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Serum Protein Change in Response to Positive Tuberculin Skin Test in Humans.* (28445)

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Although tuberculin sensitivity can be transferred passively with considerable regularity by means of leucocytes obtained from sensitive donors, results of attempts at passive transfer through the use of serum or plasma have been rather equivocal. Zinsser and Mueller(1) described passive transfer of tuberculin sensitivity to guinea pigs using serum from tuberculous rabbits. Best results seemed to prevail if the serum was taken from the donor rabbits 24 hours after they had received an intravenous injection of Old Tuberculin. Sandage and Birkeland(2) reported that blood taken from tuberculous rabbits during the response to intravenous tuberculin conferred tuberculin sensitivity to normal recipient rabbits. Blood taken from tuberculous rabbits without tuberculin treatment did not have this capacity. More recently, Cole and Favour(3) described the successful transfer of tuberculin sensitivity in guinea pigs with plasma obtained from sensitive donors. Activity resided in a new plasma fraction which they definded and named fraction IV-10. It is of interest to the present discussion that their donor animals had to be skin-tested with PPD within 48 hours of being bled in order to yield plasma capable of passive transfer.

The various authors mentioned above have postulated that the response to antigen (O.T. or PPD) by tuberculin-sensitive hosts might cause liberation into the circulating plasma of an "antibody-like" substance responsible for passive sensitization. If this is so, then it seemed reasonable to believe that such a substance might be detectable by suitable biochemical techniques. Investigation of the reactivity of a well-defined human population group to 3 types of tuberculin afforded an opportunity to determine whether there is a significant alteration in serum protein components during the response to skin tests in highly sensitive individuals.

Materials and methods. Blood samples were taken from 19 students at the Fort Sill Indian School, Lawton, Okla. The students ranged in age from 16 to 22 years, with an average of 18 years, and consisted of 15 males and 4 females. Immediately after blood samples were taken the entire group was skin tested with intermediate strength (0.0001 mg) of human, avian, and Battey tuberculins (PPD). Forty-eight hours later the skin reactions were measured and a second blood sample was taken. Analysis of the serum proteins was done on filter paper strips using a Spinco electrophoresis apparatus. All electrophoretic separations were done at room temperature at a constant current in pH 8.6 barbital buffer. At the end of migration the strips were oven dried, stained with bromphenol blue, rinsed in acetic acid, and redried. The protein composition and relative

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TABLE I. Effect of Tuberculin Test on Electrophoretic Distribution of Serum Proteins in Humans (Experimental Group).

No.	PPD-S (mm ind.)	Grams % Albumin	Alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin
1		4.77	.16	.44	.92	.84
2		4.71	.27	.42	.85	1.39
3	P	4.61	.22	.71	.87	1.16
4	r	5.06	.33	.48	.74	1.15
5	e	5.01	.27	.48	.82	1.40
6	`	4.66	.35	.45	.64	.97
7	t	3.86	.30	.60	.90	2.23
8	e	4.31	.28	.52	.81	1.97
9	s	4.60	.30	.57	.80	1.56
10	t	4.84	.29	.61	.83	1.19
11		5.01	.30	.43	.96	1.57
12		4.88	.22	.35	.58	1.73
Mean		4.69	.27	.50	.81	1.43
1	14	4.38	.29	.45	.84	1.10
2	25	4.49	.36	.36	.85	1.51
3	26	4.33	.31	.58	.82	1.40
4	12	5.25	.33	.51	.62	1.20
5	27	4.61	.26	.69	.91	1.65
6	33	4.64	.36	.52	.77	1.21
7	34	3.63	.38	.75	.99	1.89
8	16	4.31	.34	.68	.81	1.84
9	37	4.18	.42	.50	.84	1.63
10	33	5.10	.24	.54	.76	1.13
11	16	4.82	.33	.37	.80	1.46
12	34	5.22	.33	.62	1.17	2.04
Mean	25	4.58	.33	.55	.85	1.50
"t"		1.44	3.29*	1.13	.67	1.29

* Statistically significant, $P < .01$.

percent distribution was determined by scanning the papers in a Spinco Analytrol. Total serum protein determinations were made by the biuret method and the results were used to calculate absolute values for the various components.

Results. Twelve of the 19 students (Table I) responded to human tuberculin with reactions of more than 10 mm induration (mean, 25 mm). In these individuals there was a statistically significant elevation in alpha-1 globulin during their response to the skin tests as compared with the pre-test value. These same students responded to avian and Battey tuberculins with mean reactions of 11 mm and 7 mm respectively. Seven students gave reactions of less than 10 mm (mean, 3 mm) to human tuberculin and mean reactions of 9 mm and 3 mm to avian and Battey tuberculin respectively. No significant alteration in any of the serum components could be detected in this group 48 hours after application of the skin tests (Table II).

It could be argued that the increase in alpha-1 globulin noted in the first group might be attributable to the fact that they were reacting to 3 different skin-test substances simultaneously. Results of further analyses of the data, however, suggest that the effect observed was due to reactivity primarily, if not exclusively, to human tuberculin and was not necessarily complicated by the other 2 skin tests.

Discussion. These results lend further support to the growing body of evidence suggesting that the substance responsible for tuberculin hypersensitivity has the characteristics of an alpha globulin. The work of Cole and Favour has already been cited. Their newly defined plasma fraction, IV-10, which was capable of passive sensitization in guinea pigs, was contained primarily in Cohn's fraction IV, alpha globulin. It is of interest that fraction IV-10 contained all of the antibody to tuberculoprotein, and none to tuberculopolysaccharide. Jeter and associates(4) reported on Tiselius electrophoretic

TABLE II. Effect of Tuberculin Test on Electrophoretic Distribution of Serum Proteins in Humans (Control Group).

No.	PPD-S (mm ind.)	Grams % Albumin	Alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin
1	P	4.38	.23	.72	.76	1.29
2	r	4.34	.32	.53	.85	1.53
3	e	5.03	.20	.44	.64	1.34
4	`	5.04	.18	.44	.66	1.51
5	t	4.62	.29	.45	.78	.99
6	e	4.72	.46	.63	.70	1.06
7	s	5.01	.34	.23	.89	1.04
Mean	t	4.73	.29	.49	.75	1.25
1	5	4.32	.29	.62	.62	1.57
2	9	4.98	.29	.64	.49	1.37
3	0	5.15	.20	.33	.45	1.36
4	4	4.08	.30	.49	.65	1.83
5	0	4.63	.20	.50	.80	1.09
6	0	5.20	.21	.52	.59	1.12
7	0	7.06	.46	.65	1.44	1.63
Mean	3	5.06	.28	.54	.72	1.42
"t"		.94	.20	.62	.31	1.87

analyses of extracts prepared from leucocytes obtained from tuberculin-sensitive guinea pigs. A new electrophoretic component resembling an alpha-1 globulin was found in high concentration in cells from sensitive animals which was lacking in normal cells. Extracts containing this component transferred cutaneous reactivity passively to normal recipient animals. Weimer and associates(5) and Janicki(6) have noted that the earliest significant change in serum proteins in guinea pigs undergoing tuberculin sensitization is an increase in alpha-1 globulin within 5 to 8 days. This time relationship correlates well with the results of further studies by Janicki(7) demonstrating the appearance in guinea pig serum of the factor responsible for *in vitro* cytolysis of leucocytes by PPD. Lawrence and Pappenheimer(8) have shown that the factor in leucocytes responsible for passive transfer of tuberculin hypersensitivity can be released *in vitro* by simply incubating the cells in normal plasma or serum. Janicki (6), using similar techniques, found that normal serum into which transfer factor has been released *in vitro* has the capacity to render normal leucocytes susceptible to lysis by PPD. Electrophoretic analysis of the serum revealed a significant elevation only in the alpha-1 globulin.

Finally, it seems pertinent that support for the belief that gamma globulin plays no role in delayed sensitivity comes from the numerous studies of patients with agammaglobulinemia. Delayed sensitivity to foreign protein antigens can be induced with regularity in such patients, despite their inability to form classical serum antibody. Moreover, it has been reported that delayed allergy may be transferred from agammaglobulinemic subjects to normal individuals by means of peripheral blood leucocytes(9,10).

Summary. Individuals who are highly sensitive to tuberculin PPD develop a statistically significant elevation in serum alpha-1 globulin during their response to a PPD skin test. The implication of this finding with respect to the mechanism involved in tuberculin hypersensitivity is discussed.

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Nucleic Acids of Measles and Vesicular Stomatitis Viruses. (28446)

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Measles virus is classified as a myxovirus on the basis of its structure and cytopathic effect in tissue cultures(1,2,3), and therefore was tentatively assumed to be an RNA virus although the nature of its nucleic acid had not been established. Because 5-fluorodeoxyuridine (FUDR) inhibits the synthesis of DNA viruses(4,5,6), it can be used to differentiate RNA and DNA viruses. This report on the effect of FUDR on production of virus by an established line of cercopithecus monkey kidney cells (BS-C-1)(7), provides indirect evidence that measles actually is an RNA virus, and incidentally that vesicular stomatitis virus (VSV) is also an RNA virus.

A culture of the Edmonston strain of measles virus adapted to HeLa cells was used to inoculate 6 tubes containing monolayers of BS-C-1 cells. As assayed in the same cell line, the virus concentration of the inoculum was $10^{4.5}ID_{50}$ per tube. At time of inoculation the cell count was approximately 2×10^5 per monolayer. The medium used for virus growth and maintenance was Hanks' balanced salt solution containing 0.1% lactalbumin hydrolysate, 0.16% $NaHCO_3$, and 2% horse serum. In 3 of the 6 tubes the medium also contained 0.5 γ of FUDR per ml (2×10^{-6} M).

Each experiment included control cultures infected with (a) adenovirus type 3, as a known DNA virus; (b) the Leon strain of type 3 poliovirus, as a known RNA virus; and, because poliovirus is ether resistant while measles virus is ether sensitive, (c) VSV as an ether sensitive virus which we assumed to be an RNA virus until a survey of the literature revealed no proof of this. The adenovirus concentration, as assayed by BS-C-1 cells, was 10^2ID_{50} per tube. The poliovirus concentration, as assayed in primary monkey kidney cells, was $10^{7.5}$ plaque-forming units per tube. The VSV concentration, as assayed in BS-C-1 cells, was $10^{6.5}ID_{50}$ per tube.

The infected cultures were harvested for virus by one cycle of freezing and thawing at the time that all the cells were involved, which was 4 to 5 days for measles, 6 days for adenovirus, 3 days for poliovirus, and 24 hours for VSV. Yields of virus were assayed in tubes of BS-C-1 cells by the dilution end point technique.

The results of 3 experiments are given in Table I. As expected, the FUDR suppressed

TABLE I. Effect of 5-Fluorodeoxyuridine (FUDR) on Measles and Vesicular Stomatitis Virus (VSV) Yields.

Exp No.	Virus	Yield (ID_{50}/ml) No FUDR	Yield (ID_{50}/ml) FUDR (.5 γ/ml)
1	Measles	10^6	$\geq 10^{6.5}$
	VSV	10^7	$\geq 10^{6.5}$
	Controls:		
	Poliovirus (RNA)	$10^{7.5}$	$10^{7.5}$
	Adenovirus (DNA)	$10^{3.5}$	$10^{1.5}$
2	Measles	$10^{5.0}$	$10^{4.5}$
	VSV	$10^{8.0}$	$10^{9.2}$
	Controls:		
	Poliovirus	$10^{8.0}$	$10^{7.5}$
	Adenovirus	$10^{5.0}$	$10^{3.0}$
3	Measles	$10^{4.5}$	$10^{5.5}$
	VSV	$10^{8.0}$	$10^{7.5}$
	Controls:		
	Poliovirus	$10^{7.5}$	$10^{7.5}$
	Adenovirus	$10^{4.0}$	$10^{2.0}$