernatant of tbc. plasma					X	X						
Supernatant of normal plasma							X	X				
Globulin precipitate tbc. plasma									X	X		
Globulin precipitate normal plasma											X	X
Tuberculin antigen	0.1		0.1		0.1		0.1		0.1		0.1	
Saline		0.1		0.1		0.1		0.1		0.1		0.1
Total WBC												
5 min.	6,670	6,600	6,930	6,840	5,200	6,230	9,050	8,930	6,670	7,030	7,840	8,050
60 min.	5,130	6,580	6,900	6,850	5,200	6,190	8,870	8,880	4,800	7,060	7,770	8,000
% decrement	—23.0	—0.2	—0.5	+0.2	0.0	—0.7	—1.8	—0.4	—28.0	+0.5	—1.1	—0.6

* Both plasmas stored at 10°C for 10 days.

5. Tuberculous plasma, after storage at 10°C for 7 days, retains its capacity for lysing white cells in the presence of "Old Tuberculin" (Table III).

Discussion. An earlier report[6] stressed the essential role of tuberculous plasma in the one-hour *in vitro* cytolysis of human white cells. The foregoing results now indicate that the active component of such tuberculous plasma is a non-dialyzable, heat-labile fraction which remains stable after storage for 7 days at 10°C. The inactivation of such plasma by heating to 56°C for 15 minutes is apparently not due to the destruction of complement since the addition of fresh guinea pig complement did not restore the leucocyte lysing activity of the heated plasma. The precipitation of this active factor by dialyzing tuberculous plasma against distilled water seems to place this lysing component in the globulin fraction of the plasma proteins. The heat lability of this plasma substance distinguishes it from the usual precipitating and agglutinating antibodies described in tuberculous plasma. It is of particular interest that Chase[4] was able to destroy the ability of tuberculous cells to transfer sensitivity passively if such cells were heated to 48° for 15 minutes. In fact, work now in progress suggests that such tuberculous cells may be the source of this lytic promoting factor in plasma. It is not known whether the cytolytic process herein described represents an *in vitro* counterpart of the tuberculin type tissue reaction.

Summary. There is present in the plasma of tuberculous humans a heat-labile, non-dialyzable component which is responsible for the *in vitro* lysis of washed leucocytes by tuberculoprotein. Further properties of this lytic producing substance are its stability at 10°C for at least 7 days and its precipitation with the globulin fraction of the plasma proteins.

Received April 20, 1949. P.S.E.B.M., 1949, **71**.