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In vitro Lysis of Leucocytes from Tuberculous Humans by Tuberculoprotein.*

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In a study on the delayed type of hypersensitivity, it has been found that lymphocytes from tuberculous mice and guinea pigs are specifically destroyed when exposed *in vitro* to tuberculoprotein.[1] The purpose of this report is to extend this type of observation to man.

Experimental. Using a method previously described,[2] suspensions of human white cells were obtained by layering whole, heparinized blood over albumin-saline mixtures,‡ and centrifuging. The resulting cell suspensions consisted of 50-80% lymphocytes, 20-40% neutrophil leucocytes, and small percentages of the other white blood cells. Experiments were performed on the leucocytes from 3 groups of subjects: (1) tuberculin-positive (PPD) patients with bacteriologically proven, active, severe tuberculosis; (2) subjects with positive tuberculin skin tests (PPD) but no demonstrable clinical tuberculosis; and (3) healthy persons with negative tuberculin skin tests (PPD, second strength).

Cytolysis was demonstrated by adding 0.2 cc of white cell suspension (7,000-15,000 cells per cu mm in fresh normal human serum) to 0.2 cc of "antigen" solution (fresh normal human serum containing PPD-s,[3] 25 γ per cc). Directly after mixing, these preparations were incubated in a water-bath at 37°C. Samples were taken at 5, 20, 60 (and 90) minutes for total white cell counts (5-10% error), and differential counts (1000 cells).

As cell system controls, parallel counts were performed on similar cell concentrations suspended in fresh normal human serum alone. Cell preparations suspended in fresh normal serum containing 200 γ per cc of a beta streptococcal protein, similar to that used to demonstrate cytolysis in tissue culture,[4] served as "antigen" controls.

It was previously noted that phosphate or citrate ions blocked cytolysis in this type of experiment.[1] To further elucidate the role of complement in this reaction, fresh human serum heated at 56°C for one-half hour, was used in some experiments as the suspending and diluting fluid, in place of fresh normal human serum.

Experiments have been performed one or more times on at least 6 individuals in each of the 3 categories outlined.

Results. Sample protocols illustrate the results obtained in typical experiments.§

1. Twenty to 60% of the lymphocytes, and 20-90% of the neutrophil leucocytes from the peripheral blood of patients with active severe tuberculosis, are specifically destroyed within 90 minutes by contact *in vitro* with small amounts of tuberculoprotein (Table I).

2. If heat-inactivated serum is substituted for fresh normal serum as the suspending and diluting fluid in these preparations, no cytolysis occurs (Table I).

3. No cytolysis, beyond that induced by the trauma of the procedure (1-10% in 2 hours), occurs on exposure of these cells to

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‡ Sterile 35% isotonic bovine albumin solution kindly supplied by Armour and Company, Stock Yards, Chicago, Ill.

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2 Ferrebee, J. W., and Geiman, Q. M., *J. Inf. Dis.*, 1946, **78**, 173, as modified by Favour.[1]

3 Seibert, F. B., and Glenn, J. T., *Am. Rev. Tub.*, 1941, **44**, 9.

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§ In the protocols, eosinophils, basophils, and monocytes have not been recorded as they total less than 2%.

TABLE I.
In vitro Effect of PPD on Blood Leucocytes from a Patient with Miliary Tuberculosis (Tuberculin Positive).

			Lymphocytes		Granulocytes	
		Total WBC	%	Absolute No.	%	Absolute No.
PPD	5′	2430	77.1	1880	21.8	530
Normal serum	30′	1260	88.1	1080	11.0	136
	60′	810	87.5	700	11.4	92
PPD	5′	3190	66.2	2110	33.6	1070
Heated serum	30′	3270	65.2	2130	34.8	1140
	60′	3280	70.0	2290	30.0	990
Normal serum	5′	2580	78.4	2020	21.3	550
control	30′	2380	79.0	1880	20.1	480
	60′	2570	79.7	2050	19.6	500

Note: Progressive cytolysis of lymphocytes and granulocytes in the presence of PPD and fresh normal human serum. No cytolysis with heat-inactivated serum.

TABLE II.
In vitro Effect of PPD on Blood Leucocytes from a Patient with Tuberculous Pneumonia (Tuberculin Positive).

			Lymphocytes		Granulocytes	
		Total WBC	%	Absolute No.	%	Absolute No.
PPD	5′	8890	53.2	4730	46.6	4140
Normal serum	20′	8130	52.0	4220	47.7	3880
	60′	7220	56.6	4080	43.3	3120
	90′	6160	52.4	3220	46.9	2890
Strep. protein	5′	8130	57.4	4670	42.2	3430
Normal serum	20′	8440	56.0	4730	44.0	3710
	60′	8060	60.0	4840	40.0	3220
	90′	8120	56.4	4580	43.2	3500
Normal serum	5′	7790	58.0	4520	41.6	3240
control	20′	7450	59.0	4400	41.0	3050
	60′	7030	60.0	4220	40.0	2810
	90′	7320	58.2	4260	41.6	3050

Note: Progressive cytolysis of lymphocytes and granulocytes in the presence of PPD and fresh normal human serum. No cytolysis with strep. protein (200 γ per cc).

(a) fresh normal human serum alone, or (b) streptococcal protein in fresh normal serum (Table II).

4. Leucocytes from healthy tuberculin-positive or tuberculin-negative subjects are not affected by contact *in vitro* with PPD or streptococcal protein, under the conditions of these experiments.

Conclusions. The observation that lymphocytes from animals infected with certain bacteria are specifically destroyed by contact *in vitro* with protein extracts of these bacteria[1] has been extended to humans infected with *M. tuberculosis*. In the human, as in the guinea pig, the neutrophil leucocytes are also specifically lysed. It is of interest that although the lymphocytes from tuberculous subjects of all 3 species (mouse, guinea pig, human) so far studied, are specifically sensitive to tuberculoprotein, the neutrophil leucocytes are affected only in those species which show a positive delayed-type tuberculin skin test (*i.e.*, guinea pigs and humans). In the tuberculous mouse, which fails to develop delayed-type skin hypersensitivity reactions to tuberculoprotein,[5] lympholysis occurs but

[5] Gerstl and Thomas, *Yale J. Biol. and Med.*, 1940-41, **13**, 679, ref. cit.

other cell types remain unaffected.[1] Apparently, the presence of complement is necessary for the occurrence of this form of prompt cytolysis. Experiments to further elucidate the role that this type of cytolysis plays in the pathogenesis of delayed-type hypersensitivity are in progress.

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An Electrophoretic Study of the Serum Proteins in Scleroderma.

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Scleroderma (literally: "hardening of the skin") occurs in two forms: diffuse and localized. The diffuse form is a progressive systemic disease of the connective tissue throughout the body.[1,2] The localized form on the other hand appears clinically to be limited to the skin and is a much milder disease. In spite of numerous investigations, the pathogenesis of scleroderma remains obscure. Observations on the serum proteins have been limited to an occasional routine determination showing a decrease in the albumin/globulin ratio with little change in the value for total proteins.[3] It seemed possible that a more detailed analysis by the Tiselius method of electrophoresis might yield additional information of value.

Methods and Materials. Serum was obtained in the fasting state from 12 normal subjects, 5 patients with diffuse scleroderma, and one patient with localized scleroderma. Four ml of serum were dialyzed against 2 liters of barbiturate buffer (ionic strength 0.1, pH 8.6) for 48 hours at 4° C, then diluted 1:3 with buffer. Electrophoresis was carried out in the Tiselius apparatus using a double section cell and an optical system of the type described by Philpot.[4] It proceeded for 80 minutes at a potential gradient of 8 volts per cm and a temperature of 1°C. Patterns were photographed directly, enlarged 5×, and the areas measured with a planimeter. Components were delineated by vertical lines drawn from the minima of the curves to the baseline. Ascending and descending pattern areas were averaged for each component except β-globulin; here only the ascending pattern area was used. Total and nonprotein nitrogen were determined by the micro-Kjeldahl method.

Results. In diffuse scleroderma, as shown in Table I, the albumin fraction of the serum proteins decreases and the γ-globulin fraction increases, but no significant change occurs in the total protein value. The alterations in the albumin and γ-globulin fractions are statistically significant. No new components in the electrophoretic pattern and no gross alterations in mobilities are evident. While little weight can be given to data from one case of localized scleroderma, it is interesting to note that the changes are qualitatively and quantitatively similar to those occurring in the diffuse scleroderma group. This is somewhat surprising in view of the apparent absence of systemic involvement in the localized form of the disease.

Discussion. The changes described are in no way diagnostic since similar changes occur in many chronic infections, in disseminated lupus erythematodes, in liver disease, and in other conditions.[5] They may, however, give

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