PASSIVE TRANSFER OF COCCIDIOIDIN SENSITIVITY IN GUINEA PIGS WITH A LEUKOCYTE EXTRACT

by

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A Thesis Submitted to the Faculty of the

DEPARTMENT OF MICROBIOLOGY
AND MEDICAL TECHNOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN MICROBIOLOGY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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ABSTRACT

A transfer factor preparation was made from peritoneal exudate and lymph node cells of guinea pigs which had been actively sensitized with heat-killed arthrospores of Coccidioides immitis. These cells were suspended in sterile distilled water, frozen and thawed 10 times and vacuum dialyzed for 24 hours. This preparation was used to passively transfer delayed-type hypersensitivity to coccidioidin to normal guinea pigs.

The leukocyte extract was fractionated on a Sephadex G-25 Fine chromatography column and eluted with a Tris HCl buffer at pH 8.0. Five pooled fractions were distinguished by uv absorbance properties at 280 nm and 254 nm. These fractions were tested for the ability to transfer delayed-type hypersensitivity to coccidioidin to normal guinea pigs.

Transferred sensitivity was evaluated in vivo by skin test reactions to an intradermal injection of coccidioidin. Transferred sensitivity was evaluated in vitro by performing lymphocyte transformation tests on peripheral blood and by macrophage migration inhilition tests on peritoneal exudate cells from transfer factor recipient guinea pigs. The skin test results indicated that two groups of fractions, eluted at $.69V_t$ and $.90V_t$, had the ability to transfer coccidioidin sensitivity. Delayed-type hypersensitivity to coccidioidin could not be demonstrated by the in vitro tests performed on the animals in this study.

CHAPTER 1

INTRODUCTION

Delayed-type hypersensitivity is characteristically described by the slow appearance of erythema and induration in response to an intradermal injection of specific antigen and the passive transfer of sensitivity to a naive individual with sensitized leukocytes and soluble extracts from sensitized leukocytes, "transfer factor". When lymphocytes obtained from persons or animals exhibiting delayed-type hypersensitivity are stimulated in vitro by specific antigen, they produce migration inhibition factor (MIF) and lymphocyte mitogenic factor (1). Migration inhibition factor causes the inhibition of migration of macrophages in vitro (2, 3). Mitogenic factor causes the morphological enlargement of small lymphocytes to larger lymphoblasts in vitro. This enlargement is called lymphocyte transformation and is due to increased DNA synthesis by stimulated cells. The most reproducible technique for measuring the response of lymphocytes to a stimulus is an assay of total tritiated thymidine uptake by a culture as measured by a scintillation counter (4).

All of the above characteristics of delayed-type hypersensitivity can be demonstrated in individuals who have had contact with the causative agent of coccidioidomycosis, Coccidioides immitis. In 1948 C.E. Smith and co-workers developed a skin

test reagent, designated coccidioidin (CDN), which can be used to detect a delayed-type skin response in persons who have had contact with C. immitis (5). As early as 1960 Rapaport et al. (6) reported successful transfer of coccidioidin delayed-type hypersensitivity with DNase-treated leukocyte extracts. As in other transfer experiments in humans, i.e., delayed-type hypersensitivity to tuberculin, streptococcal protein and diptheria toxoid, it was found that the sensitivity of the donor and the dosage of donor material was directly related to the transferred sensitivity in the recipient.

In 1968 Kelley et al. (7) reported that human peripheral lymphocytes in tissue culture could be transformed by the addition of CDN to the medium if the cells were from a CDN sensitive donor. Subsequently this work was expanded (8) to show that leukocytes from individuals who demonstrated a negative response to an intracutaneous injection of CDN did not respond to CDN in vitro as did sensitive cells. In 1969 Zweiman et al. (9) were able to show that lymphocyte transformation by CDN in vitro was much greater in individuals who demonstrated a positive skin test with CDN than in individuals who had a negative or weak skin test reaction. They showed that the degree of transformation in skin test positive individuals was directly related to the antigen does used in the in vitro test and that the transformation was antigen specific.

In 1973 Graybill et al. (10) reported three cases in which human transfer factor from CDN skin test positive individuals was given to patients with progressive coccidioidomycosis. Preceding and following the administration of transfer factor the patients were monitored by skin test reactivity, lymphocyte transformation and macrophage migration inhibition tests. This study did not demonstrate a consistent reconstitution of a cellular immune response to CDN and only two of the three patients showed clinical improvement following administration of the transfer factor. The authors concluded that, 1) more trials would have to be made using transfer factor as a therapeutic agent and, 2) the dissociative results of the various tests of cellular immunity indicate the need for a better understanding of the specific cells, mediators and antigens involved.

In 1965 Sinski (11) reported on the use of Hartley guinea pigs as a model for the delayed-type hypersensitivity response to CDN. He reported that skin reactions to CDN in animals immunized with formalin-killed arthrospored in saline give the same type of cellular infiltrate to an intradermal injection of CDN as animals infected with an aerosol of live arthrospores. Positive skin test reactions to CDN developed in infected and immunized animals in one to two weeks after treatment with killed or live arthrospores and neither infected nor immunized animals produced precipitating or complement fixing antibodies.

In 1967 Sinski and Dalldorf (12) using Hartley guinea pigs immunized with formalin-killed arthrospores in triethanolamine oleate and saline, observed, 1) a delayed reaction to an intradermal injection of CDN occurring at about three weeks after sensitization with arthrospores in adjuvant, 2) a skin reaction to CDN consisting of a neutrophilic infiltrate at three hours and a larger mononuclear cellular infiltrate at 24 and 48 hours after the intradermal injection of CDN, 3) inability to demonstrate serum antibody by agar gel precipitation and systemic anaphylaxis with this mode of sensitization, and 4) ability to transfer CDN sensitivity to naive animals with peritoneal exudate cells (PEC) from immunized animals but not with the serum from those animals.

In 1945 Chase (13) found that tuberculin sensitivity could be transferred to naive guinea pigs with living, intact PEC from tuberculin sensitive guinea pigs. The finding was corroborated in 1947 by Kirchheimer and Weiser (14) and in 1948 by Stavitsky (15). Stavitsky was also able to demonstrate the transfer with serum from tuberculin sensitive animals suggesting that a subcellular factor elaborated by certain types of leukocytes found in PEC and serum may be involved in the transfer phenomenon.

In 1954 Jeter et al. (16) were successful in passively transferring chemical contact sensitivity in guinea pigs with sonicated leukocyte extracts, but corroboration was slow in

appearing. In 1956 Cummings et al.(17) demonstrated the transfer of tuberculin sensitivity in guinea pigs using the sonication method of Jeter (16). In 1964 Kucharsky and Favour (18)reported the transfer of tuberculin sensitivity in guinea pigs with spleen cells and the supernatant wash fluids of those cells. In addition Tsuji et al.(19) reported the transfer of tuberculin sensitivity in rabbits with sucrose gradient fractions of serum. Finally in 1966 Kochan and Bendel (20) were able to transfer tuberculin sensitivity in guinea pigs with a frozen-thawed cellular extract of PEC and spleen cells, a method previously believed to work only in the human system.

During this period there were numerous attempts to elucidate the various cellular and molecular interactions involved in cell mediated immunity using in vitro methods that could be correlated with systemic delayed type hypersensitivities. Oppenheim reported on the response of guinea pig lymphoid cells to antigen in vitro and its relation to delayed-type hypersensitivity. He noted that draining nodes responded in vitro long before the animal exhibited a skin test response. Peripheral blood lymphocytes responded to antigen in vitro later than draining node lymphocytes, but earlier than a skin test response (21, 22). It was also reported that the transformation response was antigen dose dependent and that it demonstrated the same antigen specificity as skin test responses (23).

Oppenheim was not able to report a clearly distinguishable independence between antigen induced lymphocyte transformation in vitro and in vivo antibody production. Mills (4) reported that sensitized lymphocytes from guinea pigs immunized by an intravenous route with egg albumin showed no transformation with the immunizing antigen in culture, yet were able to respond to PHA. Animals immunized with egg albumin in complete Freund's adjuvant injected into the foot pad showed, along with PHA stimulation, significant antigen induced lymphocyte transformation, but no antibody could be detected in the supernatant liquid of these cultures by passive hemagglutination, passive cutaneous anaphylaxis or agar gel double diffusion.

Oppenheim did show some transformation associated with in vivo antibody production by lymphocytes from animals with no skin test reactivity (21). However, in light of previous results indicating that antigen stimulation can precede skin test reactivity and a detectable rise in antibody titer, Oppenheim and Mills concluded that lymphocyte transformation can be used as an indicator of delayed hypersensitivity in actively sensitized animals.

David reported that the inhibition of migration of tuberculin sensitive cells by tuberculin as originally observed by Rich and Lewis (24) in 1932, could be used as a sensitive test for the detection of delayed-type hypersensitivities (2). Previous work had demonstrated that the inhibition of guinea pig PEC

is antigen specific (25) and that the inhibition followed the same specificity as dermal reactions (26). It was then reported that cells from animals which exhibited delayed-type hypersensitivity to an antigen were inhibited from migrating by that antigen whether they were producing antibody or not, and cells from animals producing antibody, but not exhibiting delayed-type hypersensitivity, were not inhibited by the antigen specific for that antibody (25).

In 1969 Spitler and Lawrence (27) reported that the production of MIF by sensitized lymphocytes in response to antigenic stimulation in vitro can be correlated with the degree of lymphocyte stimulation demonstrated by those cells. These reports lend support to the acceptance of the suggestion by Oppenheim that lymphocyte transformation and MIF production should be the defining characteristics of delayed-type hypersensitivity in lieu of the classical dermal reaction.

There have been several tissue culture systems used to demonstrate passive transfer of delayed hypersensitivities. Fireman et al. (28) reported a soluble factor elaborated in vitro by tuberculin sensitive cells that can transfer tuberculin sensitivity in vivo and in the presence of PPD induce increased mitotic activity and morphological alternation of leukocytes in cultures of peripheral blood from tuberculin negative individuals. Valentine and Lawrence (29) also reported the activity of a soluble factor that is released in the presence of antigen by

sensitive cells into tissue culture medium. Nonsensitive cells when exposed to this supernatant liquid undergo transformation as measured by the uptake of tritiated thymidine. Furthermore, nonsensitive cells in the presence of the active supernatant liquid will show an even greater stimulation in the presence of antigen.

Arala-Chaves et al. (30) reported similar findings using a frozen-thawed, sonicated, dialyzed and filtered leukocyte extract from persons demonstrating skin test reactivity to PPD and/or Candida antigen. They found that the leukocyte extract plus antigen does induce lymphocyte transformation when cultured with nonsensitive or weakly sensitized cells, but that the optimal concentration of extract varies from donor to donor. They also reported some nonspecific stimulation with the leukocyte extract in culture without antigen.

In 1969 Paque et al. (31) reported the ability of a frozen-thawed, human leukocyte extract to convert nonsensitive cells to CDN sensitive cells in vitro using migration inhibition of guinea pig PEC to evaluate sensitization. They found that cells from individuals with high skin test reactivity to a specific antigen contain a substance that transfers the ability to release MIF in the presence of that antigen to nonsensitive cells. In addition Thor and Dray (32) reported using an RNA extract of tuberculin and histoplasmin sensitive lymph node cells to induce MIF production in previously nonsensitive cells.

Salaman (33) has demonstrated significant correlation between the inhibition of normal guinea pig PEC in the presence of PPD plus a human, leukocyte dialysate and the tuberculin sensitivity of the donor from which the dialysate was prepared.

It is now well accepted that the phenomena of macrophage migration inhibition and antigen induced lymphocyte transformation are manifested by soluble factors, MIF and mitogenic factor, elaborated by small lymphocytes (1). Since the manifestations of these factors have been accepted as in vitro correlates of delayed-type hypersensitivity, these factors may play an important role in the in vivo delayed-type hypersensitivity. It has been proposed that when transfer factor comes in contact with immunologically naive lymphocytes it converts those lymphocytes to an antigen responsive state. These newly responsive cells are induced to reproduce and subsequent generations of cells maintain the specific antigen responsiveness. Subsequently, these cells will respond to that antigen by the production of various factors including mitogenic factor, migration inhibition factor and transfer factor, as if they were obtained from animals actively sensitized against the antigen.

As discussed earlier, delayed-type hypersensitivity can be transferred to a naive individual with whole leukocytes and a soluble extract of leukocytes, "transfer factor", from a sensitive donor. Successful transfer has historically been indicated by the transfer of dermal reactivity to antigen. Since at least

in the case of lymphocyte transformation, significant stimulation can be demonstrated earlier than a dermal response, it may be that the manifestation of MIF production and mitogenic factor production indicates passive sensitization in the case where there are no dermal manifestations of successful transfer of delayed-type hypersensitivity.

In this study, the passive transfer of coccidioidin sensitivity using a cellular extract of PEC and lymph node cells is reported. An evaluation of a possible method of separation of the activity of the guinea pig transfer factor by column chromatography is given and data concerning the production of MIF and mitogenic factor in passively sensitized animals is discussed. Finally, the degree of correlation of the various determinations and their relevance in predicting skin test responses were analyzed.

CHAPTER 2

MATERIALS AND METHODS

Animals

All animals in this study were Hartley strain albino guinea pigs from the departmental colony. Actively sensitized donors were either male or female weighing 600 to 700 g. Recipients were either male or female weighing 350 to 400 g.

Sensitizing Antigen

Heat killed arthrospores from C. immitis var. Silveira were obtained from Dr. Robert N. Ferebee and Dr. James T. Sinski. Live arthrospores obtained from Dr. James T. Sinski were inoculated onto Sabouraud's dextrose agar in a biological safety cabinet. Cultures were grown and the medium was allowed to dry for the purpose of obtaining maximum arthrospore production. The cultures were subsequently heat killed by autoclaving at 121 C at 15 psi for 45 minutes. The heat killed arthrospores were then scraped off the agar, ground up with a heavy glass rod, weighed and stored under dessication until use.

Skin Test Antigen

Coccidioidin without preservative was obtained from Dr. William T. Northey at Arizona State University, Tempe, Arizona.

A single batch was used for all skin testing and in vitro work in this project. This batch of CDN was standardized against standard "Batch C" CDN prepared and donated by Dr. James T. Sinski (11). By comparing skin reactions to a 0.1 ml intradermal (ID) injection of both coccidioidins on an actively sensitized animal, it was found that the Northey CDN gave smaller skin test reactions than "Batch C" CDN, but could be called approximately equivalent to the Smith standard (Lot 64D2.5, Division of Biological Standards, NIH, Bethesda, Maryland).

Sensitization of Donors

For active sensitization 20 mg of arthrospores were suspended in one ml of Freund's Incomplete Adjuvant. This mixture was emulsified in a VirTis "23" homogenizer (The VirTis Co. Inc., Gardiner, New York) until a stable emulsion was obtained. Donor animals were injected subcutaneously with 1.0 ml of the emulsion in multiple sites in the nuchal region. After four weeks, actively sensitized animals were skin tested with 0.1 ml of undiluted CDN and the skin tests were read at 18 and 24 hours. Only animals observed as having greater than 5 mm^2 erythema were subsequently used as donors (5).

Recovery of Transfer Factor from Sensitized Cells

Donor animals were injected intraabdominally with 20 milliliters of sterile mineral oil, N.F. (American Drug and Chemical Co.). Forty-eight hours later these animals were

exsanguinated and the PEC aspirated and collected in Hanks' balanced salt solution with 20% normal guinea pig serum (SHBSS). The cells were separated from the oil in a 500 ml separatory funnel and washed once with SHBSS.

Cervical, suprascapular and axillary lymph nodes were removed, trimmed of fat and a single cell suspension prepared by mincing the nodes on top of a stainless steel screen (60 mesh) and washing the cells through the screen with SHBSS. The PEC suspension and the node cell suspension were combined and a viable cell count was obtained using the trypan blue exclusion technique. Finally the cells were centrifuged at 500 g at room temperature. The supernatant liquid was removed and the cells were resuspended in a total volume of 20 to 30 ml of sterile distilled water. The final suspension was placed in a polypropylene tube that could withstand the freeze-thaw procedure.

The cells were disrupted by ten freeze-thaw cycles. The tubes containing the cell suspension were rapidly frozen in a dry-ice acetone bath. The suspension was allowed to thaw in a 37 C water bath before being refrozen. This material was clarified by centrifugation at 20,200 g for 30 minutes at 4 C. The supernatant liquid was dialyzed (VWR Dialyzer Tubing Size #8, No. 25225-204) under vacuum for 24 or 48 hours. The dialysate was stored at -70 C and quickly thawed immediately before use.

Material from 1.0×10^9 cells was tested for activity by intraabdominal injection on two consecutive days. Activity was

evaluated by skin testing 48 hours after the second injection.

Column Chromatography

A Sephadex G-25 fine (Pharmacia) column was used to fractionate the vacuum dialyzed cell extract. The K26/70 column (Pharmacia) was prepared by Frank Roinestad in May, 1975, and was repacked once during this project. The column was set up for descending flow, the head pressure was 82 cm and the sample was fed by gravity. The buffer used for washing, loading and eluting was 0.01 M Tris HCl at pH 8.0. The column was operated and stored at room temperature. The bed volume before repacking the column was 340 ml and after repacking was 330 ml. Ten ml samples were used on all column runs and ten ml fractions were collected. An inline spectrophotometer (LKB 8300 Uvicord, LKB Instruments, Inc., Rockville, Maryland) reading at 280 or 254 nm was used to monitor the position of the peaks. The flow rate was held constant at approximately 1.5 ml per minute. The eluted volume comprising each peak as determined by a spectrophotometer reading at 280 nm was stored at -70 C and thawed immediately before use.

Chemical Analysis

The protein concentration of each peak and the vacuum dialyzed material was estimated using the Lowry method (34).

The carbohydrate concentration of the dialyzed material and each peak were estimated by adding two ml of a 0.2% anthrone solution in 95% sulfuric acid to one ml of sample and boiling

for three minutes (35, p. 261). This method detects all saccharides, however, various concentrations of glucose were used for reference.

Ribonucleic acid concentration was estimated using the orcinol method of Meijbaum for pentose. This method can be used on crude material as an index of RNA since few cells contain pentose other than as RNA (35, p. 262). Xylose standard solutions were used for reference.

The concentration in micrograms per milliliter of protein, carbohydrate and RNA multiplied by the total volume of material given to each recipient guinea pig was calculated and reported as the dosage to each recipient.

Activity of Peaks In Vivo

Guinea pigs used to test activity of fractions were given 1.0×10^7 to 5.0×10^8 cell equivalents of material intraabdominally at each injection. After 48 hours these recipients were skin tested with 0.1 ml of undiluted CDN and the reaction was observed at four, 18, 24 and 48 hours. These recipients were repeatedly injected with fractioned material and subsequently skin tested with CDN until a positive skin test reaction was observed or until each recipient had received cell equivalents equal to that required to passively sensitize the same size guinea pig with dialyzed cell extract. For recipients a positive skin test was considered $5mm^2$ of erythema or greater. Control animals were given ten ml of Tris HCl buffer every time

fraction recipients were injected. Control animals were also skin tested with 0.1 ml of CDN every time fraction recipients were tested.

Forty-eight hours after the recipients of the peaks and dialyzed extract had been evaluated by skin testing, each animal was given ten ml of sterile mineral oil intraabdominally. After 72 hours PEC were collected for use in the MIF test and peripheral blood was used in lymphocyte transformation studies.

Macrophage Migration Inhibition Test

The macrophage migration inhibition test was essentially as described by Harrington and Stastny (36). PEC from recipients were collected and washed once in HBSS and once with TCM 199 (GIBCO) containing 100 u of penicillin/ml, 100 micrograms of streptomycin/ml, 0.001 M HEPES buffer and 15% normal guinea pig serum. The pH of this medium was adjusted to 7.3 with 1.0 N NaOH. Packed PEC were resuspended in an equal volume of culture medium and then diluted 1:2 with a 0.4% solution of agarose in culture medium with serum. Droplets of the cell suspension were dispensed into wells of a plastic microtiter plate (Linbro Scientific, IS-FB-96-TC) with a 25 microliter repeating syringe (Parts Nos. 702-N, PB600, Hamilton Co., Reno, Nevada).

After the droplets had set, 0.2 ml of medium or medium plus dilutions of CDN was added to each well and the plate was incubated at 37 C in 100% humidity for 24 hours. The radius of migration of cells from the edge of the droplet was measured on

an inverted microscope equipped with an ocular grid. Migration inhibition was reported as

$$\log \left(\frac{\text{average migration of test cells}}{\text{average migration of control cells}} \times 100 \right)$$

Lymphocyte Transformation

At the same time PEC were collected for MIF tests peripheral blood was collected in 0.1 ml of a 1000/ml solution of sodium heparin. The blood was diluted 1:30 in RPMI 1640 (GIBCO) tissue culture medium with 100 u of penicillin/ml, 100 micrograms of streptomycin/ml and .025 M HEPES buffer. The pH of this medium was adjusted to 7.4 with 1.0 N NaOH. Two ml of this cell suspension were dispensed into plastic culture tubes (Falcon No. 2059) to which 0.1 ml of antigen dilution or 5 micrograms of PHA-P (Difco) had been added. On day four cultures were pulsed with one microcurie of tritiated thymidine (New England Nuclear) in 0.1 ml of tissue culture medium for 24 hours. Cells were harvested on day five in a multiple filter apparatus (Millipore Cat. No. XX2702550, Bedford, Mass.). All cells were recovered on Reeve Angel glass fiber filter pads (No. 934AH, VWR), red cells were lysed with 3% acetic acid and the remaining cells were dried with absolute methanol.

The pads were removed to scintillation vials and allowed to air dry before ten ml of a toluene base scintillation cocktail were added to each vial. The vials were counted for one minute in a Packard TriCarb Liquid Scintillation Spectrometer Model 3320

(Downer's Grove, Illinois). Transformation is reported as the average of the log of replicate test counts minus the average of the log of the control counts.

CHAPTER 3

RESULTS

Figure 1 shows a typical chromatogram of the crude cell dialysate after passing through the Sephadex G-25 Fine column. The void volume (V_0 peak) of this column is 140 ml. As indicated in the figure, the next three peaks are called N_1, N_2 and N_3 respectively because they were more clearly defined by absorbances at 254 nm than at 280 nm. The final peak is called P because it demonstrated a higher absorbance at 280 nm than at 254 nm. Table 1 gives the position of the eluted peaks, the milliliters of eluate that were pooled and subsequently injected into recipient animals.

Thirty-four recipient guinea pigs were used in this project. Raw data on the biochemistry of the material given to each animal, the results of all the MIF tests and the results of all the lymphocyte transformation tests can be found in accompanying Appendices A, B and C.

Table 2 gives the skin reactions of all 34 recipient guinea pigs used in this study. Two of the three preparations from actively sensitized guinea pig leukocytes were active when the crude preparation was tested for transfer activity. The material in the void volume did not demonstrate any transfer

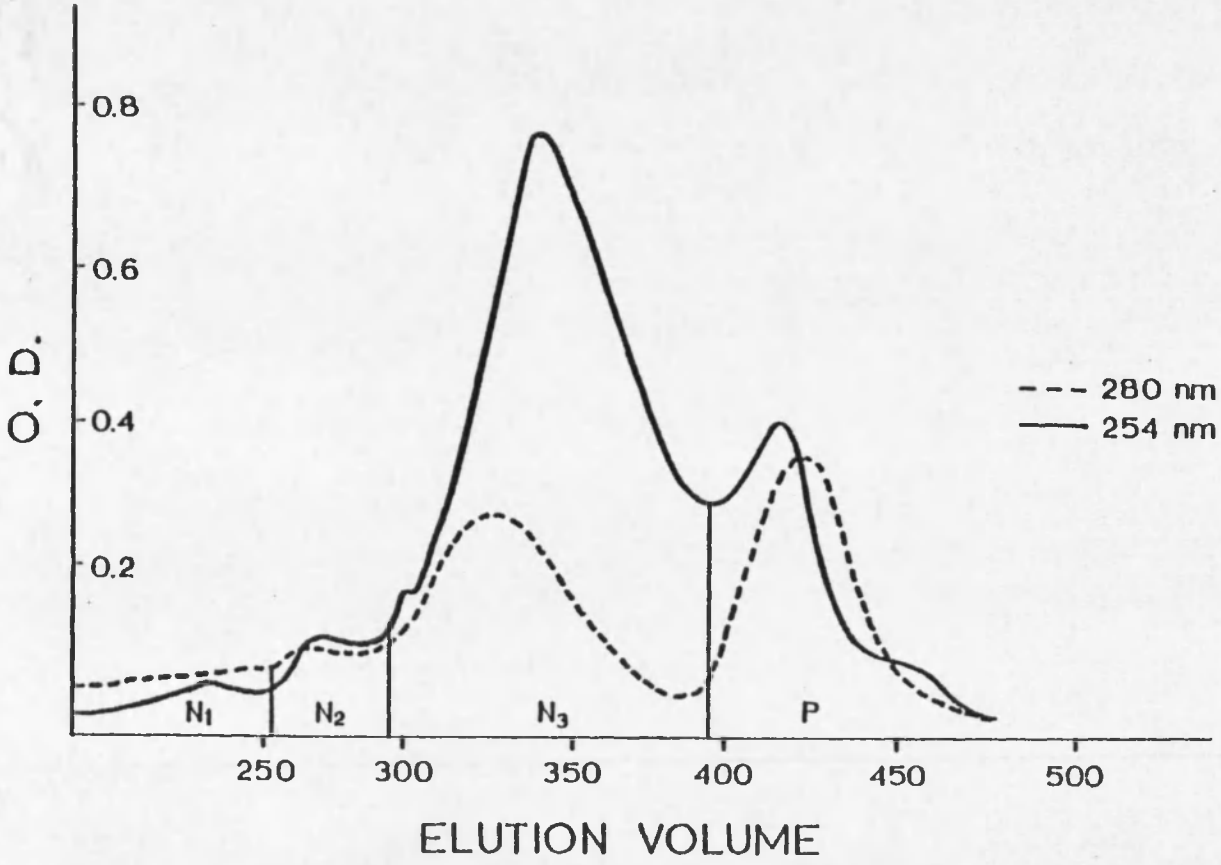

Figure 1. Chromatogram of freeze-thawed, vacuum dialyzed cell extract.

Table 1. Sephadex G-25 Fine column fractions from Coccidioides immitis sensitive transfer factor preparation.[a]

peak	Milliliters eluted	V_e/V_t[b]
V_0	130-170	.38
N_1	180-260	.69
N_2	270-290	.76
N_3	300-390	.90
P	400-500	1.12

[a] 24Å pore size dialysate of actively sensitized leukocyte extract.

[b] $\frac{\text{elution volume of peak}}{\text{total elution volume of column}}$

Table 2. Skin Test reactions[a] of recipient guinea pigs 48 hours after treatment with transfer factor preparation and its fractions.

transfer factor preparation	material injected intraabdominally					
	crude dialysate[b]	V_0[c]	N_1[c]	N_2[c]	N_3[c]	P[c]
	(4)[e]	(5)	(5)	(5)	(5)	(5)
1	6x8	-	5x5	-	5x6	-
1		-	10x8	-	4x4	-
2	5x5	-	4x4	-	6x6	-
2	5x5	-	8x8	-	10x10	-
3	-[d]	-	5x5	-	-	5x5

[a] length and width in mm of erythema 24 hours after intradermal injection of 0.1 ml CDN in a virginal site.

[b] 24A pore size dialysate prepared from actively sensitized leukocytes.

[c] Sephadex G-25 Fine pooled fractions cell dialysate.

[d] - no reaction

[e] number of animals in parentheses.

activity as measured by skin test reactions in recipient guinea pigs. Material in the fractions comprising N_1 was consistent in transferring CDN sensitivity as measured by skin test reaction. In one case the area of erythema was too small to be considered a positive reaction. Material in the N_2 fractions did not transfer any sensitivity that was detectable by a skin test reaction. In three of five guinea pigs N_3 did transfer sensitivity to CDN that could be detected by a skin test reaction. In only one instance, did P material transfer CDN sensitivity as measured by a skin test reaction. There is variation in the sizes of skin test reactions within each group of eluate recipients.

The lymphocyte transformation data are presented in Table 3. This table lists the mean transformation indices for each group of eluate recipients in response to three concentrations of CDN and to 5 micrograms of PHA. No increase in thymidine uptake is demonstrated by cultures from any group of recipients at any concentration of CDN. All groups of recipients were able to respond to PHA and the differences in response to PHA by the various groups can be noted. Recipients of the crude dialysate, V_0, N_1 and P responded to 5 micrograms of PHA to the same extent as the control animals. However, PHA stimulation was depressed in N_2 recipients and heightened in the N_3 recipients.

Table 4 contains the log mean percent of control migration for each group of recipients. PEC from recipients treated

Table 3. Lymphocyte Transformation[a] of peripheral blood from guinea pig recipients of transfer factor preparation and its fractions.

material injected intraabdominally	dilution of coccidioidin added in 0.1 ml volume to 2 ml culture			5 micrograms PHA added to 2 ml culture
	1:25	1:100	1:200	
Tris HCl (5)[b]	-.0960±.183	.0640±.234	-.0920±.281	.8700±.331
Crude dialysate[c] (3)	.0100±.237	-.1233±.303	-.1833±.362	.7267±.427
V_0[d] (4)	-.0900±.205	-.0900±.262	-.1425±.314	.7725±.370
N_1[d] (4)	.0400±.205	.0300±.262	-.0225±.314	.9125±.370
N_2[d] (4)	-.0075±.205	-.0725±.262	-.0850±.314	.4525±.370
N_3[d] (4)	.0650±.205	-.0225±.262	-.0375±.314	1.0875±.370
P[d] (5)	.1460±.183	.0520±.234	.0180±.281	.9720±.331

[a] average log cpm test cultures - average log cpm control cultures. mean ± 95% C.L.

[b] number of recipients per group in parentheses

[c] 24Å pore size dialysate prepared from cells of actively sensitized leukocytes

[d] Sephadex G-25 Fine pooled fractions of cell dialysate.

Table 4. Macrophage Migration Inhibition Tests[a] on PEC from guinea pig recipients of transfer factor preparation and its fractions.

material injected intraabdominally	dilution of coccidioidin in .2 ml of medium	
	1:25	1:100
Tris HCl (5)[b]	1.7948±.168	1.7802±.092
crude dialysate[c] (3)	1.7817±.218	1.7920±.119
V_0[d] (4)	1.8528±.188	1.9148±.103
N_1[d] (4)	1.8638±.188	1.8555±.103
N_2[d] (4)	1.8109±.188	1.9008±.103
N_3[d] (4)	1.4942±.188	1.8953±.103
P[d] (5)	1.7510±.168	1.9476±.092

[a] $\log \frac{\text{average migration of test cells}}{\text{average migration of control cells}} \times 100$

mean ± 95% confidence limits

[b] number of animals in parentheses

[c] 24 Å pore size dialysate prepared from actively sensitized leukocytes

[d] Sephadex G-25 Fine pooled fractions of cell dialysate

with column eluate fractions were tested in the presence of CDN diluted 1:25 or 1:100. Migration inhibition was demonstrated by all groups when a CDN dilution of 1:25 was used in the test. Inhibition was significantly greater in animals who had received injections of N_3 and P material than in animals from any other group when a CDN dilution of 1:25 was used. Control animals and recipients of crude dialysate showed greater inhibition with CDN 1:25 than the V_0, N_1 and N_2 recipients, but this inhibition was not nearly as great as demonstrated by the N_3 and P recipients.

Group discrimination analysis (37, p. 273) was run on seven groups of recipients: control animals, crude dialysate recipients, V_0, N_1, N_2, N_3 and P recipients. The variables used to discriminate these groups were MIF production with CDN 1:25, MIF production with CDN 1:100, lymphocyte transformation with CDN 1:25, lymphocyte transformation with CDN 1:100, lymphocyte transformation with CDN 1:200 and lymphocyte transformation with five micrograms of PHA. None of these groups could be discriminated when $P = .05$. In Table 5 the F ratios and P values are reported for each variable. The axis with the highest level of significance is illustrated in Figure 2. The centroids for each group and discriminant scores for each subject is plotted.

This same analysis was run again on the seven groups of animals, but only three variables were used to discriminate these groups. They were the skin test reaction, MIF production with CDN 1:25 and lymphocyte transformation with CDN 1:25. Group

Table 5. Statistical evaluation of in vitro variables used in group discrimination analysis of transfer factor recipient guinea pigs grouped by peak material received.

VARIABLE	F RATIO	P
MIF with CDN 1:25	1.9237	.1215
MIF with CDN 1:100	1.7541	.1553
Transformation with CDN 1:25	.8760	.5290
Transformation with CDN 1:100	.3465	.9041
Transformation with CDN 1:200	.2090	.9691
Transformation with 5 mcg PHA	1.3278	.2863

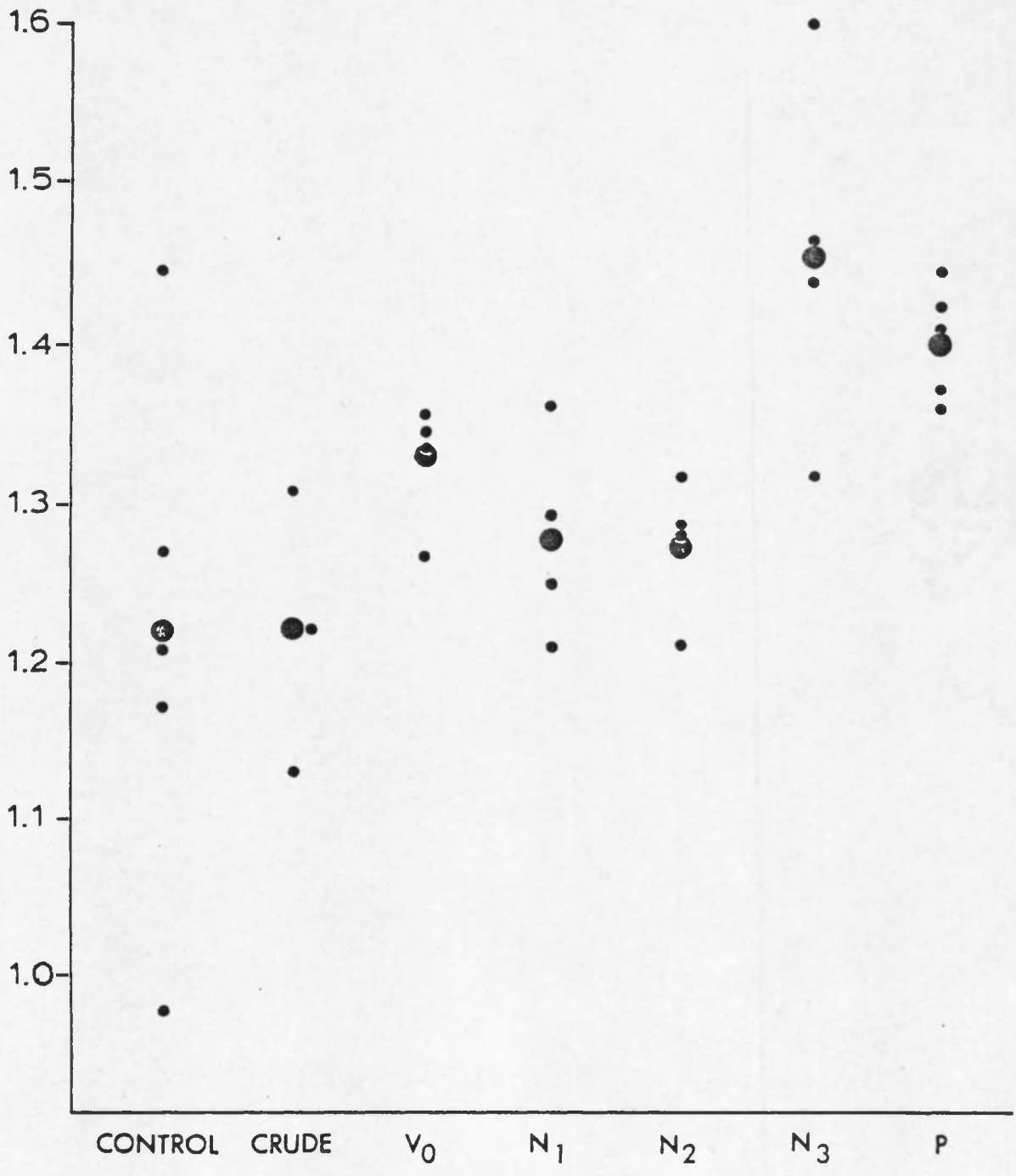

Figure 2. Centroids and Discrim Scores of transfer factor recipient guinea pigs grouped by fractions given and discriminated by MIF production with coccidioidin 1:25.

discrimination is statistically significant, P = .0001, on the discriminant axis illustrated in Figure 3. The only variable that is statistically significant in this analysis is the skin test variable, P = .0001.

To see if the tendency for MIF production with CDN 1:25 to discriminate treatment groups follows the separation by skin test reaction, the subjects were redivided into three groups: control, skin test positive and skin test negative. The variables used to discriminate these groups were MIF production at CDN 1:25 and lymphocyte transformation at CDN 1:25. The program was unable to separate these groups with the given variables. Figure 4 shows the centroids and discriminant scores determined by this analysis.

Part of the data reported here were analyzed by multiple regression analysis (37, p.295). Sixteen of the 18 variables measured for each of 33 subjects was included in this analysis. Table 6 shows the matrix of Pearson's Product Moment correlation coefficients for the following variables. The first variable refers to one of three transfer factor preparations used in this study. The next five variables indicate from which column fractions the material given to recipients was taken. The following four variables, respectively, are the amount of Lowry-positive material, orcinol-positive material, anthrone-positive material and the log of the number of cell equivalents given to each animal. Variable 11 is a dichotomous variable indicating a positive or negative skin reaction. Variable 12 is the average diameter of the skin

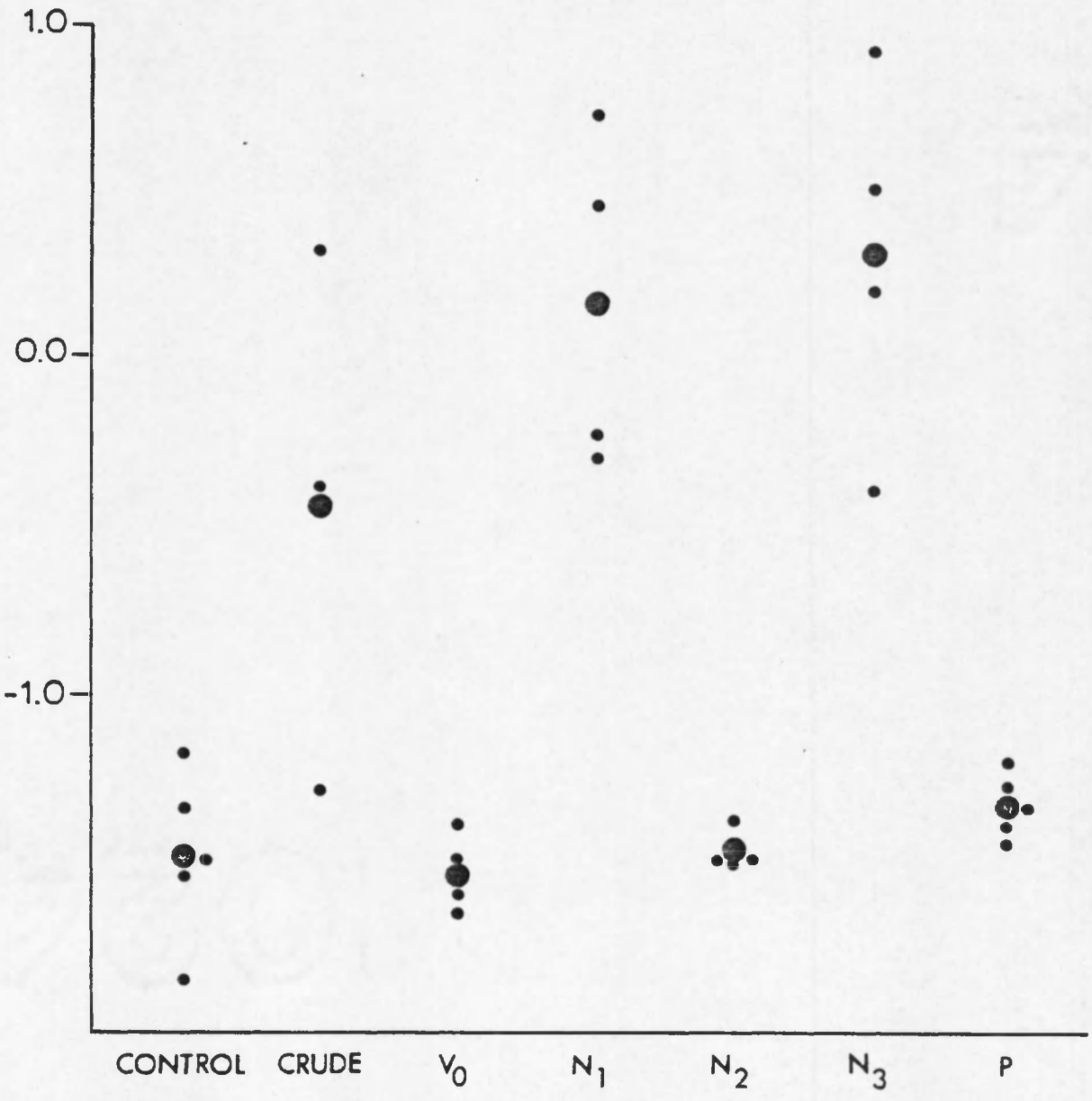

Figure 3. Centroids and Discrim Scores of transfer factor recipient guinea pigs grouped by fractions given and discriminated by skin test reaction.

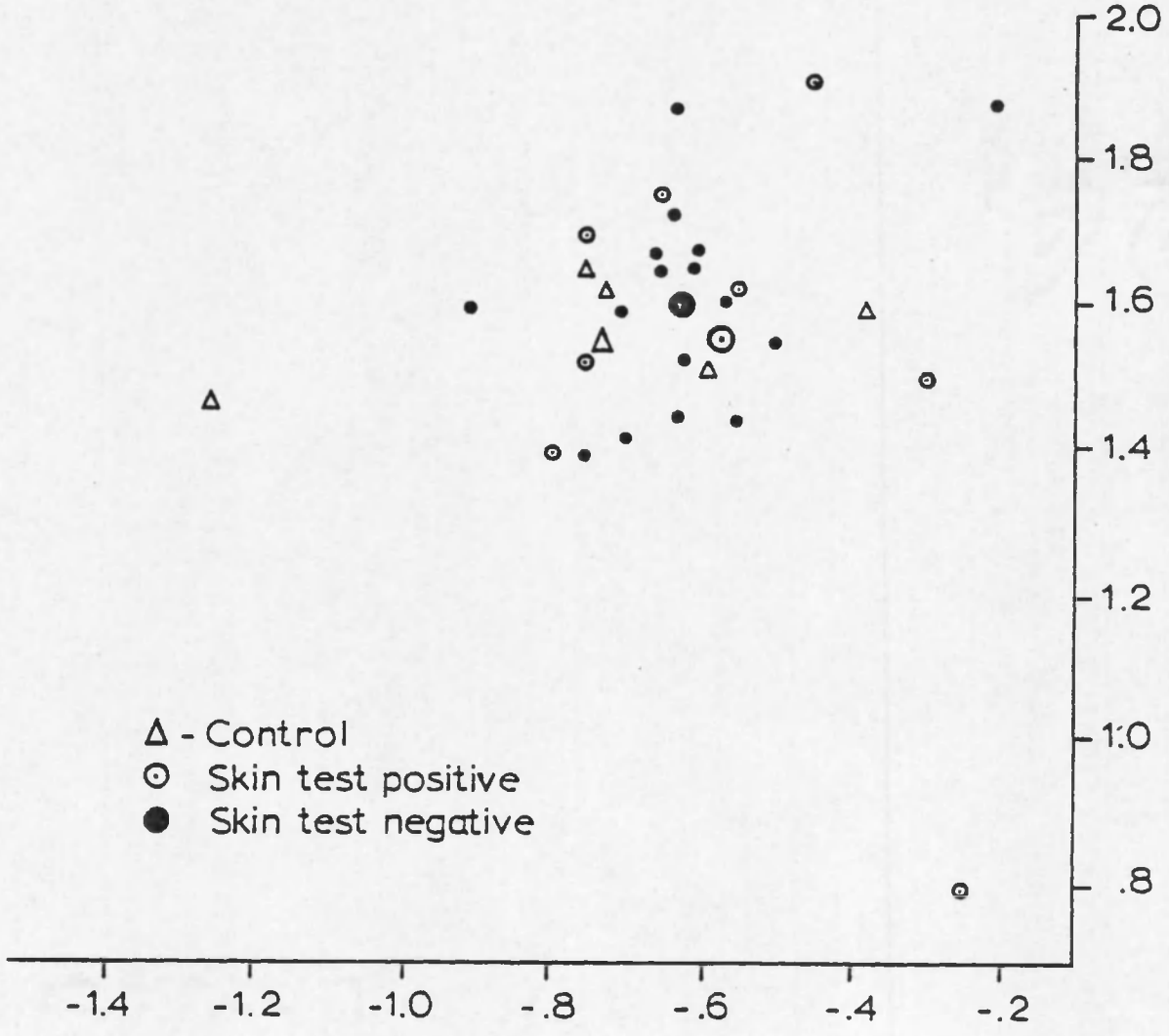

Figure 4. Centroids and Discrim Scores of transfer factor recipient guinea pigs grouped by skin test response and discriminated by MIF with coccidioidin 1:25 and lymphocyte transformation with coccidioidin 1:25.

Table 6. Matrix of Pearson's Product-Moment Correlation Coefficients for 16 variables on 33 recipient guinea pigs.

Variable No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. TF preparation	1.0000	.2514	.2514	.2514	.2514	.2514	.2692	.1700	.1515	.7722	.2240	.1734	.0931	-.3074	-.2536	.0379
2. peak V_0		1.0000	.1750	.1750	.1750	.1750	.5659	-.0931	-.0640	.2537	-.0653	-.1683	-.1480	-.2038	-.2116	-.3128
3. peak N_1			1.0000	.1750	.1750	.1750	.5245	-.0779	-.0553	.2554	.5502	.5431	.0699	.0059	-.0462	-.0308
4. peak N_2				1.0000	.1750	.1750	.4799	.5039	.5331	.2575	-.0653	-.1683	-.0390	-.1530	-.0381	-.3524
5. peak N_3					1.0000	.1750	.5465	-.0525	-.0411	.2553	.5502	.5316	.1089	-.1085	-.0760	.0466
6. peak P						1.0000	.5470	-.0904	-.0580	.2626	-.0653	-.1683	.2529	-.0195	.0080	-.1047
7. dose of protein							1.0000	.0244	.0780	.2415	.3416	.2183	-.0204	-.1031	-.1369	-.2163
8. dose of RNA								1.0000	.9954	.1753	-.0992	-.1126	-.0268	.0565	.1260	.0130
9. dose of carbohydrate									1.0000	.1711	.0934	-.1241	-.0107	.0869	.1372	.0182
10. cell dose										1.0000	.2933	.2877	.2554	-.1333	.0413	.0158
11. skin reaction (t,-)											1.0000	.8952	.1459	.0031	.0541	.1016
12. average diameter of the skin test reaction												1.0000	.0626	.0333	.0569	.1266
13. transformation with coccidioidin 1:25													1.0000	.0992	.3779	.2879
14. transformation with coccidioidin 1:100														1.0000	.6902	.2354
15. transformation with coccidioidin 1:200															1.0000	.2194
16. transformation with 5 mcg PHA																1.0000

1. Transfer factor preparation. 2^{9}Å pore size dialysate of actively sensitized leukocyte extract.
2.-6. Sephadex G-25 Fine pooled fractions of chromatographed cell dialysate.
7.-9. Dosage in mg/ animal of protein, carbohydrate and RNA given to recipient guinea pigs in 2 through 6 above.
10. Animals received 4 injections, each equivalent to approximately 5 X 10^6 PEC.
13.-15. Specific antigen stimulation by coccidioidin (CDN) on cultures containing a 1:30 dilution of peripheral blood from each recipient.
16. Nonspecific stimulation by PHA on cultures containing a 1:30 dilution of peripheral blood.

reactions. Variables 13 through 15 are the lymphocyte transformation indices when CDN dilutions of 1:25, 1:100 and 1:200, in that order, were added to the cultures. The last variable is the lymphocyte transformation index when five micrograms of PHA were added to the cultures. The average diameter of the skin test reaction is the criterion variable in every regression problem or model discussed. Variable 11, the variable indicating a positive or negative skin test reaction, is not used as a predictor variable.

The full model regression equation shown in Table 7 uses all variables to predict the average diameter of the skin reaction. The coefficient of determination is very high (.8999), i.e., 90% of the variation in the skin reactions can be explained by the variation in the rest of the data. It is obvious from the predictors' weights that N_2, the dose of carbohydrate, the dose of RNA, the cell dose and lymphocyte transformation at a CDN concentration of 1:25 are contributing little to this multiple regression equation. The variates making the largest contribution to the overall multiple regression equation are the batch of material received, V_0 and N_1. It is also interesting to note that the first three interations used N_1, N_3 and the dose of protein to find the maximum increase in RSQ. These are the variates with the highest linear correlation coefficients to the skin test variate in Table 2. With these three variates, 63.5% of the variation in the skin reactions of the recipients can be explained.

Table 7. Predictor Weights, Multiple Regression Coefficients (R_m), Coefficients of Determination (RSQ) and Regression Constants (R.C.) for 16 variables on 33 recipient guinea pigs.

Predictor	Predictor Weights					
	full model I	model Ia	model Ib	model Ic	model Id	model Ie
1. TF preparation	3.5641	3.6191	2.1638	-	-	-
2. peak V_0	3.3999	2.7954	-2.5721	-	2.7265	2.4192
3. peak N_1	4.0384	3.9855	3.9752	-	3.2464	3.6895
4. peak N_2	.9155	3.0904	-1.7160	-	3.1978	-1.4528
5. peak N_3	-1.8338	-2.6030	-2.7476	-	-2.4108	-2.7767
6. peak P	1.5786	1.9547	.8475	-	1.7258	1.4846
7. protein	-1.9282	-2.0643	-	-1.0527	-1.8014	-.8945
8. RNA	-.0068	-.1542	-	-.7375	-	-
9. CHO	0.0000	.1547	-	.1026	-	-
10. cell dose	-.4627	-.4747	-	-	-.3268	-
11. skin reaction (+,-)	-	-	-	-	-	-
12. average diameter of skin reaction..... criterion.....						
lymphocyte transformation						
13. CDN 1:25	-.4165	-	-	-	-	-
14. CDN 1:100	-1.4690	-	-	-	-	-
15. CDN 1:200	-1.9648	-	-	-	-	-
16. PHA 5 mcg	1.0511	-	-	-	-	-
R_m	.9486	.9395	.8788	.3894	.9271	.8776
RSQ	.8999	.8827	.7723	.1517	.8595	.7702
R.C.	-.4718	.4191	-.7065	8.5101	4.8345	2.4658

1. Transfer factor preparation. 24Å pore size dialysate of actively sensitized leukocyte extract.
2.-6. Sephadex G-25 Fine pooled fractions of chromatographed cell dialysate.
7.-9. Dosage in mg/ animal of protein, carbohydrate and RNA given to recipient guinea pigs in 2 through 6 above.
10. Animals received 4 injections, each equivalent to approximately 5×10^8 PEC.
13.-15. Specific antigen stimulation by coccidioidin (CDN) on cultures containing a 1:30 dilution of peripheral blood from each recipient.
16. Nonspecific stimulation by PHA on cultures containing a 1:30 dilution of peripheral blood.

To isolate the variables responsible for the high RSQ calculated for the full model, it is necessary to eliminate certain groups of variables, reformulate a regression equation and compare the two models using an F test. Reduced model a includes all data in the full model minus the lymphocyte transformation data. The RSQ for this model (.8827) is not significantly different from the full model. The variables with low predictor weights are the dose of carbohydrate, the dose of RNA and the cell dose. All other variables are of approximately equal importance. Column eluates N_1, N_3 and the dose of protein together accounted for 60% of the variation in the skin test reactions.

In model b the multiple regression equation for variables designating only the material received by the animals is given. N_2 and P have the lowest predictor weights and N_1 has the highest. Model c shows a regression equation including only data concerning the dose of protein, dose of RNA and dose of carbohydrate. The dose of protein has the highest predictor weight of these three variables, but has an RSQ of only .0477. This is not significantly different from zero. However, when data concerning the dose of pentose and carbohydrate are added to the equation, the RSQ is .1517 and is significant at the 90% level of confidence.

This set of intercorrelating data can be reduced from an array of 16 variables to an array of seven variables without showing a significant decrease in RSQ. Model d shows the multiple

regression equation for the variables representing the column eluate dose of protein and cell equivalents received by the animals. The RSQ for this model is .8595. Even though the cell dose has a very low weight in the multiple regression equation it is essential to predicting the variability in the skin test data as closely as the full model I. Model e is the same multiple regression equation as d without using the cell equivalent as a predictor. RSQ for this model is .7702.

CHAPTER 4

DISCUSSION

The passive transfer of CDN sensitivity with a leukocyte extract has been achieved. It was shown by regression analysis that the fractions of the material given, the dose of protein and the cell dose were the most important variables in predicting the skin reactions of the recipient animals. It is important to note that the particular preparation of extract did not make any contribution to the variability of the skin test reactions.

When considering the cell dose and the significance demonstrated by model d of the multiple regression analysis the treatment of the control animals should be noted. To a certain degree, the cell equivalent dosage of crude dialysate and its fractions was held constant. The control animals in this study did not receive "normal" crude dialysate and its fractions as might have been optimal. This group of animals received no cell equivalent dosage. Upon further investigation it was determined that the value of the cell dose was not important for predicting the skin test reaction of the recipient, but introduction of cell dialysate or fractions was necessary.

Since transfer factor preparations in other studies as well as this one are less than completely defined chemically,

an attempt was made to establish a measureable component that would correlate with the variation in skin test responses of the recipient guinea pigs. Of the three biochemicals measured only protein content correlated with skin test reactions. A greater number of recipients injected with a wider range of protein would be necessary to establish an effective dose range.

Many of the skin test reactions reported in this study were close to $5mm^2$, a size considered to be a minimum positive skin test mean by Smith (5). A sufficient number of guinea pigs in this study showed skin test reactions that are consistant in time of occurrence and in gross appearance with what is considered to be a true delayed-type response to CDN. The skin test was the single in vivo criterion used to establish a state of delayed-type hypersensitivity in these guinea pigs.

Histological analyses were not done on the groups of recipients analyzed here. Guinea pigs of the same colony tested with the same lot of CDN as was used in this study have been biopsied and the histology on $5mm^2$ reactions were considered to be consistent with delayed-type hypersensitivity reactions (38). One should also be advised of the antigens used in sensitizing and in skin testing these animals. There are antigenic differences among the three morphological forms of C. immitis - mycelial, arthrospore, spherule - as detected by agar gel diffusion (39) and complement fixation (40). Formalin-killed spherule vaccines induced stronger immunity in mice than arthrospore or mycelial vaccines (41). There is no evidence to suggest that

those antigenic differences would not be discernible by the cellular immune responses of a delayed-type hypersensitive guinea pig (42). It is yet to be determined just what specificity if any transfer factor has, but one must consider that transfer factor produced as the result of sensitization with arthrospores and the recipient of transfer factor and skin tested with CDN could cause the weak responses observed.

The lymphocyte transformation response to CDN in skin test positive humans has been reported (9). In humans with a very strong degree of skin test reactivity (5) large transformation indices have been demonstrated. However, weaker degrees of skin test reactivity (5) were not associated with in vitro lymphocyte response and transformation indices were not different from tests on those individuals with no CDN sensitivity (9). Graybill et al. (10) reported lymphocyte transformation in CDN transfer factor recipient humans. A transformation index of four was considered the low limit of positive lymphocyte transformation. Oppenheim et al. have reported 10 to 100 fold increases in tritiated thymidine uptake by guinea pig lymphoid cells that have been actively sensitized against PPD. They noted that peripheral blood lymphocytes responded less well than node lymphocytes and in only half the animals (22).

The transformation indices for transfer factor recipients were somewhat lower than those found in other studies (22). The reasons for this are not known, but since the transformation

indices for cultures on control animals and test animals are approximately the same the problem appears to reside in the culture technique used. The large confidence intervals reported for antigen stimulation of recipient cells may indicate the variability among individual animals and/or the failure of the culture technique.

Mean transformation indices for experiments using CDN 1:25 in culture were negative for control as well as V_0 and N_2 recipients. This may indicate that this concentration of CDN may be inhibitory to the cells in culture. One would expect that an inhibitory agent would be reduced at greater dilution; this did not prove to be the case. When CDN at a 1:200 dilution was used in lymphocyte transformation experiments the mean transformation indices for all groups except recipients of P fractions were negative.

Paque et al. (31) indicated passive transfer of MIF production by guinea pig PEC in response to CDN by a 59-66% decrease in the area of migration from the capillary tube. Graybill et al. (10) considered less than 70% of control migration positive migration inhibition in CDN transfer factor recipient humans. Using these criteria the data reported here show migration inhibition with CDN 1:25 by all groups except V_0 and N_1 recipients. Since PEC from control animals at both a 1:25 and a 1:100 dilution of CDN show positive migration inhibition, some kind of

nonspecific inhibition or toxicity is being demonstrated. Sinski and Reed reported no toxicity to guinea pig PEC with 0.01% CDN (43).

It is not a warranted conclusion that these in vitro correlates are not legitimate. It is not established, though, that results obtained in these tests when performed on actively sensitized animals or humans are the same as when these tests are performed on transfer factor recipient animals or humans. We have evidence in humans that lymphocyte transformation to CDN does not occur in weakly sensitive individuals (9). Sinski and Reed (43) have reported delayed-type hypersensitivity in guinea pigs with no migration inhibition. They suggested that this particular state of hypersensitivity which was induced by repeated skin testing lacks a necessary component to demonstrate this in vitro phenomenon. It may be that the in vitro tests for detecting sensitivity to this antigen need further improvement.

CHAPTER 5

CONCLUSIONS

It has been demonstrated that passive transfer of coccidioidin sensitivity in guinea pigs as measured by skin test reactivity can be achieved with a frozen-thawed and vacuum dialyzed leukocyte extract. We have been able to identify two groups of eluted fractions that demonstrate an ability to transfer CDN sensitivity and three groups of eluted fractions that do not have this ability. Skin test data supported by group discrimination analysis (Fig. 3) indicates that the material that transferred coccidioidin sensitivity to the recipients guinea pigs elutes in the N_1 and N_3 fractions of the G-25 Fine column described in Methods.

It was not possible to evaluate passively transferred sensitivity to coccidioidin by the in vitro tests utilized in this study. The results of the in vitro tests were insignificant in terms of predicting the skin reaction of the recipients and the individual correlation coefficients of the in vitro variables to the skin test variable were low. Groups of recipients could not be discriminated by the results of the in vitro tests. The response of the individual subject's cells in the in vitro tests varied to such a large extent that variability among the groups was insignificant.

APPENDIX A

BIOCHEMICAL ANALYSIS OF TRANSFER FACTOR PREPARATIONS AND ITS FRACTIONS

Table A. 1. Total milligrams of protein as measured by Lowry method given to recipient in four injections.

transfer factor preparation	crude[b]	V_0[c]	N_1[c]	N_2[c]	N_3[c]	P[c]
1	not done	-[a]	-	-	-	.162
		.218	-	-	-	-
2	10.980	1.816	.875	.564	1.767	.696
		1.741	1.450	.050	1.414	2.340
3	5.909	.154	-	-	-	-

[a] none detected

[b] 24Å pore size dialysate of actively sensitized leukocyte extract.

[c] Sephadex G-25 Fine pooled fractious of chromatographed cell dialysate.

Table A.2. Total milligrams of carbohydrate given to recipient in four injections.

transfer factor preparations	crude[c]	V_0[d]	N_1[d]	N_2[d]	N_3[d]	P[d]
1	not done	.088	.479	.893	.290	.234
		.145	.062	.065	.324	.306
2	17.112	.289	1.775	*[b]	2.679	1.944
		.062	1.016	*	4.177	-[a]
3	5.777	.073	.244	.514	.890	-

[a] none detected

[b] above limits of the test (200 micrograms/ml)

[c] 24Å pore size dialysate of actively sensitized leukocyte extract.

[d] Sephadex G-25 Fine pooled fractious of chromatographed cell dialysate.

Table A.3. Total milligrams of pentose given to recipient in four injections.

transfer factor preparations	crude[c]	V_0[d]	N_1[d]	N_2[d]	N_3[d]	P[d]
1	not done	.067	.251	.215	-[a]	-
		-	.033	.283	.272	-
2	10.656	.201	1.083	*[b]	1.512	.170
		1.069	4.610	*	11.272	1.560
3	8.383	1.086	1.551	1.351	2.951	1.609

[a] none detected

[b] above limits of the test (200 micrograms/ml)

[c] 24Å pore size dialysate of actively sensitized leukocyte extract.

[d] Sephadex G-25 Fine pooled fractious of chromatographed cell dialysate.

Table A.4. Log of the cell equivalents of material given to recipients in four injections.

transfer factor preparations	crude[a]	V_0[b]	N_1[b]	N_2[b]	N_3[b]	P[b]
1	8.806	8.826	8.903	9.000	8.903	9.114
		7.602	7.602	7.602	7.724	7.724
2	9.301	9.301	9.301	9.301	9.301	9.301
	9.301	9.301	9.301	9.301	9.976	9.301
3	9.477	9.301	9.301	9.301	9.301	9.301

[a] 24Å pore size dialysate of actively sensitized leukocyte extract.

[b] Sephadex G-25 Fine pooled fractions of chromatographed cell dialysate.

APPENDIX B

LYMPHOCYTE TRANSFORMATION TESTS ON TRANSFER FACTOR RECIPIENT GUINEA PIGS

Table B.1. Average of the log of the counts per minute of cultures on peripheral blood of control animals.

dilution of coccidioidin	animal numbers 1	2	3	4	5
none	2.90	2.99	2.92	3.16	3.28
1:25	3.13	2.93	2.84	3.19	3.22
1:75	-[a]	-	2.66	3.21	2.87
1:100	3.00	3.06	2.98	3.02	2.88
1:200	3.24	3.12	2.89	2.96	2.58
1:500	3.27	3.13	-	-	-

[a] not done

Table B.2. Average of the log of the counts per minute of cultures on peripheral blood of V_0 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
none	3.60	3.56	3.19	3.38	3.19
1:25	3.55	3.62	3.29	3.22	2.94
1:75	-[a]	-	3.26	3.25	2.96
1:100	3.58	3.60	3.36	3.07	2.99
1:200	3.63	3.41	3.08	3.33	2.75
1:500	3.63	3.44	-	-	-

[a] not done

Table B.3. Average of the log of the counts per minute of cultures on peripheral blood of N_1 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
none	3.05	3.18	2.99	3.13	3.14
1:25	3.11	3.23	3.09	3.02	3.31
1:75	-[a]	-	3.20	3.03	3.10
1:100	2.99	3.30	3.21	3.03	3.20
1:200	2.94	3.10	2.97	3.19	3.08
1:500	2.85	3.07	-	-	-

[a] not done

Table B.4. Average of the log of the counts per minute of cultures on peripheral blood of N_2 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
none	3.69	3.13	2.95	3.28	3.13
1:25	3.57	3.19	2.96	3.29	3.15
1:75	- [a]	-	3.08	3.19	3.17
1:100	3.64	2.92	3.08	3.32	3.06
1:200	3.75	3.02	3.21	3.20	2.92
1:500	3.70	3.00	-	-	-

[a] not done

Table B.5. Average of the log of the counts per minute of cultures on peripheral blood of N_3 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
none	3.06	3.16	2.92	3.45	3.15
1:25	3.34	3.14	3.02	3.34	3.26
1:75	- [a]	-	3.32	3.31	2.93
1:100	3.23	3.29	2.99	3.26	2.95
1:200	3.33	3.06	2.85	3.38	2.90
1:500	3.05	3.03	-	-	-

[a] not done

Table B.6. Average of the log of the counts per minute of cultures on peripheral blood of P recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
none	3.00	3.30	3.10	3.11	3.36
1:25	3.51	3.38	3.22	3.08	3.41
1:75	-[a]	-	3.04	2.88	3.24
1:100	3.44	3.41	3.24	2.90	3.14
1:200	3.79	3.32	3.21	2.57	3.07
1:500	3.70	3.41	-	-	-

[a] not done

Table B.7. Average of the log of the counts per minute of cultures on peripheral blood of crude dialysate[a] recipients.

dilution of coccidioidin	transfer factor preparation			
	1	2	2	3
none	3.13	3.51	3.39	3.49
1:25	3.21	3.63	3.32	3.51
1:75	-[b]	3.56	3.27	3.42
1:100	3.21	3.67	3.31	3.12
1:200	2.99	3.36	3.34	3.13
1:500	2.91	-	-	-

[a] 24A pore size dialysate of actively sensitized leukocytes.

[b] not done

Table B.8. Lymphocyte transformation[a] by 5 mg of PHA of peripheral blood cultures from transfer factor recipient guinea pigs.

treatment	transfer factor preparation				
	1	1	2	2	3
control	2.90	2.99	2.92	3.16	3.28
crude[b]	4.16		3.88	4.09	3.93
V_0[c]	4.07	4.12	4.41	4.10	3.87
N_1[c]	4.35	4.14	4.63	3.85	3.81
N_2[c]	3.97	3.78	4.56	3.60	3.69
N_3[c]	4.48	4.26	4.31	3.82	4.61
P[c]	3.57	4.14	4.45	4.11	4.46

[a] average of the log of the counts per minute.

[b] 24A pore size dialysate of actively sensitized leukocyte extract.

[c] Sephadex G-25 Fine pooled fractions of chromatographed cell dialysate.

APPENDIX C

MACROPHAGE MIGRATION INHIBITION FACTOR TESTS ON TRANSFER FACTOR RECIPIENT GUINEA PIGS

Table C.1. Percent of control migration of PEC from control animals.

dilution of coccidioidin	animal numbers				
	1	2	3	4	5
1:25	43	63	61	44	57
1:50	-[a]	-	75	75	61
1:100	57	58	33	33	62
1:200	67	66	-	-	-

[a] not done

Table C.2. Percent of control migration of PEC from V_0 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
1:25	63	-[a]	114	46	62
1:50	-	-	121	54	66
1:100	93	-	94	67	62
1:200	100	-	-	-	-

[a] not done

Table C.3. Percent of control migration of PEC from N_1 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
1:25	84	70	-[a]	49	82
1:50	-	-	-	55	90
1:100	70	90	-	56	60
1:200	91	94	-	-	-

[a] not done

Table C.4. Percent of control migration of PEC from N_2 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
1:25	45	80	-[a]	72	59
1:50	-	-	-	82	66
1:100	79	76	-	94	57
1:200	75	94	-	-	-

[a] not done

Table C.5. Percent of control migration of PEC from N_3 recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
1:25	35	51	-[a]	59	17
1:50	-	-	-	80	31
1:100	73	95	-	82	55
1:200	79	103	-	-	-

[a] not done

Table C.6. Percent of control migration of PEC from P recipients.

dilution of coccidioidin	transfer factor preparation				
	1	1	2	2	3
1:25	70	60	46	44	55
1:50	-[a]	-	88	61	59
1:100	99	102	80	77	70
1:200	108	102	-	-	-

[a] not done

Table C.7. Percent of control migration of PEC from recipients of crude dialysate.[a]

dilution of coccidioidin	transfer factor preparation		
	1	2	3
1:25	60	88	40
1:50	-[b]	77	40
1:100	59	67	44
1:200	65	-	-

[a] 24Å pore size dialysate of actively sensitized leukocyte extract.

[b] not done

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