

ANALYSIS OF LEUKOCYTIC EXTRACTS FROM GUINEA PIGS HYPERSENSITIVE TO TUBERCULIN AND 2,4-DINITROCHLOROBENZENE¹

W. S. JETER, K. A. LAURENCE^{2, 3} AND P. M. SEEBOHM

Departments of Bacteriology and Internal Medicine, State University of Iowa, Iowa City, Iowa

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Delayed type hypersensitivity to tuberculin and to simple chemical substances has been transferred passively with intact peritoneal exudative leukocytes by a number of investigators (Landsteiner and Chase, 1942; Chase, 1945; Stavitsky, 1948; Cummings *et al.* 1947; Kirshheimer and Weiser, 1947; Metaxas and Metaxas-Buehler, 1955). In a previous paper Jeter *et al.* (1954) reported successful passive transfer of delayed cutaneous sensitivity to 2,4-dinitrochlorobenzene in guinea pigs with extracts of leukocytes disrupted by sonic oscillations. Experiments by Lawrence (1955) showed that transfer of tuberculin and streptococcal sensitivity in human beings could be accomplished with extracts of blood leukocytes lysed by freezing and thawing and by suspension in distilled water. Attempts to transfer sensitivity in guinea pigs with extracts of cells lysed by freezing and thawing failed (Chase, 1945). Cummings *et al.* (1956) demonstrated passive transfer of tuberculin sensitivity in guinea pigs using extracts of peritoneal exudative leukocytes and splenic cells disrupted by sonic vibration.

No definitive experiments characterized the nature of the substance or substances responsible for transfer with cellular extracts. The purpose of this paper is to describe and partially identify a substance present in leukocytic extracts from tuberculin and dinitrochlorobenzene-sensitive animals which may be responsible for passive sensitization. A portion of these results was presented at the 1956 annual meeting of the Society of American Bacteriologists (Jeter *et al.*, 1956).

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² Postdoctoral fellow of the National Institutes of Health, Department of Health, Education, and Welfare.

³ Present address: Department of Microbiology and Immunology, Cornell University Medical College, New York, N. Y.

MATERIALS AND METHODS

Male albino guinea pigs weighing 350 to 450 g were used in all experiments. Sensitization to 2,4-dinitrochlorobenzene was evoked by painting daily for 1 week a shaved area of skin at the nape of the neck with a 2 per cent alcoholic solution of this chemical (Seebohm *et al.*, 1954). Tuberculin sensitivity was induced by 3 subcutaneous injections in the groin at weekly intervals of 5 mg heat killed tubercle bacilli incorporated in a mineral oil-aquabase emulsion. The strain of *Mycobacterium tuberculosis* was isolated in this laboratory and possessed a high degree of virulence for the guinea pig.

Skin testing reagents used for the determination of sensitivity were 1 per cent 2,4-dinitrochlorobenzene in olive oil and 1:50 Old Tuberculin in 0.15 M NaCl.

After a satisfactory degree of sensitivity had been achieved as evidenced by a positive skin test, donor animals in groups of 12 or 18 were given 30 ml of sterile light mineral oil intra-abdominally. Forty-eight hours later exudative cells were collected in Hanks' solution containing 0.2 per cent gelatin and 0.0008 per cent heparin. After centrifugation at 2000 rpm, cells were washed once and resuspended in the same medium. The cellular volume was estimated and leukocyte counts made. The suspended cells were treated for 10 min in a 9 kc Raytheon magnetostriiction oscillator. The vibrated material was then centrifuged for 15 min at 25,000 rpm for clarification, and the supernatant fluid was divided into 2 portions for electrophoretic analysis and transfer studies. Protein content of each extract was estimated by the quantitative biuret method (Gornall *et al.*, 1949).

Tiselius electrophoretic analyses were done in a Perkin-Elmer apparatus at 1 C. Barbitol buffer, pH 8.6, ionic strength 0.1, resistance 330 ohms, was employed for all determinations. The time of analysis was 120 min and the current 10 ma.

In passive transfer studies, recipient animals

were given injections of extracts intra-abdominally. Tests were made on chemically depilated areas of skin at either 24 or 48 hr after transfer, and readings were made daily for at least 3 days.

EXPERIMENTAL RESULTS

Electrophoretic studies. Electrophoretic analyses were carried out on cellular extracts derived from tuberculin sensitive, 2,4-dinitrochlorobenzene sensitive, and normal donor animals. The total protein content of all the extracts analyzed was adjusted to a constant value of 12 mg per ml. The protein content of the extracts ranged from 12 to 16 mg per ml. A compilation of average data for 7 to 10 experiments in each group is shown in table 1. Characteristic patterns from each of the groups are in figure 1. Six fractions were found in extracts from donors in both the tuberculin and dinitrochlorobenzene-sensitive groups, whereas only 5 components were demonstrable in extracts of cells from normal control animals. The percentage concentration was highest in fraction VI, the fraction unique to extracts from sensitive donors. On the other hand, the greatest relative

change in concentration between the normal and sensitive groups appeared as a loss in fractions II and V, with slight loss evident in other fractions.

The electrophoretic mobilities of the fractions in both experimental groups are similar to the mobilities of components present in the normal control extracts, with the exception of fraction V. This could result from the absence of fraction VI in the normal extracts coupled with a greater amount of the fifth fraction. In any event, the mobility of the fraction peculiar to extracts of cells from sensitive animals, fraction VI, is significantly higher than that of any normal component.

The last line of table 1 shows average ranges of the fractions observed in terms of components previously demonstrated by other investigators (Moore, 1945; Deutsch and Goodloe, 1945) for normal guinea pig serum and plasma. From a comparison with these data, the fraction peculiar to cellular extracts from sensitive animals, fraction VI, is similar, or identical to α -1-globulin.

Passive transfer studies. In the tuberculin group, seven passive transfer experiments were

TABLE 1

*Electrophoretic analyses of cellular extracts from sensitive and normal donor guinea pigs**

	Fraction No.					
	I	II	III	IV	V	VI
Cells from tuberculin sensitive donors						
%: Avg	7.0	11.1	8.1	9.5	15.3	49.0
Range	6.1-9.3	8.5-28.0	7.9-17.1	6.1-14.4	7.0-16.0	28.5-57.6
μ †: Avg	.66	1.23	1.75	2.51	3.70	4.97
Range	.65-.82	1.19-1.88	1.72-2.53	2.48-3.28	3.61-4.16	4.83-5.23
Cells from 2,4-D† sensitive donors						
%: Avg	8.6	17.1	9.0	11.0	12.7	40.0
Range	3.2-12.0	12.5-28.0	6.7-23.2	7.4-16.6	4.8-15.3	25.6-53.7
μ : Avg	.62	1.32	1.81	2.41	3.49	4.81
Range	.55-.78	1.18-1.41	1.64-1.99	2.31-2.86	3.29-2.97	4.60-5.26
Cells from normal donors						
%: Avg	8.9	25.9	14.3	14.3	36.6	—§
Range	5.3-11.1	22.1-39.3	10.1-17.6	11.1-17.6	26.6-41.3	—
μ : Avg	.61	1.27	2.0	2.79	4.09	—
Range	.55-.71	1.15-1.68	1.76-2.35	2.59-2.96	3.86-4.39	—
Comparative serum globulin fraction.....		γ		β	α_2	α_1

* 7-10 experiments in each group.

† μ = mobility: 1×10^{-5} cm²/r/sec.

‡ 2,4-dinitrochlorobenzene.

§ Fraction VI not present in normal cellular extracts.

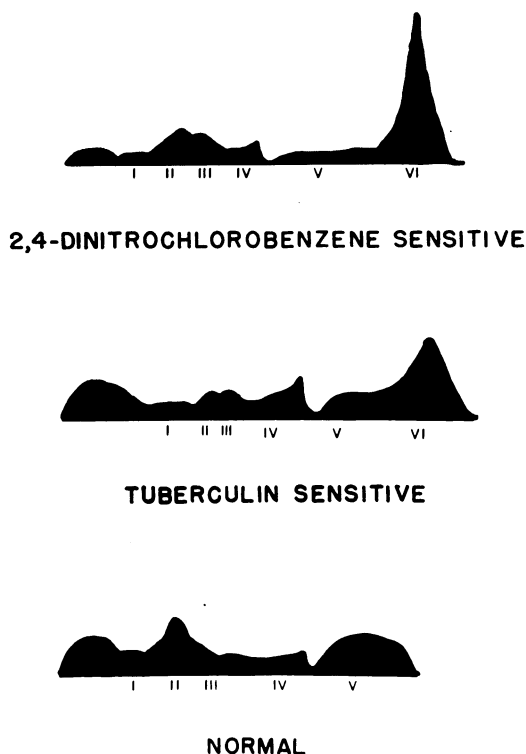


Figure 1. Typical electrophoretic patterns of extracts of leukocytes from sensitive and normal animals. Fractions are designated by Roman numerals.

done. Five recipient animals gave skin test reactions at 24 and 48 hr following testing with 1:50 Old Tuberculin showing an erythematous area between 12 and 16 mm in diameter with induration and edema. Some central blanching was evident in the reactions seen on three animals. One of the animals died before the skin test could be read. The other was negative and this negativity is unexplained. However, at least 71 per cent of the recipient animals in this group gave positive skin reactions on testing.

Nine transfer attempts were made with extracts of cells from dinitrochlorobenzene-sensitive donors in this series of experiments. Seven, or 77 per cent, of the animals gave homogeneous erythematous reactions when tested with 1 per cent 2,4-dinitrochlorobenzene in olive oil. In one of the negative experiments, a portion of the extract was lost in centrifuge breakage, the other is unexplained.

DISCUSSION

These results show the presence in cellular extracts from donor guinea pigs sensitive to tuberculin and to 2,4-dinitrochlorobenzene of an electrophoretic component which is not in extracts of cells from normal animals. It has an electrophoretic mobility in the range of guinea pig serum α -1-globulin. Extracts containing this component transferred cutaneous reactivity passively to normal recipient animals in more than 70 per cent of our attempts. Although it is impossible to attribute transferability of passive sensitization directly to this component, its consistent presence in extracts which produced passive sensitization strongly suggests that it may play a role. Of interest in this connection are the studies of Cole and Favour (1955) who demonstrated antibody to tuberculoprotein in an α -globulin-albumin fraction of plasma from tuberculin sensitive guinea pigs. This fraction was reported as being capable of passive transfer of delayed type skin sensitivity to tuberculin. Combination of this fraction with the α -globulin fraction inhibited passive transfer, however.

Further elucidation of the function of this fraction must await more precise studies on separation of the component and testing the sensitizing capacity of a pure or more nearly pure substance.

SUMMARY

Electrophoretic and passive transfer studies were done with extracts of peritoneal exudative leukocytes from tuberculin-sensitive, 2,4-dinitrochlorobenzene-sensitive, and normal donor guinea pigs. A new electrophoretic component resembling an α -1-globulin was found in high concentration in cells from sensitive donors, which was lacking in normal cells. Positive passive transfer reactions were demonstrable with aliquot portions of more than 70 per cent of extracts analyzed in both sensitive groups.

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