A METHOD FOR STUDYING DELAYED HYPERSENSITIVITY IN VITRO

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A METHOD FOR STUDYING DELAYED HYPERSENSITIVITY IN VITRO

by

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INTRODUCTION

There is a need for a simple and reliable *in vitro* test for delayed hypersensitivity. The first part of the present study was devoted to an investigation of the usefulness of the presently available *in vitro* test which involves the migration of peritoneal exudate cells in capillary tubes. Results of this investigation are presented in the appendix. In the later part of this research project an alternate *in vitro* test for delayed hypersensitivity was developed. The manuscript describing this method, along with a review of the pertinent literature are included in this thesis.

LITERATURE REVIEW

Delayed or tuberculin type sensitivity differs from immediate sensitivity in that there is a delay of 16-48 hours before the appearance of local reaction after contact with the specific antigen, whereas immediate type sensitivity reactions are visible within 5 minutes to 4 hours after contact with an antigen. Immediate sensitivity is antibody associated and can be passively transferred in serum; delayed hypersensitivity has so far been passively transferred only by living lymphoid cells. *In vitro*, mononuclear cells from animals with delayed hypersensitivity upon contact with specific antigen undergo morphologic and functional changes, viz. vacuolation of macrophages (1), a decrease in phagocytosis (2), differentiation of small mononuclear cells into macrophages (3), blast formation (4) and inhibition of macrophage migration (5).

Several *in vivo* and *in vitro* methods are used to detect or study delayed hypersensitivity. The former include an intradermal test or corneal injection. The intradermal test is most commonly used to detect the tuberculin type delayed hypersensitivity. While it is still the best test available for this purpose, it nevertheless has many limitations. For example, *in vitro* sensitivity has been demonstrated even after the loss of dermal sensitivity in animals dying of tuberculosis or suffering intercurrent infections (6). In another situation, infant guinea pigs which did not react to skin test showed specific *in vitro* cytotoxic effects (7). Lurie et al. (8) demonstrated in vitro cellular sensitivity

in the absence of skin sensitivity in estrogen treated animals. Even in cases where animals develop slow dermal sensitivity or remain negative to skin test, in vitro cytotoxicity can be demonstrated regularly (9). As a matter of fact as early as 1936, Moen (10) stated that in vitro macrophage reactivity was more reliable than intradermal test in detection of delayed hypersensitivity.

Development of a suitable in vitro model for delayed hypersensitivity is also important from the point of techniques. Delayed hypersensitivity underlies similar if not identical reactions involved in several diseases, transplantation immunity, and autoimmune states. Not only the knowledge of this phenomenon at the molecular level is lacking, but the role of various types of cells and antibodies is very uncertain (11). The lack of a suitable in vitro system has been one of the main reasons for the slow progress in this area. In short, it is important from the points of view of both basic and applied research that a satisfactory in vitro model for delayed hypersensitivity be developed.

One of the earliest in vitro studies on delayed hypersensitivity was done by Holst in 1922. He noted an inhibition of phagocytosis amond leukocytes from tuberculous patients in the presence of tuberculin (2). Renewed interest in this area was initiated by the work of Rich and Lewis in 1928-1932 (5, 12). They cultured explants of buffy coat and bone marrow from guinea pigs in autologous plasma which coagulated and provided a semi-solid medium in which the cells migrated and grew.

They demonstrated that addition of tuberculin to the explants from tuberculous guinea pigs caused increased destruction of cells and inhibited their growth and migration. Tuberculin had no effect on the explants from normal animals. These results were confirmed and extended by Aronson who reported that these cytotoxic effects were not noticeable in the case of immediate hypersensitivity (13). The explants from guinea pigs with immediate sensitivity to horse serum did not react to horse serum *in vitro*.

In 1936 Moen and Swift (14) introduced the term cytotoxic index to express the extent of cytotoxic effect. The value for cytotoxic index was obtained by dividing the area of growth of the explants in the test cultures containing tuberculin by the area of growth of the explants in the control cultures.

The specificity of the *in vitro* cytotoxicity was confirmed by many workers (15-20). *In vitro* cytotoxic effects in guinea pigs with delayed hypersensitivity to tuberculin, ovalbumin, and diptheria toxoid were elicited only by the specific antigen (19). Cells from guinea pigs sensitized to dinitrophenylated proteins were affected only by the immunizing conjugate (20).

Spleen as well as several tissue explants were explored for *in vitro* cytotoxic effects. Carpenter (21) demonstrated *in vitro* cytotoxicity in the explants of lungs and lymph nodes. Several workers explored in great detail the *in vitro* reactivity of the epithelial cells to antigen. However, the results were not uniform. Packalen, et al.

(23) found specific inhibition of epithelial growth from kidney and liver. But Jacoby and Marcks (24) reported no cytotoxic effects in the same tissues. Negative results were also reported on skin (25) and corneal epithelium (26). In vitro studies were also carried on in aggressor (target cell) cultures. Isoimmune mouse peritoneal macrophages were found to cause cytolysis of target cells in monolayer cultures (27).

In 1947 Favour (28) introduced an in vitro cytotoxic method based on the sharp decrease in the number of blood leukocytes which occurred on incubation with specific antigen. His initial observations were confirmed by many workers (29-32), but it was soon noted that similar results were obtained with various states of immediate hypersensitivity (33, 34).

Although the in vitro cytotoxicity of hypersensitive tissues in vitro cytotoxicity of hypersensitive tissues in the presence of specific antigen was firmly established, none of the several in vitro systems studied was consistent and reliable enough to be adapted as a routine model. Kapral and Steinbring using cells from sensitive animals (1) noted several morphologic changes in the peritoneal monocytes due to tuberculin. The changes included vacuolation of macrophages, loss of phagocytic ability and cell detachment. Fabrizio (35) noted a decrease in the migration from plaques of peritoneal exudate cells.

An imporved in vitro test for delayed hypersensitivity was developed by George and Vaughan (36) in 1962. They used peritoneal exudate cells from guinea pigs with hypersensitivity to tuberculin. The washed cells

were packed into capillary tubes which were placed in Mackaness chambers at 37°C. The cells migrated out of the capillary tubes in a fan-like fashion onto the surface of glass cover slips. In the presence of tuberculin their migration was markedly inhibited. The cells from normal animals migrated in the absence and presence of antigen. This procedure was further refined by David and coworkers (19, 20, 37, 38). They established that the inhibition of migration was the property of the cells and was not affected by circulating antibodies. They further found that the presence of a small number of sensitive cells (2.5%) in a population of normal cells would result in the inhibition of migration of the mixed population. A rough correlation was observed between the *in vitro* inhibition of migration and the size of the skin reaction.

The capillary method was used to great advantage by Bloom and Bennet (39). They used partially purified macrophages and lymphocytes from tuberculous animals and found that sensitive lymphocytes were necessary for inhibition of macrophage migration. They demonstrated that as few as 2% sensitive lymphocytes when mixed with normal exudate cells or purified macrophages, could induce inhibition of migration in the presence of antigen. Furthermore, they obtained an "active" cell-free supernatant from sensitive lymphocytes which could sensitize normal macrophages. They suggested that lymphocytes are active cells in delayed hypersensitivity and upon contact with the antigen, they release a soluble factor--the migration inhibition factor (MIF) which sensitized macrophages which are merely indicator cells. This hypothesis is open to question, since

in a recent report (40), both the serum and heat-eluates of macrophages from sensitized guinea pigs conferred sensitivity to migration-inhibition upon macrophages from normal guinea pigs.

M A N U S C R I P T

A SIMPLE IN VITRO TEST FOR DELAYED HYPERSENSITIVITY BY SPECIFIC INHIBITION OF MACROPHAGE MIGRATION

S. K. Quadri

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Introduction

In 1932 Rich and Lewis (1) reported that the migration of cells from splenic and lymph node explants of tuberculous guinea pigs was inhibited in the presence of tuberculin. In 1962 George and Vaughan (2) used the relative distance of migration of peritoneal exudate cells out of capillary tubes onto a glass surface in the presence and absence of antigen. This method has been used, with slight modifications, by David et al. (3) and others (4). Because the capillary technique requires a considerable amount of leucocytes, time and manipulations, we use a circular deposit of peritoneal exudate cells on a glass cover slip. The relative distances of radial migration, after attachment and incubation in the absence and presence of an antigen, are measured with a low-power microscope.

Materials and Methods

Cells: Three month old guinea pigs were sensitized by intraperitoneal (IP) injection of 5 mg (wet weight) of viable, attenuated M. bovis (BCG). An additional 1 mg of BCG was injected 8-10 weeks later. After 2-8 months, peritoneal exudate was induced by an IP injection of 6-10 ml of sterile light mineral oil. Four to 6 days later, 100 ml of Hank's balanced salt solution (BSS) were injected IP. The peritoneal fluid was collected and centrifuged at 4C for 10 minutes at 160 xG. The sedimented cells were washed twice with BSS and centrifuged for 5 minutes at 90 xG. The final cell sediment was suspended in 4-5 ml of Medium 199 (M199) containing 15% normal guinea pig serum (M199--15% NGPS). The cell concentrations in the suspensions ranged from $9\text{-}16 \times 10^3$ cells/ml.

Antigens: Tuberculin sensitivity was tested with purified protein derivative (PPD, Parke-Davis, Detroit, Michigan). Coccidiodin (Cutter Laboratories, Berkely 10, California), brucellergen (Merck Sharp and Dohme, West Point, Pennsylvania) and histoplasmin (Michigan Department of Health) were used at the concentrations prescribed for skin tests.

Migration Studies: Sykes-Moore chambers were autoclaved with the bottom cover slip and rubber ring in position. The cell suspension was drawn into a Pasteur pipette and five droplets were placed on the bottom cover slip, four around the periphery and one in the center. Each droplet was approximately 1.5 mm in diameter. The chambers were covered and incubated at 37C for 15-20 minutes. The cover slip was then floated, droplet side

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Results

The radial migration of macrophages from peritoneal exudates of tuberculin sensitive and normal guinea pigs was quantitatively measurable, Figure 1. The migration of cells from tuberculin sensitive guinea pigs was markedly inhibited by 25 ug PPD/ml. The mean percentage inhibition of migration (PIM) from 10 animals was 61.2 $\pm$ 12.5 (MEAN + Standard deviation). Cells from normal guinea pigs were not affected, with the PIM from 10 animals measured as 0.6 $\pm$ 2.1.

The inhibition was detectable at 1 hr and persisted beyond 48 hr. The mean PIM for 6 animals at 8 hr, 24 hr, and 48 hr was 47.9 $\pm$ 7.1, 57.9 $\pm$ 13.7 and 52.9 $\pm$ 11.7, respectively.

The inhibition was specific. A skin test dose of PPD (second strength) markedly inhibited the migration (mean PIM for 4 animals was 44.1 $\pm$ 4.7), but skin test doses of histoplasmin, brucellergen and coccidiodin did not (the mean PIM for 4 animals was 5.2 $\pm$ 2.6, 0.8 $\pm$ 1.4, and 3.4 $\pm$ 1.9, respectively).

Variation in the concentration of cell suspensions affected the percentage of migration but not the percentage of inhibition (Table 1). Differences in the percentage of migration for various cell concentrations in the absence of antigen corresponded to those in the presence of antigen, so that the percentage of inhibition was not affected appreciably.

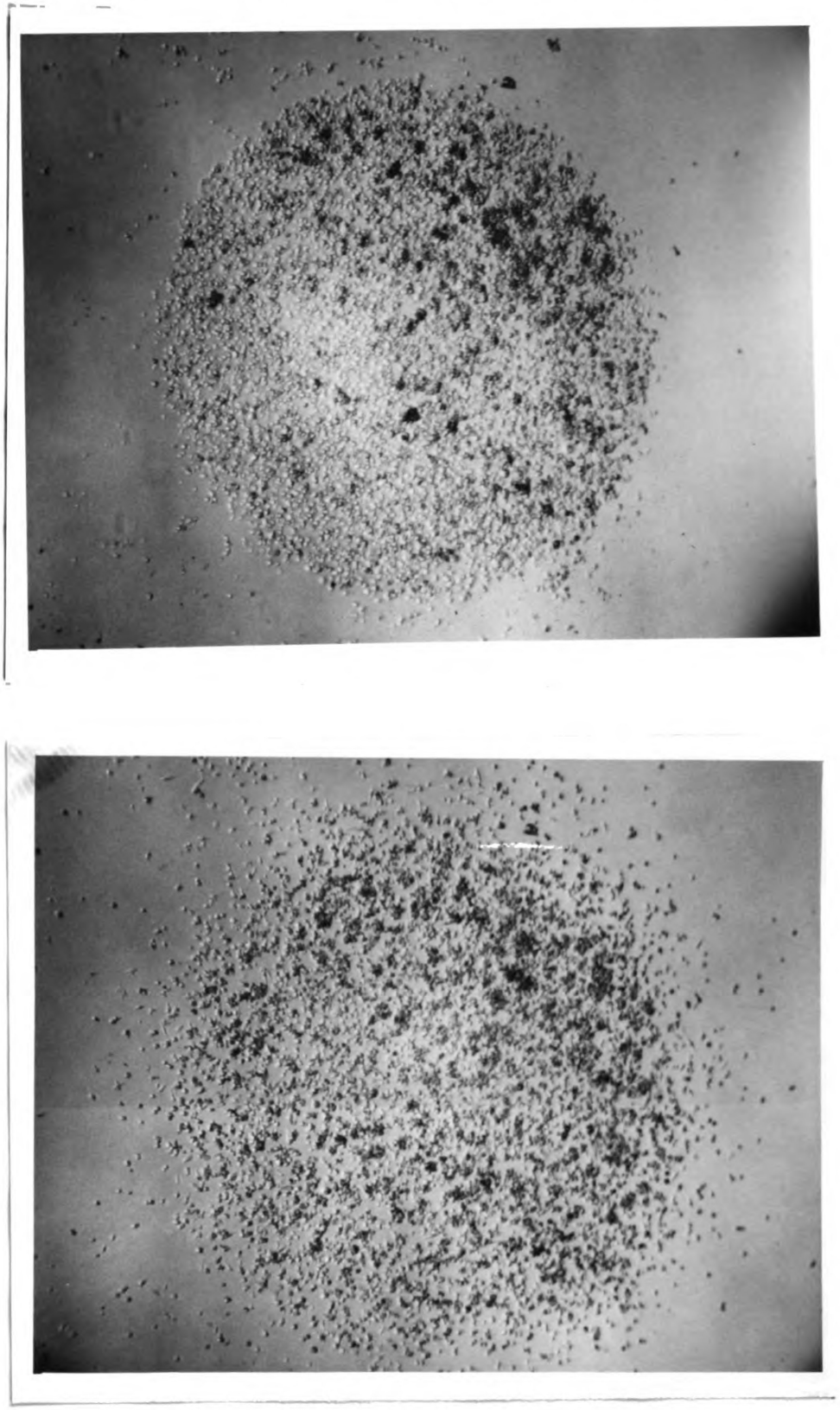
The extent of inhibition was influenced by the concentration of antigen. The mean PIM with 10 animals for 6.25 ug, 12.5 ug, 25 ug and 50 ug PPD/ml was 27.4 $\pm$ 12.8, 45.6 $\pm$ 16.2, 56.5 $\pm$ 13.0 and 64.4 $\pm$ 12.1 respectively. The lowest concentration of PPD tested and found to inhibit migration was 0.062 ug/ml.

Table 1. Effect of concentration of cells of peritoneal exudate cell suspensions on the inhibition of migration of macrophages from tuberculin-sensitive guinea pigs in the presence of 25 ug PPD/ml.

CELL SUSPENSIONS	% INHIBITION OF MIGRATION		TUBERCULIN SENSITIVE GUINEA PIGS							
			1	2	3	4	5	6	7	8
Initial cell suspension*	% Migration	M199	42.9	55.6	46.8	61.7	41.3	36.6	10.3	51.0
		PPD	9	18.1	12.0	17.9	12.5	11.9	3.9	20.1
	% Inhibition of Migration		79.8	67.5	74.4	71.0	69.8	67.5	62.2	60.6
1:2 Diln. of initial cell suspension	% Migration	M199	16.9	58.5	48.4	63.9	39.5	31.2	30.5	71.5
		PPD	3.5	20	15.2	18.1	10	10.5	12.2	21.8
	% Inhibition of Migration		79.3	65.1	68.6	71.7	74.7	66.4	60.0	69.6
1:4 Diln. of initial cell suspension	% Migration	M199	15.4	44.9	95.6	39.1	29.8	21.1	15.4	50.1
		PPD	3.3	15.7	12.8	11.9	7.7	5.9	5.1	15.8
	% Inhibition of Migration		78.6	65.1	72.0	69.6	74.2	71.7	64.9	68.5
MEAN PERCENT INHIBITION OF MIGRATION			79.2±0.6	65.9±1.3	71.6±2.9	70.7±1.0	72.9±2.6	68.5±2.7	62.3±2.4	66.2±4.9

*Cell concentration in initial suspension was 8.8×10^3 - 16×10^3/Cu mm

Figure 1. Radial migration of macrophages. A migration spot before incubation (upper) and after incubation (lower).



Discussion

These experiments have demonstrated that radial migration of macrophages from peritoneal exudates of tuberculin sensitive guinea pigs was specifically inhibited by PPD. This method for demonstrating inhibition of migration furnishes a simple means for studying factors associated with delayed hypersensitivity and has a number of economic and technical advantages over earlier methods. The inhibition was rapid and effectively accomplished by a range of tuberculin concentrations. Sensitivity was demonstrated by the inhibition of migration by as low as 0.062 ug of PPD/ml. Approximately 30-40 chambers can be made from 1 ml of peritoneal cell suspensions containg 1×10^4 cells/ml. Erythrocytes often constitute an undersirable contaminant in peritoneal exudate (3), but are easily eliminated in the present test. The test was not subject to error due to variations in the concentrations of cell suspensions.

Lymphocytes are often considered to act as mediators of delayed hypersensitivity (5). The presence of 1%-6% lymphocytes could have caused the inhibition of migration of macrophages through production of a presumed migration inhibition factor, MIF (4). However, the early inhibition (at 1 hr) would also favor the possibility of macrophages being sensitive cells after conversion from lymphocytes or from cytophilic antibody (6). Recently both cytophilic antibody and MIF have been implicated in the inhibition of migration of macrophages (7).

Altogether, the present test is believed to be relatively simple to

use, permits quantitation of migration and cell types, has a high degree of accuracy, and should lend itself readily to various manipulations for further study of delayed hypersensitivity *in vitro*.

Summary

A rapid and reproducible test was developed for detection of delayed hypersensitivity *in vitro*. The basis of test was the inhibition of radial migration of sensitive peritoneal macrophages on a glass cover slip in the presence of sensitizing antigen.

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A P P E N D I X

APPENDIX

The appendix consists of three reports submitted to the Animal Health Division, ADE, USDA. The third report describes the successful use of capillary test and confirmation of the specific inhibition of migration of peritoneal exudate cells from tuberculin-sensitive guinea pigs in the presence of PPD. In the next stage attempts were made to determine the effects, if any, of the skin test on the migration of normal and sensitive peritoneal exudate cells. The results of this and a duplicate experiment are contained in the fourth and fifth reports. The fourth report also contains procedures for isolation of migration inhibition factor and passive sensitization of normal cells.

After the extensive use of capillary test in the above experiments, it was concluded that there was need to develop a more reliable and accurate <u>in vitro</u> test for delayed hypersensitivity. The remainder of the research period was addressed to this problem and resulted in the development of the spot migration test described in the manuscript.

SUMMARY FROM THE THIRD REPORT

Capillary method of migration of peritoneal exudate cells:

Animals: Twenty four guinea pigs of mixed sexes were taken at 3 months of age from our colony. They were not tuberculin tested prior to use. The colony has been a closed colony for seven years and has remained tuberculin-negative.

Sensitization of guinea pigs: Twelve of the guinea pigs were inoculated intraperitoneally with 5 mg wet weight of viable M. bovis (BCG).

Collection and preparation of peritoneal exudate cells: Twenty ml of sterile light mineral oil per guinea pig were injected intraperitoneally. Three to five days later, 100 ml sterile Hanks Balanced Salt Solution without heparin or antibiotics (BSS) were injected intraperitoneally. The abdomen was kneaded gently and the fluid collected aseptically. The fluid was centrifuged for 10 minutes at 1200 rpm at 4 C and the supernatant fluid discarded. The cells were washed and centrifuged twice with BSS. The final sediment of cells was mixed with Medium 199-15% normal guinea pig werum to yield 10-20% cells by volume. The cell suspension was drawn into sterile capillary tubes. The tubes were sealed at one end and centrifuged for 5 min at 900 rpm. The capillary tubes were cut at the cell-fluid interface. Two or three tubes were placed in each Sykes-Moore chamber.

Migration-inhibition test: Four or more chambers containing two or more tubes of cells were prepared for each animal each time it was tested. One ml of M199-15% normal guinea pig serum was added to each of two chambers and one ml of M199-15% normal guinea pig serum containing 16 ug protein of PPD-S added to the other two. After 20 hours incubation at 37 C, the tubes and cells in the chambers were observed under the microscope. The distance of the migration of the cells linearly from the tip of the capillary tube was measured by an ocular micrometer. The distance was recorded in microns.

Results: The cells from infected guinea pigs migrated in the absence of PPD-S; their migration was inhibited by PPD-S (Table 2). The cells from non-infected guinea pigs migrated in the presence or absence of PPD (Table 3). The distance of migration for each tube in each guinea pig is given. The average for each animal is given. While variations occur, the real difference of the cells from sensitive guinea pigs plus PPD-S is readily apparent. The range of linear migration for each group was as follows:

Uninfected, no PPD: 510 to 1755 u

Uninfected, with PPD: 435 to 2040 u

Infected, no PPD: 510 to 2040 u

Infected, with PPD: 0 to 255 u

Effect of skin test on the in vitro migration of peritoneal exudate cells:

Following the above tests, six of the infected guinea pigs and six non-infected guinea pigs were tuberculin tested with 0.10 ug/0.1 ml/guinea

pig intradermally. A delayed skin reaction from 10 mm to 16 mm developed in the six infected guinea pigs. There was no reaction in the non-infected guinea pigs.

Two days after the tuberculin tests, migration-inhibition tests were begun to determine the effect of tuberculin tests on the results of migration-inhibition test. Four animals were tested daily: one infected, tuberculin tested; one infected, not tuberculin tested; one non-infected, tuberculin tested; one non-infected, not tuberculin tested. When the complete set of animals have been tested (3rd through the 10th day post-tuberculin), the tests were repeated (11th through 18th day post-tuberculin.)

Table2 . Linear migration in microns of mononuclear cells from guinea pigs infected with Mycobacterium bovis (BCG) in the presence or absence of PPD-S in vitro.

INFECTED GUINEA PIGS

	WITHOUT PPD					WITH PPD				
	Chamber # 1		Chamber # 2			Chamber # 1		Chamber # 2		
Animal #	Capillary # 1	# 2	# 1	# 2	AVERAGE	Capillary # 1	# 2	# 1	# 2	AVERAGE
1	1190	680	850	1020	935	85	0	51	0	34
2	680	1105	1020	935	935	119	119	85	39	91
3	510	510	510	850	595	68	0	102	0	43
4	1196	850	2040	1360	1362	255	170	34	0	115
5	2040	1360	680	510	1148	136	51	68	68	81
6	1360	1190	680	850	1020	0	0	85	34	30
7	680	850	1326	1360	1054	0	0	0	51	13
8	1870	1530	1955	1700	1764	0	17	0	34	13
9	1530	680	1190	1360	1190	170	34	34	34	68
10	544	765	1700	1530	1135	0	0	51	34	21
11	1360	646	1360	1360	1182	102	68	51	136	89
12	1105	680	1700	884	1092	0	85	0	0	21
AVERAGE	1172	904	1251	1143	1118	73	45	47	36	52
RANGE	510 - 2040					0 - 255				

Table 3. Linear migration in microns of mononuclear cells from normal guinea pigs in the presence or absence of PPD-S *in vitro*.

NORMAL GUINEA PIGS

	WITHOUT PPD					WITH PPD				
	Chamber # 1		Chamber # 2			Chamber # 1		Chamber # 2		
Animal #	Capillary # 1	# 2	# 1	# 2	AVERAGE	Capillary # 1	# 2	# 1	# 2	AVERAGE
1	1190	1190	1360	1700	1360	1360	1190	1615	1275	1360
2	1360	1435	1020	705	1130	1190	850	680	850	893
3	1360	1190	1156	918	1156	1020	850	1020	510	850
4	1190	1530	1190	1190	1275	1190	1360	850	1020	1080
5	1360	680	612	1156	952	1530	1190	1020	1190	1233
6	595	510	1020	1190	829	850	510	2040	680	1020
7	1020	1275	1360	765	1105	1360	1190	1530	1020	1275
8	935	1105	1020	1300	1090	765	1020	1360	1190	1084
9	850	935	1139	1190	1029	1139	850	1360	680	1007
10	1360	1755	680	1105	1225	1360	1020	510	1020	973
11	680	1445	1020	850	999	1105	1020	510	1020	914
12	1360	1190	1150	1020	1180	1530	435	1190	2040	1299
AVERAGE	1105	1187	1061	1091	1111	1200	957	1140	1041	1083
RANGE	510 - 1755					435 - 2040				

SUMMARY FROM THE FOURTH REPORT

Passive sensitization of mononuclear cells in vitro:

When lymphocytes from sensitive animals are treated with tuberculin, the supernatant fluid passively sensitizes monocytes from normal animals. Lymphocytes from normal animals or monocytes from normal or sensitive animals do not possess the passive sensitizing factor. We will attempt to isolate the factor. We hope from this study to not only gain an understanding of the mechanism of the phenomenon of delayed hypersensitivity but also to lead to the development of an *in vitro* test employing normal mononuclear cells or some primary or secondary cell cultures as index cells.

In the passive sensitization *in vitro* study, peritoneal exudate cells were obtained from guinea pigs as described in the Third Semi-Annual Report. The cells were washed twice with Hank's balanced salt solution and then suspended in Medium 199 containing 15% normal guinea pig serum. The cells were then incubated for 2 hours at 37°C in a glass petri dish. During this period the macrophages attached to the glass surface whereas the lymphocytes remained non-adherent and were removed with the fluid after gently shaking the plates. This cell suspension was carried through the above procedure for 3 more times. During this time, polymorphonuclear cells are generally lysed, monocytes remain attached to the glass and therefore, the cells remaining in the fluid were primarily lymphocytes. In addition, fresh medium was added to the plates to which

the monocytes attached and were incubated 2 hours. The fluid was removed after gently shaking the plates and discarded. The cells attached to the glass were released by adding ethylenediaminetetraacetate (1:5000) for 5 minutes. The released cells were monocytes.

In the next step, the possibility of the elaboration by sensitized lymphocytes of a sensitizing factor was tested. The sensitized lymphocytes obtained in the manner described above were cultured in the absence or presence of various quantitites of PPD. After 20-24 hours the cell suspension were centrifuged gently and the cells removed. The cell-free supernatants were further clarified by centrifugation at 250 r.p.m. for 15-20 minutes. The peritonea cells or macrophages obtained from normal donors were suspended and centrifuged in capillary tubes, placed in chambers which were filled with M199 with serum and various quantitites of the supernatants.

Thus far, the results indicate that supernatants from sensitized lymphocytes cause inhibition of normal macrophages or normal peritoneal exudate cells in the presence of PPD whereas supernatants from normal lymphocytes fail to produce the same results.

Data on the effect of skin test on the <u>in vitro</u> migration of peritoneal exudate cells is presented in Table 4. However, a duplicate experiment is also in progress.

4. Linear Migration of Peritoneal Monocytes *in vitro* from Normal Guinea Pigs and Tuberculin Sensitive Guinea Pigs after Skin-Testing.

ER NAL IN.	BCG INFECTED GUINEA PIGS				NON-INFECTED GUINEA PIGS			
	Skin Tested		No Skin Test		Skin Tested		No Skin Test	
	no PPD	with PPD	no PPD	with PPD	no PPD	with PPD	no PPD	with PPD
2	1105	340	850	340	1105	1020	1190	1020
	1360	255	1105	204	850	680	1360	952
3	1190	51	1020	102	1190	1190	1020	850
	1530	68	1360	0	1020	1190	850	850
4	1020	510	1870	340	850	1020	1360	1020
	680	425	2040	510	680	680	1020	850
5	850	170	850	170	680	850	952	850
	1105	187	1020	204	850	680	1020	850
6	680	51	1105	170	680	850	1105	1020
	850	102	850	153	1020	1105	935	765
7	680	170	850	340	1190	1020	1105	952
	935	187	1105	102	1105	680	1360	1105
8	1190	255	1207	170	22100	1190	1870	1700
	1020	272	1190	0	22117	1530	1870	1955
9	1530	0	1190	170	1207	1190	1530	1190
	1020	136	1207	255	1190	1020	1207	1020
10	1207	136	1207	170	1190	680	1190	1020
	1020	170	1190	255	1207	850	1020	850
11	1190	170	1207	0	1207	680	1700	1190
	1207	255	1207	255	1190	340	1207	1207
12	1190	0	510	0	850	680	1190	1190
	1190	0	680	0	1105	340	850	85
13	1700	340	---- Died ----		1020	1020	850	1020
	1207	510			1207	850	1190	850

The range of linear migration prior to tuberculin testing:

Uninfected, no PPD *in vitro*:	510 - 1755 u
Uninfected, with PPD *in vitro*:	435 - 2040 u
Infected, no PPD *in vitro*:	510 - 2040 u
Infected, with PPD *in vitro*:	0 - 255 u

SUMMARY FROM THE FIFTH REPORT

Effect of skin test on the in vitro migration:

As reported in the Fourth Semi-Annual Report, an experiment was being performed on more animals to determine the effect of intradermal tuberculin tests on the in vitro migration-inhibition of macrophages from normal and sensitized animals prior to the collection of macrophages.

Twelve BCG infected guinea pigs and 12 non-infected guinea pigs were tuberculin tested with 0.5 ug/.1 ml/guinea pig intradermally. Delayed reactions from 9-15 mm developed at the site of all infected guinea pigs.

The in vitro migration tests were conducted on the tuberculin tested animals 2-6 days after the intradermal tuberculin injection. One animal from each group viz, sensitized non skin tested, sensitized skin tested, non-sensitized-nonskin tested, non-sensitized-skin tested, was tested on these days. The in vitro tests were repeated on these animals in the same order from day 8 to day 13 so that animals tested on day 2 were again tested on day 8, and animals tested on day 3 were tested again on day 9 and so on. The results are presented in the following table.

TABLE 5. Linear migration of peritoneal monocytes *in vitro* from normal and tuberculin-sensitive guinea pigs after skin testing.

'S AFTER 'RADERMAL 3ERCULIN TEST	BCG INFECTED GUINEA PIGS				NON-INFECTED GUINEA PIGS			
	Skin Tested		No Skin Test		Skin Tested		No Skin Test	
	no PPD	with PPD	no PPD	with PPD	no PPD	with PPD	no PPD	with PPD
Day 2	850	221	850	68	1190	1020	1190	1190
	1105	340	1190	51	1275	935	1190	1020
Day 3	1020	170	1190	136	1360	1190	1360	1190
	1360	153	1020	153	1020	935	1530	1326
Day 4	680	0	1530	136	1020	680	1020	1105
	935	170	1495	170	850	935	1190	1360
Day 5	1020	340	1530	255	1190	906	935	1105
	1105	255	1615	170	1020	765	1020	1190
Day 6	765	221	1326	65	1020	595	1020	1360
	850	289	1530	204	1156	850	1190	1445
Day 7	1870	170	1070	323	1190	1105	1020	680
	1700	255	935	306	850	1190	1190	1020
Day 8	1190	340	1530	153	1190	1190	1360	680
	1360	306	1360	136	1190	1020	1105	1020
Day 9	1870	510	850	255	1360	1190	1360	1190
	1530	255	1360	170	1020	1020	1530	1020
Day 10	680	136	680	102	1530	1020	1020	1190
	935	102	1020	170	1020	765	850	850
Day 11	1700	255	1360	102	1360	1360	680	1020
	1360	204	1615	170	1020	1020	265	850
Day 12	765	136	850	340	1190	1190	850	1020
	1020	170	1360	510	1020	1020	1360	1105
Day 13	1190	119	1360	136	1020	1020	1190	1360
	1360	102	1020	85	1360	1156	1020	1530

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