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Leucocyte Blockade of *in vitro* Tuberculin Cytolysis.*

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It has recently been shown that a portion of the white blood cells taken from a person acutely ill with tuberculosis are "lysed" by amounts of tuberculin not toxic for white blood cells taken from normal subjects or from individuals suffering from a variety of other acute illnesses.[1] It has also been found that this cytotoxic effect of tuberculin involves only the lymphocyte in the mouse,[2] a species which shows no intracutaneous sensitivity to tuberculin[3] whereas both lymphocytes and granulocytes are destroyed *in vitro* in the blood of man and guinea pigs showing *in vitro* cytolysis. A possible explanation for this discrepancy may be a difference in the affinity of human and mouse white blood cells for tuberculin. The experiments to be described are presented to show that indeed human white blood cells do and mouse white blood cells do not adsorb tuberculin.

Materials. 1. Mouse tuberculosis. Six-week-old albino Swiss mice were inoculated intravenously with 0.1 cc of a 6 to 10 day culture of H37Rv grown in synthetic media.[4] In this experiment 60% of the animals died within 6 weeks. Those surviving at 2 to 4 months were individually exsanguinated from the heart and the spleens from selected mice with gross evidence of advanced pulmonary tuberculosis removed and minced in an 0.85% saline solution containing 200 mg% glucose. Cell suspensions free of tissue fragments were washed three time by centrifugation and resuspension in the same solution and the cell count adjusted to 200,000 cells per cu. mm.

2. *Human tuberculosis patients* hospitalized for acute tuberculous infections were bled without stasis from the antecubital vein into a syringe containing 1 ml (10 mg) of heparin solution (Roche Organon). The 20 ml of blood was allowed to stand at room temperature in a test tube placed at an angle of 20-30°. Within 20 minutes the sedimentation was sufficient to give an almost quantitative separation of red cells from the plasma. The plasma, containing the majority of the white blood cells, was removed and centrifuged lightly to sediment the leucocytes. The supernatant containing most of the platelets was removed, centrifuged for 20 minutes at 2000 RPM to remove the platelets and its supernatant used to resuspend the homologous white blood cells. The cell concentration was adjusted to 200,000 cells per cu. mm.

3. *Normal mouse* white cells were obtained in the same fashion as tuberculous mouse white cells. Normal human white cells were obtained in a similar manner to that used for cells from tuberculous patients. A rapid sedimentation rate was induced in the normal human blood after heparinizing by adding 2 ml of a physiological solution of fibrinogen solution† per 20 ml of blood.

4. *Tuberculin.* O. T. obtained from the Massachusetts Department of Health was dialyzed against three changes of 0.85% saline during 3 days and the volume adjusted so that the concentration used represented a 6-fold dilution of the original material.

Heparinized blood from normal and tuberculous mice and humans was obtained by cardiac and venipuncture respectively immediately prior to setting up the experiment.

Method. A sample protocol illustrates the

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

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

3 Gerstl, B., and Thomas, R. M., *Yale J. Biol. and Med.*, 1940-41, **13**, 679, ref. cit.

4 Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.

† Fifteen mg of the lyophilized Fraction I of Cohn per ml of distilled water.

	Normal			Tuberculous		
	T_1	S_1	P_1	T_2	S_2	P_2*
Normal cells	.8 ml	.8				
" plasma			.5			
Tuberculous cells				.8	.5	
" plasma						.5
O.T.	.2		.2	.2		.2
Saline		.2			.2	

Incubated at 37°C for 30 min.
T_1, S_1, T_2, S_2 centrifuged 10 min. at 2000 RPM.
The supernatants removed and used in procedure below.

	I	II	III	IV	V	VI	VII	VIII	IX
Man:									Normal blood
Heparinized tuberculous blood	.4 ml	.4	.4	.4	.4	.4	.4	.4	.4
Supernatant T_1	.1								
" S_1		.1							
" P_1			.1						
" T_2				.1					
" S_2					.1				
" P_2						.1			
O.T. 1:2							.1		.1
Saline								.1	
WBC 5′	4530	4020	4440	4670	4520	4290	4000	4020	3140
WBC 60′	4400	4000	2880	4550	4340	3430	2610	3940	3010
% difference	−3.0	0	−35.1	−2.5	−3.8	−20.7	−34.7	−2.1	−2.1
Mouse:									Normal blood
Spleen cells	5020	4280		5100	4390		5100	4770	2700
	3390	4340		3450	4420		3360	4770	2700
	−33.5	+1.2		−32.3	−0.8		−34.0	0	0

* T = tuberculin + cells.
S = saline + cells.
P = tuberculin + plasma.
1 = normal.
2 = tuberculous.

method of experimentation. The tubes and counting procedures used were the same as described in a recent communication.[5] In the experiments using human blood, subjects with the same blood groups were used.

The illustrated experiment has been carried out 2 or more times on 4 different subjects and 3 separate pools of mouse spleen cells with essentially identical findings. In many other experiments done to explore the optimal concentrations of various ingredients, it has been found that the cell concentrations, amounts of O.T. used, and the duration of incubation are critical. Although complex, the experiment can be repeated regularly when the indicated precautions are observed.

"Full strength" O.T. is used in the first section to equal in final dilution that obtained with O.T. 1:2 in the second section.

Results. Inspection of the table indicates that the plasma suspension of white blood cells of normal and of tuberculous people blocks tuberculin when an *in vitro* biological assay method for the detection of tuberculin is used. White cells from the spleen of a tuberculous mouse do not so block tuberculin. In neither species does the presence or absence of tuberculosis in the donor host alter the type of *in vitro* cell-tuberculin-blockade. It is interesting, however, that the serum of a normal

[5] Favour, C. B., and Fremont-Smith, P., to be published.

person does not inactivate tuberculin whereas the tuberculous individual's blood does partially block the subsequent *in vitro* cytotoxic effect of tuberculin.

Comment. It has long been assumed that the tuberculin which is placed in the skin or other tissues and which produces a delayed tuberculin reaction is localized in that area. The very nature of the tuberculin reaction itself is evidence for this belief. In the experiments reported here some direct evidence for this belief is presented. The leucocytes of man which are the classic model of host cells of the tissue culture worker are capable of reacting with tuberculin in such a way that it no longer has cytotoxic effects on sensitized white cells from tuberculous subjects. Mouse leucocytes, on the other hand, do not react with tuberculin. It is perhaps not a coincidence that the tuberculous mouse shows no intracutaneous reactions to tuberculin[3] as do tuberculous rabbits, guinea pigs and humans. These findings suggest that there is a primary "affinity" for tuberculin of the tissues of some species and not for others. This "affinity" does not depend on the presence of a tuberculous infection but is present in those species capable of developing a tuberculin reaction. This phenomenon may be compared to other tissue tropisms, for example the neurotropic viruses. It also helps to explain some of the differences in cell damage produced in the tuberculin type reaction as contrasted to the cellular changes in anaphylactic phenomenon.

Summary. 1. Suspensions of human white blood cells in homologous fresh plasma will adsorb tuberculin (O.T.) from the plasma.

2. White blood cells from normal persons with a negative intracutaneous reaction to tuberculin as well as white cells from persons with active tuberculosis show this phenomenon.

3. Suspensions of mouse white blood cells in homologous fresh plasma will not adsorb tuberculin from the plasma.

4. There is no difference in the lack of tuberculin adsorption by white blood cells from normal or tuberculous mice.

5. Normal human plasma does not whereas plasma from patients with active tuberculosis does partially inactivate tuberculin under the conditions of these experiments.

6. Tuberculin has been assayed by its *in vitro* cytotoxic effect on white blood cells from tuberculous subjects.

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Failure of Phenosulfazole to Influence the Course of Infection with Murine Poliomyelitis Virus in Mice.

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Sanders, SubbaRow, and Alexander[1] presented evidence to show that a new sulfonamide compound (N-(2-thiazolyl)-phenol sulfonamide, abbreviated as phenosulfazole, trade name "Darvisul") which was synthesized at the Calco Chemical Division of the American Cyanamid Company acted as an effective antiviral substance in mice infected with the Col. SK strain of murine poliomyelitis virus. Drug treatment instituted 24 hours following intraperitoneal injection with virus at various levels of potency apparently brought about a regular and striking reduction in the mortality rate when compared with that among untreated controls. As judged from the protocols, however, the therapeutic effect showed no clear gradations with respect to amount of virus used for infection or

[1] Sanders, M., SubbaRow, Y., and Alexander, R. C., *Texas Rep. on Biol. and Med.*, 1948, **6**, 385.