tissues diseased with tobacco mosaic and with other viruses to see what further information can thus be gained concerning the focus and mechanism of virus proliferation.

Summary. Electron micrographs of sections cut through tobacco leaves infected with tobacco mosaic indicate that the virus is often present in fibrous masses associated with chloroplasts which are severely affected. Virus can clearly be seen in the cytoplasm of the diseased cells sometimes as single filaments. The nuclei seem unaltered by the infective process and have appeared free of virus. The great length of these filaments, much in excess of 2800A, indicates an end-to-end arrangement of the rods *in situ*.

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Increased *in vitro* Tuberculin Leucocyte Cytolysis Following a Tuberculin Skin Test.* (17598)

CUTTING B. FAVOUR, BARBARA A. HARRISON, AND CHARLES K. OSGOOD
(Introduced by J. H. Mueller)

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

It has recently been shown that leucocyte suspensions prepared from the blood of animals showing tuberculin hypersensitivity are injured when exposed to tuberculin.(1,2) Further analysis of this cell-tuberculin-system has shown that a serum factor, present in the euglobulin portion of serum from tuberculous subjects,(3,4) is capable of destroying portions of the white cells of the tuberculous as well as the non-tuberculous guinea pig and man, if first these cells are exposed to tuberculin.(5) A convenient way of demonstrating this serum factor consists in following the white blood counts in samples of heparinized whole blood to which appropriate amounts of tuberculin have been added.(6)

The purpose of this report is to describe an increase in *in vitro* tuberculin-leucocyte cytolysis in blood of tuberculin sensitive guinea pigs following an intracutaneous tuberculin reaction. This study was prompted by earlier findings that a positive tuberculin test in man may be followed by a similar increase in *in vitro* tuberculin cytolysis.(6)

Materials and Method.

Materials. Guinea Pigs. Four hundred to 900 g animals were inoculated one to 3 times at 2-month intervals in both groins and pectoral regions with 10 mg of heat-killed tubercle bacilli (H37Rv) suspended in 1 ml of Bayol F (0.25 ml at each site). Animals were selected for study who showed hard masses 1 to 2 cm in diameter in the above areas and who also demonstrated cutaneous induration greater than 1 cm in diameter at 48 hours from a second strength PPD (5 γ in 0.1 cc) skin test.(7) Normal tuberculin-negative guinea pigs were used as controls. No skin tests were done more recently than 3 weeks before the experiment began.

Leucocytes. Two ml blood samples were taken by cardiac puncture into a syringe containing 0.2 ml heparin (Liquaemin), mixed,

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TABLE I.

In vitro Effect of PPD upon the Leucocyte Count on Animals Before and After Receiving an Intracutaneous Tuberculin Test. Animals 1 through 8 are experimental; Animals 9 through 16 are controls.

	Before PPD Skin Test. Date 7/19.				After PPD Skin Test. Date 7/21.				
G.P. No.	Avg WBC	% fall Saline	% fall PPD	PPD decrement	Skin test at 48 hr, mm	Avg WBC	% fall Saline	% fall PPD	PPD decrement
1	4760	+ 0.5	—14.4	—14.9	12x13	7680	0	—28.8	—28.8
2	5720	— 1.1	— 1.1	0	10x10	10630	—0.2	—23.7	—23.5
3	8870	— 0.6	— 9.7	— 9.1	11x11	12700	—0.6	—17.6	—17.0
4	6350	—27.3	—24.8	+ 2.5	12x12	11330	—0.5	—25.8	—25.3
5	8980	— 0.3	—13.3	—13.0	11x11	10780	0	—19.6	—19.6
6	6420	+ 0.5	— 1.6	— 2.1	18x16	8370	—1.0	—29.1	—28.1
7	4850	— 0.3	—13.4	—13.1	20x15	7260	+0.8	— 8.4	— 9.2
8	4920	— 0.7	+ 1.0	+ 0.3	17x16	12690	—0.2	—24.5	—24.3
9	3650	— 0.5	— 3.3	— 2.8	neg.	3810	+0.6	— 1.5	— 2.1
10	3640	+ 1.7	0	— 1.7	”	6250	—1.3	— 0.7	+ 0.6
11	6190	+ 0.3	+ 0.2	— 0.1	”	4710	—1.5	— 1.5	0
12	4940	— 1.1	+ 0.6	+ 1.7	”	4950	+0.5	— 0.3	— 0.8
13	2300	— 1.0	+ 0.5	— 0.5	”	3330	—0.2	+ 0.5	+ 0.7
14	3650	— 0.7	— 1.2	— 0.5	”	4380	—1.0	— 1.3	— 0.3
15	10300	+ 0.6	+ 0.1	+ 0.1	”	11170	0	0	0
16	7070	+ 0.3	+ 0.5	+ 0.2	”	8760	—0.7	— 0.5	+ 0.2

PPD—5 γ/0.1 cc—planted intracut. 0.1 cc per guinea pig.

and the white cells counted by the method described elsewhere.(5,6)

Tuberculin. PPD used was freshly prepared by dissolving one tablet of commercial second strength PPD(7) in 0.5 ml of 0.85% saline and using 0.1 ml for the test. This resulted in a concentration of 50 γ per ml of cell suspension. Old Tuberculin used was obtained from the Massachusetts Department of Health. The crude O.T. was dialized against 3 changes of 0.85% saline and used as a 1:10 dilution or a concentration of 1.5 mg per ml of cell suspension.

Method. To 0.4 ml heparinized blood in each of 2 siliconed Wassermann tubes was added 0.1 ml of PPD solution and 0.1 ml of 0.85% saline respectively. After adequate mixing leucocyte counts were performed (5-minute counts). The tubes were then rolled at 100 RPM in an incubator for 1 hour before the second leucocyte count. The PPD decrement is the per cent fall in the tuberculin tube minus the per cent fall in the saline control tube.

Results. In Table I are given the cytolysis tests on a group of 8 tuberculin-sensitive and 8 normal guinea pigs which were studied just before and 48 hours after receiving 5 γ of PPD intracutaneously. This dose resulted in a typical indurated reaction 1 to 2 cm in diameter in all experimental animals. Inspection of Table I shows that 7 out of 8 experimental and no one of the control animals showed a greater decrement following the cutaneous tuberculin reaction. In a subsequent experiment essentially similar results were obtained on blood samples from the same animals using O.T. as a skin testing antigen. Animal 4 in Table I was bled with some difficulty and experienced a mild "alarm reaction."(6) The size of the skin test did not correlate with the cytolysis test.

Discussion. In an attempt to transfer passively tuberculin hypersensitivity to normal rabbits by the injection of whole blood from tuberculous rabbits, Sandage and Birkeland(8) noted that this could be done successfully only when the blood was used from animals during their response to an intravenous injection of tuberculin. Observations reported here indicate that a recent tuberculin reaction alters *in vitro* tuberculin cytotoxic responses as well. Accordingly, passive transfer of the tuberculin reaction with whole blood is a more complex event than that with washed cells using the method of Chase.(9)

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Summary. Guinea pigs which have been sensitized to tuberculin by the injection of heat-killed tubercle bacilli in paraffin oil show an increased *in vitro* tuberculin leucocyte cytolysis within 48 hours after a positive intracutaneous tuberculin reaction.

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Appearance of Protein Tagged with Radioactive Iodine in Thoracic Duct Lymph.* (17599)

HARVEY KRIEGER, WILLIAM D. HOLDEN, CHARLES A. HUBAY, MURRAY W. SCOTT, JOHN P. STORAASLI, AND HYMER L. FRIEDELL

From the Departments of Surgery and Radiology, Western Reserve University, School of Medicine, Cleveland, Ohio.

When plasma protein or albumin is tagged with radioactive iodine (I-131) and the combination is injected intravenously into dogs or humans to determine the plasma volume, there is a progressive decrease in the measurable radioactivity of blood samples withdrawn periodically.(1,2) This suggests the passage of the iodinated protein into extravascular spaces. It is known that protein circulating in the blood stream passes with considerable facility into the thoracic duct lymph. The concentration of protein in lymph varies in different parts of the body. Field *et al.*(3) reported the plasma protein concentration in 13 dogs to be 6.25 g per 100 cc whereas the concentration in thoracic duct lymph was 4.00 g per 100 cc. The average concentration of albumin in the plasma and thoracic duct lymph of the same animals was 3.61 and 2.45 g per 100 cc respectively. The purpose of the present experiments was to determine the rapidity with which the intravenously injected radioactive protein appeared in thoracic duct lymph. The amount of radioactivity in lymph 10 minutes following the intravenous injection was of special interest, since this period is the commonly accepted interval between the injection of either radioactive protein or T 1824 and withdrawal of blood for the determination of this plasma volume.

Method. Eleven healthy adult fasting dogs, weighing 8 to 16 kilos, were used. Each dog was anesthetized with intravenous sodium pentobarbital .030 g per kilo of body weight. Through a left cervical approach the junction of the thoracic duct and the left subclavian vein was exposed. The duct was ligated close to the subclavian vein and, depending upon its size, a number 20 or 18 gauge needle was inserted into the lumen of the duct and fixed with a No. 000 black silk ligature. The needle was irrigated with a few drops of heparin to prevent clotting. A femoral vein and artery were exposed and isolated to be used for injection of the iodinated protein and collection of blood samples. Twenty cc of iodinated protein containing less than 1 g of albumin and approximately 40 microcuries of radioactivity were injected into the femoral vein. Simultaneous samples of blood and lymph were collected from the femoral artery and thoracic duct 10, 30, 60, and 120 minutes following the injection. The animals were divided into two groups. Group I consisted of 6 dogs into which radioactive iodinated human albumin was injected. Group II consisted of 5 dogs into which was injected canine plasma protein activated with the iodine. Technical difficulties prevented the collection of some of the lymph samples in the first

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