

Effect of Intravenous Injection of Tuberculin on Leucocytes of Normal and Tuberculous Rabbits.* (17889)

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The specific cytotoxic effect of tuberculin for certain cells of the tuberculous animal, first shown by Rich and Lewis(1), has been confirmed by others(2-4) using tissue cultures. Recently Favour and his group(5,6) have reported that in the presence of tuberculin, leucocytes of tuberculous animals and humans are lysed *in vitro*. It now appears that this phenomenon is referable to a plasma factor, since leucocytes from normal as well as tuberculous humans react in the same fashion when suspended in plasma from tuberculous individuals(7). Similar phenomena occur *in vivo*(8,9). Furthermore, the significance of changes in leucocytic formulae in tuberculosis has long been recognized by clinicians. Cunningham and Sabin(10), Sabin and Doan(11), and Wiseman and Doan(12) focused attention on changes in the monocyte-lymphocyte ratio, and showed that an increase

in lymphocytes is associated with the healing stage of active tuberculosis, while a depression in relative numbers of lymphocytes denotes progression of the disease.

This peculiar role of the lymphocyte, and its importance in the passive transfer of tuberculin sensitivity(13) has prompted as to study the effect of tuberculin *in vivo* on the lymphocytes of tuberculous animals.

Experimental. Male albino rabbits were inoculated subcutaneously with the Ravenel strain of *Mycobacterium tuberculosis* var. *bovis* propagated on Petraghani's medium. At the time of use, about two months after infection, each rabbit had begun to lose weight and showed a definite cutaneous sensitivity to tuberculin. These rabbits and normal rabbits were given 0.25 or 0.50 mg P.P.D. (Purified Protein Derivative) intravenously. Differential and total leucocyte counts were made from a free-flowing drop of blood from the marginal ear vein. Certified pipettes were used in diluting the blood for the total leucocyte count and both chambers of the Neubauer Brightline Counting Chamber were counted. The smears were stained immediately with Field's stain and 200 cells in various parts of the slide were counted. Counts were made at appropriate intervals during the day preceding the injection of P.P.D. to control the effect of the normal daily fluctuation in total leucocyte and in differential white cell numbers which is accentuated in tuberculosis.

Results. Inspection of the table indicates a leucocytopenia due to the sharp drop in the number of circulating lymphocytes. The studies show that this reaction to tuberculin is progressive because, while the number of circulating lymphocytes had begun to decrease in 3 hours, the maximum decrease was not reached until 6 to 9 hours after the intra-

* This work was supported by a grant from The Ohio Tuberculosis and Health Association.

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TABLE I.
Effect of P.P.D. on Hematological Picture of Normal and Tuberculous Rabbits.

Group	Hr after tuberculin	WBC* ×100	Neutrophiles ×100	Lymphocytes ×100	% Neutrophiles	% Lymphocytes	% Monocytes
I	0	110	24.	80.5	24	75	1
	3	84	39.4	44.5	47	53	0
	6	46	27.6	18.4	60	40	0
	9	70	43.5	26.6	62	38	0
	13	116	61.5	55.5	53	47	0
	28	116	25.5	86.6	22	74	4
II	0	154	57.	78.5	37	51	12
	6	48	31.2	16.3	65	34	1
	9	70	45.	25.2	64	36	0
	24	186	82.	78.	44	42	14
III	0	252	116.	93.	46	37	17
	6	158	120.	23.8	76	15	9
	12	250	160.	45.	64	18	17
	24	206	82.5	101.	40	49	11
IV	0	92	30.	61.	33	67	0
		118	46.	72.	39	61	0
	6	122	39.	83.	32	68	0
		134	61.5	72.4	46	54	0
	9	120	28.	89.	24	74	2
		114	42.1	71.9	37	63	0
	13	100	29.	71.	29	71	0
		118	43.6	73.	37	62	1
	26	108	35.6	72.5	33	67	0
		120	39.6	79.1	33	66	1

Group I. Four tuberculous rabbits with 2 mo. infection. .5 mg P.P.D.

Group II. Four tuberculous rabbits with 2 mo. infection. .5 mg P.P.D.

Group III. Four tuberculous rabbits with 3 mo. infection. .25 mg P.P.D.

Group IV. Two normal rabbits.

* Avg count of all the animals in each group.

venous injection of P.P.D. Between 75-80% of the lymphocytes disappear from the circulating blood during this period, after which they increase slowly and reach or may exceed their previous level in 24 hours. That the leucocytopenia is due entirely to the disappearance of the lymphocytes is indicated by the fact that the number of circulating neutrophiles shows little decrease. In fact, there is a sharp rise in the total number of neutrophiles between 9 and 12 hours after P.P.D. is injected. Such changes do not occur in normal animals given the same treatment.

Discussion. Favour(5) found that lysis by tuberculin of both lymphocytes and neutrophiles occurs *in vitro*, while our results indicate that little or no observable destruction of neutrophiles occurs. However, since a dynamic state exists *in vivo* that is not duplicated *in vitro*, a rapid production of

neutrophiles under these conditions may mask their actual destruction. Moreover, analysis of Favour's data suggests that although both cell types are lysed *in vitro*, the lymphocytes are destroyed at a greater rate than are the neutrophiles.

In active tuberculosis, a certain amount of autoinoculation with tuberculo-protein probably occurs continuously, and it is possible that this is responsible for the relative lymphopenia characteristic of progressive tuberculosis.

Summary. Following the intravenous injection of P.P.D. into tuberculous rabbits there is a decrease in the total number of white cells and lymphocytes. The maximum decrease occurs in 6 to 9 hours and is followed by a gradual return to normal. There is also frequently a delayed increase in neutrophiles.

Received April 25, 1950. P.S.E.B.M., 1950, v74.