THE LYMPHOCYTIC ORIGIN OF A PLASMA FACTOR RESPONSIBLE FOR HYPERSENSITIVITY IN VITRO OF TUBERCULIN TYPE*

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In a series of earlier investigations by others (Rich and Lewis, 1932, Moen and Swift, 1936, Heilman, Feldman, and Mann, 1944) and preliminary reports from this laboratory (Favour, 1947, Favour, Fremont-Smith, and Miller, 1949) methods have been described for the study *in vitro* of tuberculin-type hypersensitivity. One feature of the tuberculin effect *in vitro* is the affinity of leucocytes from both tuberculin-negative as well as tuberculin-sensitive hosts for tuberculin (Favour, 1949). When white cells from non-tuberculous subjects are sensitized with tuberculin and exposed to a factor in the plasma from tuberculin-sensitive subjects, these cells undergo lysis (Miller, Favour, Wilson, and Umbarger, *a*, 1949). The lytic "plasma factor" is present in the euglobulin portion of blood, is heat labile (Miller, Favour, Wilson, and Umbarger, *b*, 1949), and requires complement for its specific effect (Miller, Vaughan, and Favour, 1949).

In the present report experiments are described which indicate that "plasma factor" can be derived from the lymphocytes of the circulating blood of an appropriately sensitized host.

Materials and Methods

Subjects.—Guinea pigs weighing 400 to 600 gm. were inoculated in the groins and pectoral regions with a total of 2.5 mg. of heat-killed tubercle bacilli (H37Rv) suspended in light mineral oil (Bayol F). The organisms were grown on the surface of a liquid medium described by Dubos and Middlebrook (Dubos and Middlebrook, 1947), the culture heated at 60°C. for 1 hour, and the bacteria lyophilized following three washings and centrifugations from distilled water. After 2 months the animals were reinjected with a similar quantity of heat-killed organisms. Individual guinea pigs showing palpable masses at the sites of inoculation and exhibiting intradermal induration of more than a centimeter in diameter 48 hours following the injection of 5 γ of PPD (Seibert and Glenn, 1941) were chosen for study. Normal uninoculated guinea pigs shown to be tuberculin-negative served as controls.

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Human subjects were chosen for study who showed bacteriological evidence of an active tuberculous infection and whose white cells in whole blood suspensions were found to undergo lysis when exposed to tuberculin *in vitro*. It should be noted that the white cells of many persons with active tuberculosis do not show this phenomenon (Favour, Fremont-Smith, and Miller, 1949). Healthy human beings who did not react to the intracutaneous injection of second strength PPD served as human controls. When suspensions of leucocytes which had not been entirely freed of red blood cells were suspended in heterologous plasma, donors of the same blood type were used. This precaution was taken to avoid red cell agglutination.

In the data given below "normal cells" and "normal plasma" refer respectively to white blood cells and plasma obtained either from uninoculated guinea pigs or from tuberculin-negative human beings. Similarly, "tuberculous cells" and "tuberculous plasma" refer to materials from tuberculin-sensitive subjects selected as described below.

Tuberculin.—Old tuberculin was obtained from the Massachusetts Department of Health and was dialyzed shortly before use against three changes of distilled water and one change of 0.85 per cent sodium chloride. It was used as a 1:8 dilution based on the original volume of crude O.T. or as 50 mg. per ml. of O.T. solution.

Leucocyte Suspensions.—Guinea pigs were bled from the heart into a syringe containing 0.5 ml. heparin (liquaemin-Roche), for each 9.5 ml. blood. Human subjects were bled without stasis from the antecubital vein in a similar manner. The heparin and blood were gently equilibrated and the mixture transferred to silicone-coated (Jaques, Fidlar, Feldsted, and MacDonald, 1946) centrifuge tubes. Subsequent cell manipulations were carried out in similarly coated glassware.

The guinea pig blood under observation had a slow sedimentation rate. Accordingly, 15 ml. lots were centrifuged at 200 R.P.M. for 15 to 30 minutes and the plasma and buffy coat carefully pipetted into a second tube. Individual experiments were done on mixed leucocyte suspensions obtained after pooling four to six 10 ml. blood samples taken from as many animals.

The human blood from tuberculous subjects exhibited a spontaneously rapid sedimentation rate. By placing the 15 ml. centrifuge tubes of heparinized blood at an angle of 60° for 45 to 90 minutes, the upper portion of the blood was largely freed of red cells. When removed carefully by pipetting the upper one-fourth of the plasma layer gave satisfactory suspension of lymphocytes. The lower one-fourth of the plasma layer supplied excellent suspensions of neutrophils. A similar method of handling cells has been described by Buckley (Buckley, Powell, and Gibson, 1949). This worker employed fraction I of Cohn to induce a rapid sedimentation rate. Since commercial preparations of fraction I contain sodium citrate, this material was not used in the present studies. Individual experiments were performed on 50 ml. blood samples taken from single patients.

Platelets were found to be troublesome in subsequent cell counting procedures. For this reason the majority of the platelets were removed by centrifuging plasma-cell suspensions at 200 R.P.M. for 10 minutes and resuspending the cell sediment in supernatant plasma freed of platelets by more rapid centrifugation. Erythrocyte counts on the cell suspensions were less than 200,000 per c.mm. No effort was made to remove all the red cells.

Fresh plasma containing sufficient complement was obtained from the supernatant fluids of leucocyte suspensions. Prior to the incubation of cells with tuberculin, all operations involved in the preparation of the cell suspensions were carried out at room temperature. Usually 3 to 4 hours elapsed between blood letting and the start of final cell incubation. Study of preparations stained by Wright technique and direct observations of ameboid motion under the phase microscope during incubation indicated that the leucocytes remained in good condition during these manipulations. Much of this we attribute to the remarkable ability of silicone to minimize cell damage.

Plasma Factor.—Plasma factor was studied in whole fresh plasma and after dialysis of

fresh plasma for 48 hours against three changes of distilled water in order to precipitate the euglobulins which contain the active plasma factor. Details of this preparation are given elsewhere (Miller, Favour, Wilson, and Umbarger, 1949,*b*). In experiments using such plasma factor obtained by dialysis complement was supplied as 0.05 ml. of fresh guinea pig serum added to 0.35 ml. of a saline suspension of the precipitated plasma factor.

Incubation.—Lymphocytes, neutrophils, tuberculin, and plasma of normal and tuberculous subjects were mixed in various combinations as indicated in the sample protocols. Within 5 minutes white blood counts and smears were prepared and the mixtures placed in stoppered siliconized Wassermann tubes on a roller-tube rack revolving at 100 R.P.M. in a 37°C. incubator Usually 0.1 ml. O.T. (5 mg.) was added to 0.4 ml. of plasma cell suspension. Proportional multiples of these amounts were also used (*vide infra*). Where incubation for longer than 1 hour was contemplated, 100 mg. per cent glucose, 10 units of sodium penicillin G, and 50 μg. of streptomycin base per ml. were added to the plasma.

White Cell Counts.—Since the results and significance of this report rest upon the accuracy with which leucocyte counts were performed, extraordinary measures were taken to standardize the counting technique. Three hundred or more cells were counted in all instances in order to minimize the importance of minor decrements of cell numbers as compared with major changes in cell populations produced by the plasma factor. Calibrated pipettes, mechanical pipette fillers, and mechanical pipette shakers were used. The usual accuracy of leucocyte counts under optimal conditions is given as 10 per cent. Since only experimental changes in counts greater than 10 per cent were considered valid, a reasonable margin of significance has been maintained. Sample experiments reported in detail below illustrate the attention which has been paid to counting methods.

RESULTS

When tuberculin is added to the whole blood of hosts properly sensitized to tuberculin, some lysis of leucocytes takes place (Favour, Fremont-Smith, and Miller, 1949). This effect begins within 20 minutes and usually reaches its end-point within 1 hour (Favour, 1947). Under the conditions of this type of procedure, periods of observation up to several hours will show a continued survival of the leucocytes which escape initial destruction. These observations first led us to consider the 1 hour lysis of cells by tuberculin as an expression of some property of the leucocytes in these suspensions (Favour, 1947).

As indicated earlier, however, further examination of this cell system has shown that "immediate" cell lysis is dependent not on the cells but upon a factor in the plasma. If this plasma factor is removed from the cell suspension and the washed leucocytes from properly sensitized hosts suspended in normal plasma, tuberculin does not bring about an "immediate" cell lysis (*vide infra*). It will be shown below, however, that after a period of 3 to 5 hours lysis of tuberculous cells in the absence of tuberculous plasma can take place, suggesting that the cells themselves are the remote source of plasma factor.

In the following typical experiment on guinea pig blood, normal cells, normal plasma, tuberculous cells, and O.T. were mixed in various combinations as illustrated in Table I. Leucocyte counts were performed within 5 minutes after the mixtures were prepared and at 1, 3, 5, and 7 hours after incubation in roller tubes.

The experiment described in Table I has been repeated 4 times on human beings and 4 times on guinea pig blood with essentially the same findings.

The preceding protocol indicates that the leucocytes from a tuberculous host, when washed free of their surrounding plasma by means of saline, are not affected by tuberculin within the first hour of exposure as they may be in whole tuberculous blood. Following more prolonged exposure to tuberculin, in this case more than 3 hours, there is the same rapid cell breakdown of a portion of the cells and the same plateau effect in subsequent counts as that which is seen in whole blood preparations. Since normal leucocytes in the presence of

TABLE I

The Delayed Lytic Effect of Tuberculin on a Portion of the Washed Leucocytes from a Tuberculous Subject

Tube No.		I	II	III	IV	V	VI
Normal cells in normal plasma, *ml.*		1.2	1.2	—	—	1.5	—
TB cells in normal plasma, *ml.*		—	—	1.2	1.2	—	1.5
O.T., *ml.*		0.3	—	0.3	—	—	—
Saline, *ml.*		—	0.3	—	0.3	—	—
Leucocytes/mm.^{3}	5 min.	12,530	11,890	14,200	15,000	16,420	16,660
Per cent change	60 min.	−0.7	−0.6	−0.5	−0.4	+0.5	+0.3
	180 min.	+0.3	−0.3	−0.5	0	−0.2	0
	300 min.	−0.3	0	−28.6	−1.1	−1.3	0
	420 min.	+1.0	−0.2	−31.5	−0.3	−1.3	0

tuberculin are lysed by a "plasma factor" (Miller, Favour, Wilson, and Umbarger, 1949,*a*), this experiment suggests that the cells themselves may slowly liberate the "plasma factor" into normal plasma, which once present in appropriate amounts, initiates a chain reaction carrying lysis to completion within the next hour of cell contact.

On the supposition that tuberculous cells release plasma factor into normal plasma, the type of experiment given in Table I was adapted to test this possibility. The tube (No. IV) which contained tuberculous cells in normal plasma and in which no visible cell destruction took place during 7 hours of incubation was freed of cells and the supernatant fluid used as a source of shed plasma factor. The tube (No. V) which contained normal cells in normal plasma and also showed no cell destruction in 7 hours was freed of cells and the supernatant used as a control for shed plasma factor. Each of these supernatants was then used as a suspending fluid for a new preparation of normal cells. Appropriate controls were included and leucocyte counts done at 5 minutes and after incubation in roller tubes for 60 minutes. Data for this experiment are given in Table II.

The experiment in Table II was performed using guinea pig blood. Repeated studies with both human and guinea pig blood yielded similar results. It was found that, unless carefully removed by thorough centrifugation or by Seitz filtration through a syringe filter, cell debris carried along in the supernatants nullified the subsequent effect of tuberculin. This is a corollary of the affinity of tuberculin for leucocytes described elsewhere (Favour, 1949).

TABLE II

The Prompt Lytic Effect of Tuberculin on a Portion of the Leucocytes of Normal Subjects in the Presence of Shed Plasma Factor from a Tuberculous Subject

Tube No.	I	II	III	IV
Normal cells in normal plasma, *ml.*	0.1	0.1	0.1	0.1
Supernatant of TB cells, *ml.*	0.3	—	0.3	—
Supernatant of normal cells, *ml.*	—	0.3	—	0.3
O.T., *ml.*	—	—	0.1	0.1
Saline, *ml.*	0.1	0.1	—	—
Leucocytes/mm.^{3} 5 min.	10,800	9,930	9,980	10,770
Per cent change 60 min.	−0.5	−0.5	−18.0	−0.7

TABLE III

The Survival of Leucocytes during 7 Hours' Incubation in a Roller Tube

Tube No.	I	II
TB cells in normal plasma, *ml.*	9.0	—
Normal cells in normal plasma, *ml.*	—	9.0
Leucocytes/mm.^{3} 5 min.	27,200	31,000
420 min.	28,000	30,500

The data in Table II indicate that tuberculous cells, and not normal cells, under the conditions of this experimental method, release a factor into normal plasma. This release occurs even when the cells appear to retain their characteristic morphology. Furthermore, this plasma factor is capable of causing the destruction of normal cells in the presence of tuberculin. In order to determine whether the shed plasma factor from cells is identical with that obtained from tuberculous plasma, a large quantity of the supernatants described above was prepared using the same proportion of constituents as in the previous experiment. After 7 hours incubation the cell-free supernatant was dialyzed against distilled water, the precipitated euglobulin separated, and the sediment redissolved according to the method described for study of plasma factor from tuberculous blood (Miller, Favour, Wilson, Umbarger, 1949*b*). Tables III illustrates the method. Table IV illustrates the same changes on aliquots of Table III as was observed in the experiment described in Table I.

The white cells and plasma were collected separately from 50 ml. of normal and 50 ml. of tuberculous guinea pig blood. These cells were suspended in fresh normal plasma as indicated in Table III and incubated in roller tubes for 7 hours. Leucocyte counts were performed at 5 minutes and again at the end of the period of incubation.

Parallel aliquots of the same cell suspensions with the leucocyte counts adjusted to approximately 10,000 per c.mm. to facilitate cell counting were set up as indicated in Table IV and also incubated in roller tubes for 7 hours.

Following incubation the 9.0 ml. tubes of Table III were centrifuged, the supernatants filtered through a Seitz filter, and the filtrate divided into two portions. The first portion was stored at 4°C. and the second dialyzed for 48 hours in the cold against multiple changes of

TABLE IV

The Delayed Lytic Effect of Tuberculin on the Leucocytes in an Aliquot Leucocyte Suspension from the Tuberculous Subject Studied in Table III

Tube No.		I	II	III	IV
Normal cells in normal plasma, *ml.*		0.8	0.8		
TB cells in normal plasma, *ml.*				0.8	0.8
O.T., *ml.*		0.2		0.2	
Saline, *ml.*			0.2		0.2
Leucocytes/mm.^{3}	5 min.	9170	9160	8070	8040
Per cent difference	60 min.	−0.3	−0.5	0	−0.3
	300 min.	−0.3	−0.7	−29.7	0
	420 min.	−1.7	−1.2	−32.2	+0.3

distilled water. The insoluble material that precipitated during dialysis was removed by centrifugation and taken up in a quantity of saline equal to the original supernatant volume. The supernatant from the dialysate was then dialyzed for 48 hours against multiple changes of 0.85 per cent saline. At the end of this time, usually 7 to 10 days later, all three materials were tested against a fresh normal cell system as indicated in Table V.

The experiment given in Tables III, IV, and V was repeated twice each on human and guinea pig blood with essentially the same findings.

The foregoing experiment indicates that tuberculous cells suspended in normal plasma release an active substance into this plasma during a 7 hour period of incubation. When this plasma is then freed of cells and is dialyzed against distilled water, the active factor precipitates with the euglobulins leaving no cytolytic activity in the remaining albumin fraction. This shed plasma factor, like plasma factor derived from tuberculous plasma, will also cause destruction of normal leucocytes in the presence of tuberculin.

Recent emphasis on the lymphocyte as a source of antibodies (Dougherty, 1944, Harris and Ehrich, 1946) suggested the desirability of differentiating which of the circulating leucocytes was the probable source of plasma factor production. To this end the previous experiment was repeated using relatively pure suspensions of lymphocytes and of neutrophils.

TABLE V

The Prompt Lytic Effect of Tuberculin on a Portion of the Leucocytes of a Normal Subject in the Presence of the Shed Plasma Factor (Table II) and Its Euglobulins (Table VII) Derived from the Leucocytes of a Tuberculous Subject

Tube No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Normal cells in normal plasma, *ml*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Supernatant plasma of normal cells, *ml*	0.3						0.3					
Supernatant plasma of TB cells, *ml*		0.3						0.3				
Supernatant dialysate normal cells, *ml*			0.3						0.3			
Supernatant dialysate TB cells, *ml*				0.3						0.3		
Plasma precipitate normal cells, *ml*					0.3						0.3	
Plasma precipitate TB cells, *ml*						0.3						0.3
O.T., *ml*	0.1	0.1	0.1	0.1	0.1	0.1						
Saline, *ml*							0.1	0.1	0.1	0.1	0.1	0.1
Leucocytes/mm.^{3} 5 min.	6680	5276	6070	6070	6710	4130	6160	4550	6290	6030	5820	5700
Per cent change 60 min.	+0.2	−25.7	−0.5	+0.5	−0.6	−31.5	+0.3	+0.5	0.6	0	−0.8	−0.3

Fifty ml. samples of tuberculous human blood was processed by the method described earlier in this report to yield suspensions of lymphocytes and neutrophils. Somewhat larger samples of normal human blood which did not have an accelerated sedimentation rate were centrifuged at 200 R.P.M. for 30 to 45 minutes and the lymphocytes and neutrophils recovered for the control cell suspensions. Guinea pig leucocytes were not used for this experiment because the slow red cell sedimentation rate of their blood necessitated excessive amounts of blood. The data below were obtained from one of two studies on the role of the lymphocyte as a source of shed plasma factor. Essentially similar figures were obtained in both human blood experiments. The procedure was the same as in the previous experiments. Figures are given in Tables VI to IX.

Table VII describes parallel observations on the effect of O.T. on aliquots of each tube in Table VI. The proportions of ingredients are the same although the cell concentrations were changed to fit the small absolute cell counts obtained by fractional harvest of plasma.

TABLE VI

The Survival of Neutrophils and Lymphocytes during 7 Hours' Incubation in a Roller Tube

Tube No.	I	II	III	IV	Smear	
					Lym.	PMN
					per cent	*per cent*
Normal neutrophils in normal plasma, *ml.*	5.0				20	80
Normal lymphocytes in normal plasma, *ml.*		5.0			90	10
TB neutrophils in normal plasma, *ml.*			5.0		7	93
TB lymphocytes in normal plasma, *ml.*				5.0	89	11
Leucocytes/mm.^{3} 5 min.	18,400	5,300	11,000	4,900		
420 min.	20,100	5,400	15,100	4,700		

TABLE VII

The Delayed Lytic Effect of Tuberculin on a Portion of the Washed Lymphocytes from a Tuberculous Subject

Tube No.	I	II	III	IV	V	VI	VII	VIII
Normal PMN in normal plasma, *ml.*	—	—	0.15	0.15	—	—	—	—
Normal lymphocytes in normal plasma, *ml.*	0.2	0.2	—	—	—	—	—	—
TB PMN in normal plasma, *ml.*	—	—	—	—	—	—	0.15	0.15
TB lymphocytes in normal plasma, *ml.*	—	—	—	—	0.2	0.2	—	—
O.T., *ml.*	0.15	—	0.15	—	0.15	—	0.15	—
Saline, *ml.*	—	0.15	—	0.15	—	0.15	—	0.15
Normal plasma, *ml.*	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Leucocyte/mm.^{3} 5 min.	3,300	3,200	9,770	10,130	3,190	3,480	6,160	5,880
Per cent change 60 min.	+1.0	−0.3	0	−0.3	−1.1	−0.7	+0.5	+0.5
180 min.	−0.3	−0.7	−0.7	−0.6	−2.5	+2.0	+0.3	−0.7
300 min.	−0.6	−1.9	+0.5	−0.5	−24.3	−1.5	−0.5	+0.3
420 min.	−1.1	+0.7	+0.1	0	−26.4	−2.2	0	+0.5

At the end of 7 hours the master tubes (Table VI) containing cells in normal plasma were freed of cells and the supernatant fluids dialyzed first against distilled water and then against

TABLE VIII

The Prompt Lytic Effect of Tuberculin on a Portion of Normal Leucocytes in the Presence of Undialyzed Plasma Containing Plasma Factor Shed by Lymphocytes

Tube No.	I	II	III	IV	V	VI	VII	VIII
Normal cells in normal plasma, *ml.*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Supernatant normal PMN, *ml.*	0.3				0.3			
Supernatant normal lymphocyte, *ml.*		0.3				0.3		
Supernatant TB PMN, *ml.*			0.3				0.3	
Supernatant TB lymphocyte, *ml.*				0.3				0.3
O.T., *ml.*	0.1	0.1	0.1	0.1				
Saline, *ml.*					0.1	0.1	0.1	0.3
Leucocytes/mm.^{3} 5 min.	7220	8020	6970	7540	7300	6370	7010	6540
Per cent difference 60 min.	+0.6	+0.8	0	−14.9	−1.3	−0.8	−1.1	−2.2

TABLE IX

The Prompt Lytic Effect of Tuberculin on a Portion of Normal Leucocytes in the Presence of a Euglobulin Shed into Normal Plasma by Lymphocytes Derived from a Tuberculous Subject

Tube No.	I	II	III	IV	V	VI	VII	VIII
Normal cells in normal serum, *ml.*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sediment normal PMN supernatant, *ml.*	0.3				0.3			
Sediment normal lym. supernatant, *ml.*		0.3				0.3		
Sediment TB PMN supernatant, *ml.*			0.3				0.3	
Sediment TB lym. supernatant, *ml.*				0.3				0.3
O.T., *ml.*	0.1	0.1	0.1	0.1				
Saline, *ml.*					0.1	0.1	0.1	0.1
Leucocytes/mm.^{3} 5 min.	6230	6380	5840	6000	6280	6640	6670	5930
Per cent difference 60 min.	−1.1	−1.0	−0.2	−17.3	+0.2	+0.5	−0.7	−0.7

saline; the euglobulin sediments were suspended in saline and finally studied with a fresh suspension of normal leucocyte in normal serum. Tables VIII and IX give the data of this experiment.

Inspection of the data in Tables VI to IX indicates that in the course of 7 hours' incubation, tuberculous lymphocytes liberate a shed plasma factor which is lytic for normal white cells in the presence of O.T. Similar incubation of neutrophils from normal and tuberculous subjects does not yield plasma factor. When dialyzed against water, this material liberated by the tuberculous lymphocyte precipitates with the euglobulin fraction of plasma; it can be redissolved in saline and it will cause the lysis of normal leucocytes in the presence of tuberculin in quite the same manner as plasma factor derived from the circulating plasma of the tuberculous subject. These experiments indicate that under such conditions of *in vitro* observation washed lymphocytes from the tuberculous subject slowly release into plasma a substance which is responsible for the type of specific tuberculin cell damage observed *in vitro* with hypersensitive cell models.

DISCUSSION

The classic concept of tuberculin hypersensitivity is based on the early tissue culture work of Rich and Lewis (Rich and Lewis, 1932). Belief that tuberculin hypersensitivity is a result of a sessile antibody attached firmly to tissue cells has been further strengthened by many studies (Rich, 1944) in which tuberculin allergy could not be transferred passively by the standard techniques used in the transfer of anaphylactic and pollen allergy. More recently the cellular nature of tuberculin allergy has been fortified by the work of Chase (Chase, 1945) who showed that the delayed type tuberculin allergy could be transferred passively by cell suspensions from highly sensitized hosts. Although cells, and not serum, were used in effecting such transfers, the transferred allergy was not of long duration. Similar observations on the passive transfer of tuberculin allergy in human beings have been made recently (Lawrence, 1949).

It should be pointed out that the cell suspensions used in tissue culture as well as in the passive transfer by cells were not only free of homologous plasma but, more important, were rich in cells of the lymphoid series. In fact, an excellent source of cells for passive transfer studies is the regional lymph node adjacent to a deposit of sensitizing tuberculous antigen. Furthermore, transfer cells also can be obtained from the peripheral blood. In view of the mounting evidence for the lymphocytes as the origin of many antibodies this might be expected to be the case.

In the experiments described in this paper an enlightening series of events were followed. It was found that the lymphocytes from appropriate tuberculin-sensitized hosts will shed into normal plasma a factor which can be recovered

in the euglobulin fraction of the plasma proteins. This material is specifically toxic for a portion of the leucocytes, only if such cells are also exposed to tuberculin. Neutrophils do not elaborate this shed plasma factor nor is the factor intrinsically toxic for normal or tuberculous leucocytes in the absence of tuberculin.

The experiments herein described still do not bridge the gap in our information concerning the ability of certain cells passively transferred from the sensitized host to bring about slow tissue destruction in the normal host at the site of tuberculin deposition. However, there is now a suggestion that a similar plasma factor, resembling an antibody, following the pattern of an antigen-antibody reaction and requiring complement, may be a part of the tuberculin reaction in the intact host.

Preliminary experiments to determine whether the cytolytic phenomenon is produced by antibovine albumin–albumin systems have been negative. This might be expected to be true since tissue cultures from anaphylactically sensitized hosts are not injured by homologous antigen (Meyer and Loewenthal, 1927). A full consideration of this problem is beyond the scope of the present report.

SUMMARY

Methods are described for the evaluation *in vitro* of the effect of tuberculin on the leucocytes of peripheral blood.

Washed leucocytes from a tuberculin sensitive host suspended in normal plasma are not lysed by tuberculin until after several hours of contact.

Washed leucocytes from a tuberculin-sensitive host slowly release into normal plasma a factor which will cause the lysis of normal leucocytes exposed to tuberculin.

Dialysis of normal plasma containing shed plasma factor causes the latter to precipitate with the euglobulins.

Shed plasma factor can be recovered from normal plasma which has been incubated with lymphocytes from tuberculin-sensitive hosts. Suspensions of neutrophils do not yield shed plasma factor.

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