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Tuberculin Reaction. II. Cytotoxicity of Tuberculin for Splenic Explants of Desensitized Tuberculous Guinea Pigs.*

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Important differences between tuberculin sensitivity and other types of sensitivity have been reviewed in a previous publication.[1] One of the differences is that tuberculin exerts a specific cytotoxic action on tissue explants of tuberculin-sensitive animals, whereas, such specific cytotoxic effects of antigen are not present in other types of sensitivity. This "*in vitro* sensitivity" is not limited to tuberculin-sensitive tissues but apparently includes all sensitivities of the tuberculin type. For example, Moen[2] has noted that *in vitro* cytotoxic action can be produced in case of the tuberculin type sensitivity which develops in guinea pigs as a consequence of infection with β hemolytic streptococci. Extracts of the causative organism were found to exert a specific cytotoxic action on tissue explants of infected guinea pigs.

Heilman and Seibert[3] in studies on the *in vitro* action of purified fractions of tubercle bacilli on tuberculin-sensitive tissues demonstrated that, in common with the cutaneous and systemic tuberculin reactions, specific *in vitro* cytotoxic action can be produced with tuberculoprotein, but not by other components such as polysaccharide and nucleic acid fractions.

Lasfargues, Bouquet and Delaunay[4] express doubt that the *in vitro* cytotoxic action of tuberculin is specific. They believe that the toxicity observed is simply the expression of a non-specific heightened vulnerability of the cells of the tuberculous animal. However, this is not consistent with the observation of Heilman, Feldman and Mann[5] that the tissues of animals sensitized with heat-killed tubercle bacilli react in a similar manner, for, it is not probable that the tissues of such non-infected healthy animals would possess a non-specific heightened vulnerability. It seems logical that the specific *in vitro* cytotoxic action of tuberculin is as much a manifestation of tuberculin sensitivity as are the cutaneous and systemic tuberculin reactions and, moreover, that the systemic tuberculin reaction is the summation of an "*in vivo* cytotoxic action" of tuberculin on sensitive cells analogous to the cytotoxic action which occurs *in vitro*.

In a previous publication[1] we have reviewed the evidence indicating that tuberculin type sensitivity is dependent upon antibody. The object of the present investigation was to provide additional evidence on the existence and nature of tuberculin antibody.

It was our opinion that if the *in vitro* cytotoxic action of tuberculin is dependent upon antibody the combination of such antibody with antigen by desensitization treatment with tuberculin should render the tissues of the animal less susceptible to the *in vitro* cytotoxic effect of tuberculin than the tissues of sensitive controls. Furthermore, it seemed probable that the *in vitro* studies proposed would greatly reduce the possible interference of the humoral blocking antibodies (antituberculins, anticutins) which have been assumed by some investigators[6-8] to be responsible for the high

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1 Kirchheimer, W. F., and Weiser, R. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 166.

2 Moen, J. K., *J. Exp. Med.*, 1936, **64**, 355.

3 Heilman, D. H., and Seibert, F. B., *Am. Rev. Tuberc.*, 1946, **53**, 71.

4 Lasfargues, E., Boquet, P., and Delauney, A., *Ann. de l'Inst. Pasteur*, 1947, **73**, 169.

5 Heilman, D. H., Feldman, W. H., and Mann, F. C., *Am. Rev. Tuberc.*, 1945, **52**, 65.

6 Pickert, M., and Löwenstein, E., *Dtsch. Med. Wochenschr.*, 1908, **34**, 2262.

tolerance to tuberculin observed in some animal species and in tuberculous humans following tuberculin treatment.

Methods. Adult male and female guinea pigs averaging approximately 600 to 800 g in weight were infected with the B.C.G. strain of *Mycobacterium tuberculosis* variety *bovis*. Approximately 5 to 10 mg of a young culture were given either subcutaneously or intraperitoneally. Within 4 to 5 weeks after infection most of the animals responded with a necrotic cutaneous tuberculin reaction to Old Tuberculin 1:100.† The animals were tuberculin-tested from time to time and only those that gave necrotic reactions to O.T. 1:100 were used in the experiments.

Desensitization was accomplished with progressively increasing intraperitoneal doses of O.T. given over a period of 3-4 days. The initial dose was 0.5 cc of O.T. 1:10,000. Desensitization treatment was continued until the animals failed to react to O.T. 1:10 after which they were given a final desensitizing dose of 1 to 2 cc of O.T. 1:2. The animals were used for tissue culture the day following the final desensitizing dose of tuberculin.

The methods of tissue culture used were essentially the same as those employed by Rich and Lewis.[9] Splenic tissue was scissored in cold Locke's solution and matched pieces approximately 1 mm in diameter were transferred to a second bath of cold Locke's solution containing the concentrations of O.T. or glycerine broth present in the plasma clots used in the respective experiments. The explants were finally transferred to large coverglasses with a capillary pipette and a few drops of fresh, cold heparinized guinea pig plasma containing the respective concentration of tuberculin or glycerine broth was added. The final concentration of O.T. and of glycerine broth in the plasma clots ranged from 1:40 to 1:80. A drop of molten paraffin-vaseline mixture was then deposited at each corner of the coverglasses and embryo culture slides inverted over the preparations. The slides were righted and the edges of the coverglasses sealed with molten paraffin. The cultures were incubated as hanging drops at 37.5°C ± 0.2°C in a specially constructed electric incubator. After 24 hours of incubation the mean radius of the zone of cell migration of each explant was calculated from measurements of the radius made at several points with an ocular micrometer using a magnification of X32.

Two animals were employed in each experiment; one sensitive and one desensitized. Several explants from the spleen of each animal were made in tuberculin and in glycerine broth. It was necessary to include glycerine broth control explants for each animal because of the great variation in potential cell migration in explants of different animals. Both animals were killed at the same time and explants from the sensitive and from the desensitized animal were made alternately so as to insure uniform treatment. The time which elapsed between the killing of the animals and the beginning of the incubation of the explants was approximately 1 hour. The averages of the mean radii of the zones of cell migration in the explants containing tuberculin and in the controls containing glycerine broth were determined. The extent of the cytotoxic action of tuberculin on the explants from each animal was determined by calculating the percent alteration in cell migration using the values of the explants in glycerine broth as 100%.

Results. The results of the principal experiments are presented in Table I. They show that the cytotoxic effect of tuberculin, although not absent, is uniformly and significantly less in the explants from desensitized animals than in those from sensitive animals. Additional experiments, not included in the table because of minor variations in technic, all showed a similar result.

In order to determine whether the results with explants from the desensitized animals were due to specific desensitization 2 control

[7] Jadassohn, W., and Martenstein, H., *Klin. Wochenschr.*, 1919, **26**, 1210.

[8] Pinner, M., *Am. Rev. Tuberc.*, 1931, **23**, 175.

† We are indebted to the Cutter Laboratories of Berkeley, Calif., who kindly supplied us with the Old Tuberculin used in these experiments.

[9] Rich, A. R., and Lewis, M. R., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 115.

TABLE I.
Comparison of Effect of Tuberculin on Migration of Cells in Splenic Explants from Sensitive and Desensitized guinea pigs infected with the B.C.G. strain of *M. tuberculosis*.

Exp. No.	No. of explants				Conc. of O.T. or gl.	Avg of the mean radii of the zones of cell migration in mm				% alteration of cell migration	
	d + O.T.	s+O.T.	d+gl.	s+gl.		d+O.T.	s+O.T.	d+gl.	s+gl.	d+O.T.	s+O.T.
I.	4	4	2	2	1:80	1.20	0.37	1.38	0.80	—14	—54
II.	4	3	2	2	1:50	0.85	1.02	0.92	1.84	— 8	—45
III.	4	4	4	4	1:40	0.36	0.23	0.51	0.50	—30	—52
IV.	4	4	4	4	1:50	0.86	0.73	0.78	1.18	+10	—39
V.	4	4	4	4	1:50	0.89	0.48	1.01	0.83	—12	—43

The meanings of the abbreviations used in the various tables are as follows: d = specifically desensitized; "d" = non-specifically desensitized; gl = glycerin broth; s = sensitive; O.T. = Old Tuberculin.

TABLE II.
Effect of Tuberculin on Migration of Cells in Splenic Explants from Guinea Pigs Infected with the B.C.G. Strain of *M. tuberculosis* and Given Desensitization Treatment with "Smega" Tuberculin.

Exp. No.	No. of explants		Conc.of O.T. or gl.	Avg of the mean radii of the zones of cell migration in mm		% alteration of cell migration
	"d"+O.T.	"d"+gl.		"d"+O.T.	"d"+gl.	
I.	10	6	1:50	0.60	1.26	—53
II.	4	4	1:50	0.49	1.20	—58

See Table I for definitions of abbreviations.

TABLE III.
Comparison of Effect of Tuberculin on Migration of Cells in Splenic Explants from Sensitized and Desensitized Guinea Pigs Infected with *M. tuberculosis* H37 Rv.

Exp. No.	No. of explants				Conc. of O.T. or gl.	Avg of the mean radii of the zones of cell migration in mm				% alteration of cell migration	
	d + O.T.	s+O.T.	d+gl.	s+gl.		d+O.T.	s+O.T.	d+gl.	s+gl.	d+O.T.	s+O.T.
I.	4	4	4	4	1:50	0.46	0.23	0.53	0.46	—14	—50
II.	4	4	4	3	1:50	0.57	0.46	0.81	0.75	—29	—39
III.	6	4	4	4	1:50	0.16	0.23	0.35	0.92	—55	—75

See Table I for definitions of abbreviations.

experiments were conducted with explants from animals given desensitization treatment with tuberculin prepared from *Mycobacterium smegmatis*. The results of these experiments are presented in Table II. They show that the depression of cell migration by O.T. in explants from these animals was of the same order of magnitude as the depression shown by explants of untreated sensitive animals thus indicating that the results with the tissues of animals desensitized with O.T. are due to specific desensitization.

Several experiments were also conducted in which explants from guinea pigs infected with *Mycobacterium tuberculosis* H37 Rv were employed. Considerable difficulty was experienced in securing uniformity in the explants because of the variable and often extensive splenic lesions in the animals. In addition the rapid desensitization treatment employed resulted in death of many of the animals. The results of 3 of these experiments are shown in Table III. They are similar to the results obtained with B.C.G. sensitized animals as shown in Table I.

It is to be noted that the tissue culture method employed in the various experiments only permitted observations on the initial migration of cells from the explants. The cellular elements participating in this migration were polymorphonuclear leucocytes, lymphocytes and to a lesser degree monocytes. However, the inactivity of monocytes is not significant since the high concentration of

tuberculin employed exerted a marked toxicity on the monocytes of all preparations including explants from normal animals. This confirms the observations of Shwartzman.[10]

Discussion. It is a reasonable assumption that if the *in vitro* cytotoxic effect of tuberculin on the tissues of sensitive animals is the outcome of the interaction of a sessile antibody with specific antigen, specific desensitization should accomplish saturation of antibody and consequent abolition of this cytotoxic sensitivity.

The fact that the desensitization treatment used in the present experiments did not completely abolish *in vitro* cytotoxic sensitivity does not necessarily militate against the theory that this sensitivity is dependent upon antibody. One possibility is that desensitization of the animals was not complete. It would be of interest to determine whether more complete desensitization of the animals can be accomplished and also whether desensitization of tissues could be accomplished *in vitro* by exposure to a series of increasing concentrations of tuberculin.

It does not seem likely that a blocking antibody was responsible for the results obtained in the present experiments in view of the speed with which a refractory state developed in the tissues of the animals given desensitization treatment. Above all, the specificity of the effect of desensitization makes it quite certain that antibody is responsible for the *in vitro* tuberculin sensitivity of tissue explants of tuberculin-sensitive animals.

Summary. Experiments are presented which show that the *in vitro* effects of tuberculin in suppressing the initial migration of leucocytes from splenic explants of tuberculous guinea pigs is markedly reduced by specific desensitization.

This finding indicates that the cytotoxic effect of tuberculin on tissue explants of tuberculin-sensitive animals is the result of the interaction of a sessile antibody with specific antigen.

[10] Shwartzman, G., *Arch. Path.*, 1928, **6**, 773, 790.

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Purification of Mouse Poliomyelitis Virus by Methanol Precipitation at Low Temperatures.*

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Purification of viruses, which is essentially the separation of viral protein from non-viral protein, has been the object of a large number of investigations. In an extensive review Beard[1] has critically examined the present status of the purification of animal viruses which principally involve ultracentrifugal procedures. Cox, Van der Scheer, Aiston, and Bohnel[2] were the first to report the purification and concentration of influenza and other viruses by using organic solvents to precipitate the proteins from solutions. Recently Gollan[3] has adapted the procedures of Cox *et al.*[2] to the purification of a mouse strain of MM poliomyelitis virus from mouse brain.

This investigation concerns the purification of Theiler's GDVII strain of mouse encephalomyelitis virus by methanol precipitation, and the comparison of methanol precipitates from infected mouse brain with those obtained from

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[1] Beard, J. W., *J. Immunol.*, 1948, **58**, 49.

[2] Cox, H. R., Van der Scheer, J., Aiston, S., and Bohnel, E., *J. Immunol.*, 1947, **56**, 149.