This dissertation has been
microfilmed exactly as received

66–14,209

DUNN, Donald J., 1934–

ISOLATION, PARTIAL PURIFICATION, AND BIOLOGICAL ACTIVITY OF GUINEA PIG LEUKOCYTIC TRANSFER FACTOR.

The University of Oklahoma, Ph.D., 1966
Bacteriology

University Microfilms, Inc., Ann Arbor, Michigan

Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/2075

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ISOLATION, PARTIAL PURIFICATION, AND BIOLOGICAL ACTIVITY OF GUINEA PIG LEUKOCYTIC TRANSFER FACTOR

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

DONALD J. DUNN

Oklahoma City, Oklahoma

1966

ISOLATION, PARTIAL PURIFICATION, AND BIOLOGICAL ACTIVITY

OF GUINEA PIG LEUKOCYTIC TRANSFER FACTOR

APPROVED BY

Robert A. Patnode

Florence C. Kelly

Richard M. Hyde

Nyanne Green

Ronald L. Coleman

DISSERTATION COMMITTEE

ACKNOWLEDGMENT

The author is deeply indebted to Dr. Robert A. Patnode for his guidance throughout the performance of this investigation and for his constant advice and encouragement. Appreciation is extended to the University of Oklahoma Medical Center and its Department of Microbiology for the use of equipment and facilities.

The technical assistance of Dr. J. Hill Anglin, Dr. Yu-Teh Li, Dr. Ronald Coleman, Mrs. Daphene Furra, Mrs. Ruth Allin, and Gary Tuck is sincerely appreciated.

Financial assistance was provided by National Institutes of Health, Graduate Training Grant 5 T1AI162 and a National Science Foundation Science Faculty Fellowship.

I am particularly grateful to Dr. L. V. Scott, Chairman, and other members of the Faculty of the Department of Microbiology for their help, interest, and encouragement.

TABLE OF CONTENTS

Page

LIST OF TABLES v

LIST OF FIGURES vii

Chapter

I. INTRODUCTION AND LITERATURE REVIEW 1

II. METHODS AND MATERIALS 23

III. RESULTS 44

IV. DISCUSSION 94

V. SUMMARY 106

BIBLIOGRAPHY 108

LIST OF TABLES

Table		Page
1.	Relative Protein Content of Buffy Coat Mononuclear Cells	45
2.	Relative Alpha Globulin Content of Peripheral Blood Leukocytes	46
3.	Glycoprotein Content of Cell Lysates	48
4.	Electrophoretic Analysis of Glycoprotein Content of Cell Lysates	49
5.	Effect of PPD Skin Test on Electrophoretic Distribution of Serum Proteins in Guinea Pigs	50
6.	Glycoprotein Content of Normal Leukocytes Incubated in Presence of Sensitized Cell Lysate or Sephadex G-50 Fraction III	60
7.	Passive Transfer of Tuberculin Sensitivity	61
8.	Passive Transfer of Tuberculin Sensitivity with Sensitized Cell Fractions	66
9.	*In Vitro* Capillary Tube Migration of Buffy Coat Leukocytes from Tuberculin Sensitive and Normal Animals	68
10.	Skin Test Results in Guinea Pigs Injected with 5 μg PPD Mixed with Viable Cells from Sensitized or Normal Animals	70
11.	The Effect of Antisera on the *In Vivo* Assay of Cell Sensitivity	71
12.	Skin Test Results in Guinea Pigs Injected with Normal Cells Incubated with Normal and Sensitized Cell Lysates	72
13.	*In Vitro* Capillary Tube Migration of Buffy Coat Leukocytes Incubated with Normal and Sensitized Cell Lysates	73
14.	Skin Test Results in Guinea Pigs Injected with Normal Cells Incubated with Dialyzed Serum from Normal or Sensitized Animals	75

LIST OF TABLES--Continued

Table		Page
15.	In Vitro Capillary Tube Migration of Buffy Coat Leukocytes Incubated with Dialyzed Serum from Normal or Sensitized Animals	76
16.	Skin Test Results in Guinea Pigs Injected with Normal Cells Incubated with Subcellular Fractions and Sephadex Fractions from Sensitive Cells	77
17.	In Vitro Capillary Tube Migration of Buffy Coat Leukocytes Incubated with Subcellular Fractions and Sephadex Fractions from Sensitive Cells	79
18.	Skin Test Results in Normal Guinea Pigs Injected with 5 μg PPD Mixed with Peripheral Blood Leukocytes from Passively Sensitized or Normal Guinea Pigs	82
19.	In Vitro Capillary Tube Migration of Buffy Coat Leukocytes from Passively Sensitized or Normal Guinea Pigs	83
20.	In Vitro Capillary Tube Migration of Buffy Coat Leukocytes from Tuberculin Sensitive and Normal Humans	85
21.	Glycoprotein Content of Cell Lysates	86
22.	In Vitro Capillary Tube Migration of Normal Human Buffy Coat Leukocytes Incubated with Subcellular Particles and Sephadex Fractions from Sensitized Human Leukocytes	87
23.	In Vitro Capillary Tube Migration of Normal Human Buffy Coat Leukocytes Incubated with Various Sensitized Guinea Pig Leukocyte Extracts	90
24.	In Vitro Capillary Tube Migration of Normal Guinea Pig Buffy Coat Leukocytes Incubated with Various Sensitized Human Leukocyte Extracts	92

LIST OF FIGURES

Figure		Page
1.	Photograph of Reaction Between Antiserum Against Sensitized Cell Lysates (Center Well) and Normal Cell Lysates (Upper and Lower Outer Wells) and Sensitized Cell Lysates (Lateral Wells)	52
2.	Photograph of Reaction Between Antiserum Against Sensitized Cell Lysates (Center Well) and Dilutions of Sensitized Cell Lysates; Undiluted Lysates in Well 1 and 2, 1:10 Dilution in Well 3, and 1:100 Dilution in Well 4. Magnification X 4	53
3.	Photograph of Reaction Between Anti-Normal Leukocyte Serum (Center Trough) and Normal Cell Lysate (Upper Well) and Sensitized Cell Lysate (Lower Well). Magnification X 2	55
4.	Photograph of Reaction Between Anti-Sensitized Leukocyte Serum (Center Trough) and Normal Cell Lysate (Upper Well) and Sensitized Cell Lysate (Lower Well). Magnification X 2	56
5.	Ultraviolet Absorption Spectrum of Fraction IV Obtained by Sephadex G-200 Filtration	57
6.	Ultraviolet Absorption Spectrum of Fraction IV Separated by Sephadex G-50 Filtration	58
7.	Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received Cells from a Normal Guinea Pig. Magnification X 430	62
8.	Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received Cells from a Sensitized Guinea Pig. Magnification X 430	63
9.	Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received Cell Lysates from a Sensitized Guinea Pig. Magnification X 430	65
10.	Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received G-50 Fraction III of Cells from a Sensitized Guinea Pig. Magnification X 430	67

ISOLATION, PARTIAL PURIFICATION, AND BIOLOGICAL ACTIVITY OF GUINEA PIG LEUKOCYTIC TRANSFER FACTOR

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Soon after the acceptance of the basic observation that the toxicity or infectivity of a microbe may be diminished by the host's previous contact with it, it was noted that the introduction of some substances which had no toxicity for normal subjects would result in a marked deleterious effect in the previously exposed host. This unique aspect of acquired immunity is known as hypersensitivity.

The hypersensitive reaction was described clearly for the first time by Edward Jenner in his studies on the development of the local reaction to the inoculation of cowpox virus in persons who had been vaccinated previously or who had smallpox (Rich, 1944). It was not until a similar observation was made by Robert Koch (1891) during his experimental studies on reinfection in tuberculosis that interest in the phenomenon became aroused. In 1906, von Pirquet recognized it as an altered reactivity phenomenon and termied it "allergy." This terminology implies a modification of the host in such a manner that the subsequent contact of the agent with the host tissues produces a greater than normal inflammatory response, a more rapid and severe reaction than that which

occurred as a result of the first exposure. It was after detailed reinvestigation of the observations of Jenner and Koch that von Pirquet (1909) developed the diagnostic tuberculin test.

In general two features have served as a basis of a broad classification of the various types of hypersensitive reactions. On the basis of the events leading to the induction of the hypersensitive state, it is evident that some hypersensitivities may be established simply by entrance of the sensitizing antigen into the tissues, while others require the presence of the entire infectious agent. In the type which is established by simple sensitization, subsequent responses to the same antigen occur very quickly and are referred to as immediate reactions. In contrast, the reactions induced by the presence of the infectious agent show a characteristic delay in their appearance following introduction of the agent. This provides a convenient basis for the classification of the hypersensitive state into two types, the immediate and the delayed.

The immediate hypersensitive state was first observed by Portier and Richet (1902) when they described the serious illness and sometimes death of dogs after reinjection of derivatives of sea anemones and mussels. They introduced the term "anaphylaxis" for this phenomenon. Arthus (1903) described an increased capacity to react locally at any tissue site at which the sensitizing agent was reinjected repeatedly. In this case, the local reaction, instead of exhibiting the short-lived wheal and erythema characteristic of the anaphylactic reaction, increased in size and intensity until hemorrhage and necrosis appeared which were frequently followed by sloughing of the site.

Following these observations in experimental animals the clinical

aspects of hypersensitivity received considerable attention. Von Pirquet and Schick (1905) described "serum sickness," the reaction occurring following primary and secondary injections of horse serum in man. The clinical conditions such as food and drug allergy, pollen sensitivity, and contact dermatitis have been considered to be manifestations of this type of hypersensitivity (Raffel, 1953). In each of these conditions the reaction of the sensitive individual following exposure to the responsible material is much more rapid and severe than that observed in the non-sensitive individual.

The different types of immediate hypersensitivity have several basic features in common, including the rapidity of response, a relationship of reactivity to demonstrable circulating antibody, and the participation of histamine or histamine-like substances in the development of the specific response. However, there are subtle differences in some of these types of immediate hypersensitivity. In the anaphylactic or Arthus type, the responsible antibody generally is heat-stable and precipitable by the specific antigen. The antibodies involved in some drug, food, and pollen sensitivities are generally heat labile and are not precipitated by antigen, although they can be demonstrated by passive transfer techniques described by Prausnitz and Kustner (1921). Also, the concept of the role of histamine and histamine-like intermediates in some forms of immediate hypersensitivity still require clarification (Raffel, 1953). However the similarities are much greater than the differences and the immediate reactions are believed to be dependent upon interaction of antibody and specific antigen followed by the release of histamine or similar substances and their subsequent effect on vascular endothelium and

involuntary muscle (Rich, 1944), (Waalken et al., 1960), and (Riley, 1963).

The delayed type of hypersensitivity includes the so-called "allergy of infection" developing from bacterial, viral, and fungal infections, as well as some forms of plant and chemical contact dermatitis. The tuberculin reaction is the classical example and is perhaps the most familiar form of delayed hypersensitivity. However, this form of hypersensitivity may also be associated with glanders, tularemia, whooping cough, chancroid, syphilis (Chase, 1948), pneumococcal pneumonia (Julianelle, 1930), hemolytic streptococci (Zinsser, 1925), and diphtheria (Uhr, 1957). There is evidence that a similar state exists as a result of infection with smallpox, influenza, mumps, measles, herpes simplex, and lymphogranuloma venereum (Raffel, 1953). In fungal infections, hypersensitivity has been shown to occur in histoplasmosis, coccidiomycosis, blastomycosis, and trichophytosis. Similarly, cases of helminthic infections, such as schistosomiasis, echinococcosis, filiariasis, trichinosis, and Ascariasis have been shown to develop delayed hypersensitivity.

The most apparent feature of the delayed reactivity is the characteristic delay in the appearance of the local reaction when the specific sensitizing antigen is introduced into the suitably sensitized host. Passive transfer of delayed sensitivity with serum from a sensitive donor has not been generally successful, probably because this form of hypersensitivity has been shown to have no relation to circulating antibody. Antihistamine drugs do not influence the delayed reaction and this is considered to be evidence for the lack of participation of histamine or similar substances in this form of reactivity (Pepys, 1955). Rich (1944) showed that the sensitizing antigen has a marked cytotoxicity in tissue

culture for tissue from the delayed hypersensitive host and that typical delayed reactions can be produced in the avascular cornea. These demonstrations supports the suggestion that the delayed reaction can occur in the absence of circulating antibody and factors analogous to histamine.

Although the characteristic features of delayed hypersensitivity may suggest that the phenomenon is removed from the classical immune response, it has been generally accepted as an antigen-antibody reaction. The facts that the reaction is highly specific, requires a sensitizing period for its induction, shows an anamnestic response, and can be abolished by specific desensitization are persuasive reasons for believing that this type of hypersensitivity is mediated by a specific sensitizing material. Interest in this form of sensitivity is high mainly because of its close association with chronic infections.

One of the foremost contributions from recent research is the recognition of the fundamental role of the leukocyte in delayed hypersensitivity. The current belief is that the sensitizing antibody responsible for this form of hypersensitivity is closely associated with the leukocyte. The specific antibody responsible for tuberculin hypersensitivity has been under investigation for many years. The earliest approach to this problem was concerned with attempts to transfer passively tuberculin sensitivity with extracts of organs from tuberculous donors to normal recipients. In 1909, Helmholz reported positive skin reactions in normal guinea pigs two to six days after he had injected them with defibrinated blood collected from tuberculous guinea pigs which had been skin tested previously with tuberculoprotein. In the same year, Bail (1909) injected a tissue emulsion from tuberculous guinea pigs into normal guinea pigs

and twenty-four hours later obtained positive skin reactions. The animals were killed after intraperitoneal injection of tuberculin.

The belief that cellular antibody is involved in tuberculin hypersensitivity has received further support from several other passive transfer experiments. In 1942, Landsteiner and Chase reported the transfer of delayed-type skin sensitivity to picryl chloride from sensitive donor guinea pigs to normal recipients using leucocytes which were harvested from artifically induced peritoneal exudates. Chase (1945) also demonstrated that delayed skin hypersensitivity to tuberculin could be transferred to normal guinea pigs by the injection of leukocytes isolated from peritoneal exudates, spleen, lymph nodes, and blood of sensitized animals. In 1949, Lawrence reported finding that tuberculin sensitivity could be transferred passively in humans with intact viable leukocytes from tuberculin positive individuals.

The observations of Chase were confirmed by Cummings, Hoyt, and Gottshall (1947), Stavitsky (1948), and Metataxas and Metataxas-Buhler (1948) using primarily peritoneal exudate cells, and by Kirchheimer and Weiser (1947) using spleen cells and peritoneal exudates. In addition, Kirchheimer, Weiser, and VanLiew (1949) demonstrated that systemic, as well as cutaneous sensitivity to tuberculin was conferred upon recipient animals following cell transfer. Further experimentation with this technique revealed that leukocytes from sensitive donors were capable of transferring sensitivity to 2-4 dinitrochlorobenzene in guinea pigs (Haxthausen, 1951), Brucella abortus in guinea pigs (Metataxas-Buhler, 1951), cat scratch antigen in man (Warwick, et al., 1956), 2-4 dinitrochlorobenzene and tuberculin in guinea pigs (Skog, 1956), Mycobacterium lepraemurium

in rats to guinea pigs (Wallace, 1958), Cryptococcus neoformans in rabbits (Lomanitz and Hale, 1963), Mycobacterium tuberculosis in mice (Azar and Sprunt, 1965), and tuberculin in human sarcoid patients (Urbach et al., 1952). Paterson (1960) passively transferred allergic encephalomyelitis in rats by means of lymph node cells. Boughton and Schild (1962) used lymph node cells to transfer sensitization to testicular antigen to normal animals. The recipients developed a maximum delayed reaction six days following passive transfer. Moschos and Serri (1963) accomplished cellular passive transfer of allergic eczematous contact sensitivity in guinea pigs but not in man. Baer et al. (1952) also reported negative results in man. Chase (1953) has suggested that intact, viable cells are required for successful passive transfer. In all of these earlier reports viable cells were used.

Several attempts have been made to identify the particular cell type involved in passive transfer activity. Haxthausen (1947) reported that thymic cells were successful transmitters of hypersensitivity in allergic eczema but that lymphocytes were the most effective cell type. Stavitsky (1948) found that lymphocytes from lymph nodes conferred the greatest sensitivity. Wesslen (1952) was successful in transferring tuberculin sensitivity using almost pure suspensions of lymphocytes from the thoracic duct of rabbits and guinea pigs. Crowle (1959) was successful in transferring sensitivity in mice using thymic cells, while Sever (1960) used exclusively monocytes. Kirchheimer, Hess, and Spears (1951) reported that guinea pig peritoneal exudates composed exclusively of granulocytes do not confer tuberculin hypersensitivity. Fong et al. (1962) obtained similar results using granulocytes and found that the most

effective cell type was the tissue macrophage (histiocyte) and, to some degree, the lymphocyte. Hussey (1961) found that in artifical immunity in experimental tuberculous infections the lymphocytes increase with onset of hypersensitivity and generally parallel the level of sensitivity. These findings would lead one to believe that the capacity to transfer delayed hypersensitivity resides chiefly in the lymphocytes and monocytes.

In addition to the results seen with passive transfer techniques, several other types of evidence are available to implicate the lymphocyte and monocyte as the cell type most likely involved in delayed hypersensitivity. Wielheml et al. (1958) experimentally depleted the mononuclear cell lines in hypersensitive guinea pigs by injection of leukocytic antisera prepared in rabbits and hereby abolished the delayed allergic reaction. Waksman et al. (1961) prepared rabbit antisera against lymphocytes obtained from guinea pig lymph nodes and found that when this material was injected into guinea pigs it produced a depression in the level of blood mononuclear cells. Repeated injections of the antisera produced a marked lymphopenia and when the animals were tested for delayed allergy to tuberculin and contact sensitivity to 2-4 dinitrochlorobenzene, and were examined for the development of allergic encephalomyelitis and the rejection of homografts, they all showed a marked depression in reactivity. Long and Jeter (1961) inhibited the ability of leukocytic extracts to passively transfer sensitivity to 2-4 dinitrochlorobenzene in guinea pigs by incubating the extracts with leukocytic antisera prepared in rabbits. Pincus and Flick (1963) were able to inhibit the delayed reaction to vaccinia in guinea pigs using anti-mononuclear cell serum and were successful in inhibiting the reaction as long as the animals were receiving antisera.

Further evidence for the involvement of the lymphocyte and monocyte in delayed allergy was presented by Salvin and Smith (1959) who reported that x-irradiation of Hartley guinea pigs extended the delayed reaction period and that if the leukocyte count fell below one hundred there was no response to the antigen. Arnason et al. (1962) reported the suppressive effect of thymectomy at birth on reactions of the delayed cellular type in rats and noted a reduction in the number of circulating small lymphocytes, although plasma cell and gamma globulin levels were normal.

Contrary to the opinion of Chase (1953) that intact, viable cells are required for transfer, Jeter, Tremaine, and Seebohm (1954) were able to sensitize passively normal guinea pigs to 2-4 dinitrochlorobenzene using donor leukocytes which were disrupted by sonic vibration. Using similar techniques, Cummings, Patnode, and Hudgins (1956) transferred tuberculin hypersensitivity in guinea pigs with sonic-disrupted leukocytes and spleen homogenates. In addition, delayed hypersensitivity to tuberculin (Lawrence, 1954), streptococcal M substance (Lawrence, 1955), diphtheria toxin (Lawrence and Pappenheimer, 1956), and coccidioidin (Rapaport et al., 1959), as well as homograft sensitivity (Lawrence et al., 1960), have been passively transferred in man with extracts of disrupted leukocytes from sensitive donors. Freedman et al. (1957) were able to transfer tuberculin sensitivity in humans using leukocyte extracts. They were unable to transfer sensitivity to ragweed pollen, penicillin, and horse serum. Jensen et al. (1962) accomplished simultaneous, multiple, passive transfer of hypersensitivity to human, Battey, and avian tuberculins in man using stored (-20C) leukocytes.

Transfer of cutaneous hypersensitivity to tuberculin in guinea

pigs was examined further by Kucharski and Favour (1964) using cell free extracts of spleenic tissue. Kind et al. (1965) demonstrated the passive transfer of chemical sensitivity to paraphenylenediamine or 2-4 dinitrochlorobenzene in guinea pigs using sonic disrupted cells. Eisen (1959) observed that sonic disruption of lymphoidal cells abolished their capacity to transfer sensitivity to 2-4 dinitroflorobenzene. This conflicts with the results of Jeter et al. (1954). However, Eisen was able to transfer sensitivity using whole cells, as was Haxthausen (1951), Skog (1956), and Kind et al. (1965).

Attempts to transfer passively tuberculin hypersensitivity, as well as other delayed type reactions, using serum from sensitized donors has generally been unsuccessful. However, some degree of success has been obtained by Ekami et al. (1956) and Bovornkitti (1961). In further studies, Bovornkitti et al. (1963) were able to transfer skin reactions to tuberculin in twenty-two of twenty-five trials using serum from two selected cases of active tuberculosis. Seven control individuals received serum from healthy, tuberculin negative subjects. Labelling the serum with I_{131} did not alter its capacity to transfer sensitivity. Sensitivity persisted in the recipients for fifteen days. Tsuji et al. (1964) were able to transfer delayed reactivity to tuberculin in rabbits using dialyzed serum.

Two different views as to the function of the transferred cells have developed as a result of these fundamental observations. On the one hand, the cells may elaborate antibody or an antibody-like material which subsequently sensitizes the cells of the recipient. This concept is supported by demonstration of the ability of transferred lymphocytes to synthesize and release antibody, as reported by Harris et al. (1954) and

Roberts and Dixon (1955). Evidence that an antibody or antibody-like material may be responsible for tuberculin reactivity was presented by Cole and Favour (1955) who showed that plasma collected from tuberculin positive guinea pigs within forty-eight hours following a skin test with PPD could be fractionated to yield an active transfer factor which was contained in a subfraction of the alpha globulin. They felt that the active material, which they called IV-10, is not normally present in the circulation but may be released from leukocytes during the development of the cutaneous reaction. In further studies, Cole et al. (1959) accomplished passive transfer with plasma from which the fibrinogen, gamma globulin, and beta globulin had been removed by alcohol fractionation. Ehrenkranz and Waksman (1956) were unable to confirm the work of Cole and Favour. Results of studies with agammaglobulinemic patients support the contention that the transfer factor is not a gamma globulin. Porter (1955) showed that such patients, despite their inability to synthesize gamma globulin, may be sensitized to tuberculin by BCG vaccination and, further, that their leukocytes are capable of passively transferring tuberculin reactivity to normal recipients. More recently, Good and Zak (1956) and Good et al. (1962) also showed that while agammaglobulinemic patients cannot form detectable gamma globulin they do develop delayed sensitivity to various substances and this delayed allergy can be passively transferred. Jeter, Laurence, and Seebohm (1957) analyzed extracts of leukocytes of normal guinea pigs and guinea pigs hypersensitive to tuberculin and 2-4 dinitrochlorobenzene. They showed that an electrophoretic component resembling an alpha globulin was found in high concentration in cells from sensitive animals but was lacking in normal cells. In addition, Lawrence

and Pappenheimer (1957) showed that the factor on leukocytes responsible for passive transfer of tuberculin hypersensitivity in humans is released in vitro from the cells during incubation in normal plasma or serum in the presence of PPD. The leukocytes remained morphologically intact during the release of the transfer factor and the resulting cell-free supernatant fluid passively transferred tuberculin sensitivity to normal recipients. Kantor and Smith (1965) found that, in animals sensitized to picryl chloride conjugated to guinea pig albumin, exposure of leukocytes to homologous antigen resulted in densitization and reduced successful passive transfer of delayed sensitivity from 80% in controls to 33% in the desensitized group.

Electrophoretic studies of serum proteins in tuberculous patients and experimental animals support the concept that the transfer factor might be an alpha globulin (Baldwin et al., 1953, Hudgins et al., 1956, and Freigang et al., 1963). Bovornkitti (1962) analyzed serum specimens from fifty-eight tuberculous patients and noted an increase in the alpha-2 globulin fraction.

In contrast to the concept that the transferred cells release a sensitizing material in the recipient, some investigators believe that the transferred cells themselves participate in the subsequent reaction in the recipient. Metaxas and Metaxas-Buhler (1955) have shown that typical local, systemic, and corneal reactions to tuberculin can be produced in normal guinea pigs when tuberculin is injected simultaneously with, or even before, the intravenous injection of cells from sensitive donors. They concluded that, in this case, the donor leukocytes did not have sufficient time to release a sensitizing material and thus it was

the leukocytes themselves that participated in the reaction in the recipient. They also interpreted the twenty-four hour delay in the appearance of reactivity as the time required for the transferred cells to reach the circulation. Further support for the concept that transferred sensitive cells may actually migrate to the skin test site was provided by Hagerman (1954) who labelled guinea pig lymphocytes with fluorescent dye and then injected them into normal animals. Fluorescent lymphocytes appeared in the skin of recipient guinea pigs within one hour after intravenous injection and two hours after intraperitoneal injection. In addition, Najarian and Feldman (1961) passively transferred tuberculin sensitivity with tritiated thymidine labelled lymphoidal cells and found that labelled lymphocytes were specifically attracted to the skin test sites. Labelled sensitized cells did not accumulate in non-specific inflammatory lesions. Najarian and Feldman (1963b) obtained similar results in passive transfer of tritiated thymidine labelled cells obtained from animals sensitive to 2-4 dinitroflorobenzene. The maximum reaction appeared in twenty-four hours and from one-fourth to one-third of the labelled cells were found in the epidermal skin sites. However, in the tuberculin system Feldman and Najarian (1963) found that only 1% of the cells at the skin test sites were labelled. Evidence against the specific attraction hypothesis is also contained in the work of Turk (1962) and McCluskey et al. (1963). Hamilton and Chase (1962) determined by quantitative assay techniques that labelled cells were found in the skin test site more frequently than other cells.

The mechanism involved in the induction of the delayed hypersensitive state has been the subject of a number of investigations. Raffel

(1948) found that tuberculoprotein would regularly induce only immediate hypersensitivity, but if the protein were injected with a chloroform-soluble lipopolysaccharide waxy component of the tubercle bacillus delayed hypersensitivity resulted. Similar results were obtained by Raffel and Forney (1948) when they mixed picryl chloride with the wax. The wax was non-antigenic when injected without protein. The role of the wax in inducing delayed hypersensitivity is believed to be a general biological phenomenon since a similar substance has been found by Raffel (1954) in streptococci and in vaccinia virus. These results have been considered as evidence suggesting that the wax directs the host's response to the development of delayed hypersensitivity.

Several other methods of evoking a state of delayed hypersensitivity have been described. Uhr et al. (1957a) showed that when small amounts of antigen-antibody complexes precipitated in antibody excess were injected into guinea pigs the animals showed delayed reactions to diphtheria toxoid two to three weeks before detectable circulating antibody was present. They found that complete or incomplete adjuvant was not necessary, but if it were present the degree of sensitivity was increased. Dienes (1928), using killed tubercle bacilli, first reported on the importance of adjuvant in his studies on the sensitization of guinea pigs to egg white and timothy pollen. Salvin (1958) showed that the use of very small amounts of antigen in incomplete Freunds adjuvant resulted in delayed hypersensitivity in guinea pigs. The sensitivity appeared five to six days after immunization and preceded the appearance of circulating antibody.

As discussed previously, contact sensitivity of the delayed type can be effectively induced with simple chemicals which are able to combine

with proteins of the epidermis either a priori or after being modified by the host. Salvin and Smith (1961) reported that the intradermal injection of a simple hapten resulted in contact sensitivity to topical application of the same material. Intradermal injection of hapten (picryl chloride or 1-fluoro-2,4-dinitrobenzene) conjugated to guinea pig serum induced first a delayed response and then an Arthus reaction to the conjugate but there was no evidence of contact reactivity to the hapten alone. When a conjugate of hapten and solubilized guinea pig skin was used as a sensitizing antigen, both contact hypersensitivity to hapten and delayed hypersensitivity to the conjugate developed. These observations are consistent with the hypothesis that the specificity of contact sensitivity is directed towards some particular protein of the skin that is modified by combination with the hapten. Benacerraf and Gell (1959a) found that delayed sensitivity could be elicited through the use of simple chemical compounds conjugated to native protein, denatured protein, and low molecular weight polypeptides such as salmine. Several authors, including Benacerraf and Gell (1959b) and Benacerraf and Levine (1962), have found that the specificity of the delayed reaction involves the protein carrier and not the hapten. This is illustrated by the fact that sensitivity to guinea pig albumin-m-azobenzene sulfonate does not cross react with conjugates of the same hapten with ovalbumin (Gell and Silverstein, 1962). Gell and Benacerraf (1959) observed some cross reaction with horse and bovine serum albumin. However, no antibody to the protein carrier could be detected. Benacerraf and Levine (1962) found that delayed hypersensitivity was induced equally well with either lightly or heavily substituted conjugates. Animals sensitive to either lightly or heavily substituted

conjugates exhibited strong delayed reactions to both materials, but more intense reactions to the immunizing conjugate. The sensitive animals gave a milder reaction to conjugates of the same hapten with a different carrier and to conjugates differing from the immunizing conjugate by having an additional type of hapten interposed between the immunizing hapten and the protein carrier. Salvin and Smith (1961) found that guinea pigs sensitized to either hen, duck, or goose egg albumin showed delayed hypersensitivity, followed by Arthus reactions, to homologous antigen. These animals demonstrated only weak responses to heterologous antigens. Sensitization with protein conjugated with the hapten picryl chloride resulted in delayed reactions to homologous conjugates, homologous protein, homologous protein conjugated with heterologous haptens, and mild reactions to homologous hapten conjugated with heterologous protein. In contrast with these findings, Leskowitz (1963a, 1963b, 1963c) and Jones and Leskowitz (1964) reported that, while the carrier protein is important, most of the specificity is directed toward the hapten, even when the hapten is coupled with an unrelated protein. They also found that guinea pigs injected at birth with complete antigen fail to develop hapten-specific delayed hypersensitivity when they reach adulthood. Salvin et al. (1962) obtained similar results in neonatal guinea pigs using purified soluble antigens. However, they were able to induce experimental allergic encephalomyelitis in the animals. Warwick et al. (1960) found that new born human infants are unable to manifest delayed hypersensitivity or accept passively transferred sensitivity. Uhr (1960) obtained similar results in guinea pigs.

Various factors and circumstances influence the expression of

the hypersensitive state. The response is depressed during measles (Gruner, 1909), polio, diphtheria, and varicella (Mitchell et al., 1928), mumps (Nolbant, 1937), scarlet fever and infectious mononucleosis (Bentzon, 1953), sarcoidosis (Hoyle, 1954), and hypothyroidism (Kallos and Kentzler, 1932). Various drugs and hormones have also been found to depress the reaction. Harris and Harris (1950) and Gell and Hinde (1951) reported that cortisone depresses activity in laboratory animals. Carey et al. (1950) and Long and Favour (1950) obtained similar results in man using ACTH and corisone. Derbes et al. (1950) confirmed these observations in guinea pigs. Skin sensitivity is also suppressed by administration of estrogen (Lurie et al., 1949), nitrogen mustard and thorium dioxide (Cohen et al., 1954) and 6-mercaptopurine (Hoyer et al., 1962). Salvin and Smith (1959) found that x-irradiation extends the delayed reaction period and if the leukocyte count falls below one hundred there is no skin response. Uhr and Scharff (1960) observed that the delayed response is not suppressed by mild x-irradiation.

An entirely new approach to the study of delayed hypersensitivity was provided by the demonstration of a specific in vitro cytotoxicity of tuberculin for tissues of the tuberculous host. The first report in this regard was that of Holst (1922) who noticed an inhibition of migration of leukocytes from the plasma-buffy coat interface when blood from tuberculous individuals was incubated in capillary tubes in the presence of tuberculin. Using this technique he found that tuberculin had no effect on leukocytes from non-tuberculous donors, nor were the white cells of anaphylactically-sensitive individuals affected by specific sensitizing antigen. Meyer and Loewenthal (1927) confirmed the latter observation

when they showed that horse serum did not have any deleterious effect upon explants of tissues from animals which had been sensitized to horse serum.

Rich and Lewis (1928, 1932), using tissue culture techniques, showed that washed cells from spleen, bone marrow, and buffy coats of tuberculous guinea pigs were inhibited and killed by amounts of tuberculin that were entirely harmless to the cells of normal animals. Comparisons were made of the effect of tuberculin upon normal and hypersensitive cells cultured on the plasma of normal and hypersensitive animals. Regardless of the source of the plasma on which the cells were grown, the washed cells of tuberculous guinea pigs were consistently affected by tuberculin and the cells of normal animals were not. Moen and Swift (1936) also observed that the effect of tuberculin on the cells of the hypersensitive individual was independent of the presence of serum antibody. In contrast to the finding of Rich and Lewis (1932), Baldridge and Kligman (1951) found no inhibition of growth of tissue cultures. However, they noted that their guinea pigs were not extremely sensitive. Tuberculin is reported to have no effect on the oxygen consumption of neutrophilic leukocytes from sensitive animals (Marks and James, 1953), however, Allison et al. (1965) reported a depression in metabolic enzymes in a number of different tissues. Merchant and Chamberlain (1952) showed that the phagocytic activity of leukocytes from guinea pigs sensitive to streptococcal and pneumococcal extracts was diminished when the cells were exposed to the sensitizing antigen in vitro. Patnode and Hudgins (1959) compared the fragility of sensitized and normal monocytes and found that the former were more resistant to disruption by sonic vibration than the latter.

Further investigation of the effect of tuberculin on leukocytes

in vitro was begun in 1947 by Favour when he described the lympholysis or cytolysis phenomenon. In this case, the blood leukocytes from the tuberculin sensitive host are lysed *in vitro* when exposed to tuberculin. These results were confirmed by Waksman (1953a). Miller *et al.* (1949a & b) and Miller and Favour (1951) observed the presence of a heat-labile, non-dialyzable factor in the plasma of tuberculous humans which was responsible for *in vitro* lysis of leukocytes by tuberculoprotein. It was precipitated with the globulin fraction of plasma proteins and washed leukocytes from tuberculin sensitive hosts slowly released the factor into normal plasma. Patnode and Hudgins (1959) rendered normal guinea pig leukocytes susceptible to lysis by PPD by suspending them in serum collected from sensitized guinea pigs forty-eight hours after a tuberculin skin test. The cytolysis phenomenon appears to differ from tissue culture inhibition. The latter seems to be closely associated with the degree of skin sensitivity, as suggested by Waksman (1953 and 1959). On the other hand, the cytolysis phenomenon is not completely correlated with skin sensitivity, according to the reports of Favour *et al.* (1950) and O'Neill and Favour (1957).

The early observations on the cytotoxic effect of tuberculin on tuberculin sensitive tissues have been confirmed and extended by Heilman *et al.* (1944, 1946), Kirchheimer and Weiser (1948), Favour *et al.* (1950), Favour (1951), Fabrizio (1952), Waksman (1953a), O'Neill and Favour (1955), Gangarosa *et al.* (1955), Shea and Morgan (1957), O'Neill and Favour (1957), Hall and Scherago (1957), Citron (1958), Marks (1958), Heilman (1962), and Johnson and Scherago (1963). Each of these authors used the tuberculin system in working with either spleen explants, lymph

node explants, macrophages, lymphocytes, or peripheral blood leukocyte cultures from mice, guinea pigs, or rabbits. Tissue culture inhibition has also been observed by Moen (1936) in guinea pigs cells sensitive to hemolytic streptococci, by Johnson and Scherago (1960) and Moore and Scherago (1964) using guinea pig leukocytes from animals infected with Histoplasma capsulatum, by Heilman et al. (1958) in Brucella hypersensitivity, gy Glasgow and Morgan (1957) with guinea pig tissue sensitive to mumps virus, and by Wasserman (1962) and Lubaroff and Ritts (1964) in picryl chloride sensitivity. Similar techniques were used by David and Paterson (1965) to study cellular sensitivity in allergic encephalomyelitis.

Waksman (1959) believes that the degree of skin hypersensitivity of the donor animal is a critical factor in these techniques and that significant tissue culture inhibition can be demonstrated only with cultures from highly sensitive animals which show grossly necrotic skin reactions. Marks (1958) feels that the tissue culture cell migration technique is too susceptible to uncontrolled variation in cultural conditions to serve adequately in quantitative studies in tuberculin hypersensitivity. Despite these difficulties, the tissue culture inhibition phenomenon is believed to be a reaction of antigen with cellular antibody and supports the concept that the antibody responsible for tuberculin hypersensitivity is actually present in the tissues of the sensitive host. Warnatz and Scheiffarth (1964), using a fluorescent labelled antiglobulin test, found that cellular fixed antibody exists and that it reacts specifically with the corresponding antigens involved in transplantation immunity. Turk (1960) calculated the uptake of antigen in vitro

by the lymphocytes of sensitive and normal guinea pigs. He found a consistent increase in uptake of tuberculin in the sensitive cells. Ritts and Favour (1955) measured the uptake of I_{131}-PPD and found that it was firmly absorbed to the leukocytes during the first four hours. However, this was followed by a decrease in uptake. Barker et al. (1963) found that fluorescein labelled PPD was absorbed to both sensitive and normal cells.

Several other in vitro and in vivo techniques have been devised to study cellular hypersensitivity. Waksman and Matoltsky (1958b) and Metaxas and Metaxas-Buhler (1955) utilized a quantitative in vivo technique that involved mixing of sensitive cells with PPD and injecting the mixture into either the skin or cornea of normal guinea pigs. Wasserman and Packalen (1959) and Carpenter (1963) measured cellular sensitivity to various specific and non-specific bacterial products using the inhibition of migration of buffy coat leukocytes in capillary tubes as an index of toxicity. David et al. (1964) also measured cellular sensitivity to various specific and non-specific bacterial products using the inhibition of migration of buffy coat leukocytes from the end of capillary tubes into the surrounding media in the presence of specific sensitizing material. George and Vaughn (1962) measured degrees of cellular sensitivity by mixing antigen and sensitized cells and inoculating them intradermally into normal recipients. Bloch and Nordin (1960), using a similar technique, were able to sensitize guinea pigs by injecting them with normal peritoneal exudate cells which had been incubated with PPD.

The major objectives of the present research were to isolate, partially purify, and study the biological activity of guinea pig leukocytic

"transfer factor." This work involved biochemical analysis, antigenic studies, and passive transfer of various purified fractions from leukocytes of normal and tuberculin sensitive guinea pigs.

CHAPTER II

METHODS AND MATERIALS

Experimental Animals

Male, albino, Hartley strain guinea pigs (Cavia porcellus), weighing approximately 300-400 g were used as both donors and recipients in the experiments. Male, albino, New Zealand rabbits (Oryctolagus cuniculus) weighing approximately 3 kg were used for the preparation of antisera. The animals were obtained from commercial sources[1,2] and maintained on a diet of commercial pellets and cabbage.

Organism

A virulent strain ($H_{37}Rv$) of the human type of Mycobacterium tuberculosis was used throughout these studies. A large quantity of heat-killed, dried bacilli was prepared for subsequent use. For this purpose, four-week old cultures grown on a modified Proskauer and Beck medium (Youmans and Earlson, 1947) were killed by autoclaving at 120 C for 1 hr and then were filtered on a Buchner funnel. They were washed with distilled water until the washings were colorless. The washed bacilli were

---

Guinea Pigs -

[1]Camm Research Institute Incorporated, 414 Black Oak Ridge Road, Wayne, New Jersey.

Rabbits -

[2]Roy Allen Rabbit Ranch, Oklahoma City, Oklahoma

then transferred to a Petri dish and dried at 110 C for 1 hr. The dried bacilli were sealed in the Petri dish and stored at 4 C. For animal injections, the bacilli were weighed, ground to a fine powder in a motar, and adjusted to a final concentration of 5 mg per ml by dilution with light paraffin oil (Fisher).[3] The suspension was transferred to serum vials, autoclaved at 120 C for 15 min, and stored at 4 C until needed. Another sample of heat-killed, dried organisms was obtained through the courtesy of Dr. B. W. Janicki, Veterans Administration Hospital, Washington, D. C.

Sensitization of Guinea Pigs

Normal guinea pigs were rendered hypersensitive to tuberculin by the intramuscular injection of 1 ml of sterile paraffin oil containing 5 mg of heat-killed tubercle bacilli. Four weeks later the animals were tested for tuberculin hypersensitivity by the intradermal injection of 0.1 ml (5 μg) of PPD (Merck Sharp & Dohme)[4] into the shaved dorsal skin. The injection sites on animals were varied so that the location of the site would not influence the results. The diameter of induration, and the presence of erythema and/or central necrosis, were recorded 24 hr following the application of skin tests.

Collection of Guinea Pig Peripheral Blood Leukocytes

Whole blood was obtained by cardiac puncture using heparin (Upjohn),[5] at a final concentration of 0.4 mg per ml of blood, as an

---

[3]Fisher Scientific Company, Fairlawn, N. J.

[4]Merck Sharp & Dohme, West Point, Pennsylvania.

[5]The Upjohn Company, Kalamazoo, Michigan.

anti-coagulant. One ml of 4% polyvinylpyrrolidone (PVP) or 1 ml of a 20% dextran solution in physiological saline was added to 2 ml of blood to facilitate rouleaux formation and separation of erythrocytes. The mixtures were stored for 30 min at 4 C in the syringes in which they were collected, in an upright position, to allow the erythrocytes to settle out. A polyethylene tube was attached to the end of the syringe for removal of the leukocytes and supernatant plasma. Peripheral blood leukocytes were also isolated from heparinized whole blood by centrifugation at 3,000 rpm (1,500 X g) for 10 min. The buffy coat and plasma supernatant were then carefully removed with a capillary pipette (Scientific Products).[6]

Collection of Human Peripheral Blood Leukocytes

Sophomore medical students at the University of Oklahoma Medical School served as donors of normal and sensitive leukocytes. All potential donors were skin tested with 5 μg of PPD. Induration of 15 mm in diameter or greater in 24 hr was considered as sufficiently intense for the individual to serve as a donor of hypersensitive cells. Those individuals negative to this strength PPD were used as donors of negative cells. Tuberculosis patients at the Veterans Administration Hospital, Oklahoma City, Oklahoma, were also used as donors of hypersensitive cells. Twenty-five to fifty ml of blood was collected from each donor by venipuncture. A liquid oxalate solution containing 1.5 g ammonium oxalate and 1.0 g potassium oxalate in 100 ml of distilled water was used as an anti-coagulant at a final concentration of 0.4 ml of oxalate solution per

[6]Scientific Products, 2505 Butler, Dallas, Texas.

10 ml of blood. The buffy coat leukocytes were collected from the blood samples after centrifugation at 3,000 rpm (1,500 X g) for 30 min.

Collection of Guinea Pig Splenic Cells

When large numbers of guinea pig leukocytes were desired animals were sacrificed by cervical dislocation, the spleens were removed aseptically, and each one was placed in 5 ml of 199 tissue culture medium[7] in a Petri dish. Fat and blood vessels were dissected away and discarded. The spleens were then placed in a second sterile Petri dish containing 10 ml of 199 tissue culture medium and minced into small pieces using scissors. The tissue fragments were further disrupted into a cloudy suspension by aspiration with a pipette in such a manner as to avoid bubbles and marked pressure changes. At this point the suspension was passed through a 40 gauge wire screen which allowed the lymphoidal cells to pass and the fibrous stroma of the spleen to remain on the screen. The cell suspension was then centrifuged at 800 rpm (147 X g) for 10 min at room temperature and the packed cells were used in various experiments. These techniques are modifications of those described by Harris et al. (1962) and Janowsky et al. (1964).

Removal of Erythrocytes from Buffy Coat and Splenic Cells

The erythrocytes were removed from the whole blood buffy coat preparations and the suspensions of spleen cells by the following procedures. The spleen cells present in 199 tissue culture medium were centrifuged at 3,000 rpm (1,500 X g) for 10 min at room temperature. The

[7]Difco Laboratories, Detroit, Michigan.

supernatant was decanted and saved. To the packed cells was added 10 ml of .35% NaCl and the cells were then resuspended for 1 min. This resulted in lysis of the erythrocytes. The cells were quickly centrifuged again for 1 min at room temperature and the supernatant was discarded. The cells were then resuspended in 0.9% NaCl to restore physiological tonicity. The cells were again centrifuged and the supernatant was discarded. Finally, the original supernatant was added back to the packed cells. The supernatant for the buffy coat leukocytes was the plasma in which they were present. This technique is a modification of those described by Christlieb et al. (1962) and Shelly (1963).

Preparation of Lymphocyte-Monocyte Suspensions

Lymphocyte-monocyte suspensions were prepared from buffy coat and splenic leukocytes by methods patterned after those of Johnson and Garvin (1959) and Rabinowitz (1964). Super Brite glass beads (size 110-5005)[6] were added to a 50 cm glass tube supported by a ring stand. The tube was 1 cm in diameter and the beads were held in place with a small piece of glass wool at the bottom of the tube. For each 9.5 cm of packed beads 1 ml of previously described cell suspensions were added and allowed to stand for 5 minutes. The column was then drained. By this technique, the lymphocytes and monocytes pass through the column while the granulocytes adhere to the glass beads. The effluent was centrifuged at 3,000 rpm (1,500 X g) for 10 min and the cells were resuspended in 199 tissue culture medium such that the final concentration was 1×10^8 to 1×10^{11} cells per ml.

[6]Scientific Products, 2505 Butler, Dallas, Texas.

Collection of Guinea Pig Peritoneal Exudate Cells

Peritoneal exudates which consisted mainly of mononuclear cells were induced in guinea pigs by the intraperitoneal injection of 30 ml of sterile paraffin oil. Two days later the animals were killed by cervical dislocation. A small incision was made in the peritoneum and 30 ml of Ringer's solution[7] containing 0.005% heparin was introduced into the cavity. The incision was then closed with a hemostat and the animal was gently rotated in order to mix the oil and Ringer's solution. The peritoneal cavity and viscera were then washed with 10 ml of the heparinized Ringer's solution and this material was pooled with the original exudate. The exudate was then transferred to a separatory funnel and the aqueous layer was removed. The oil layer was washed with 30 ml of Ringer's solution and the washings were collected and pooled with the aqueous layer. The aqueous cell suspension was then centrifuged at 3,000 rpm (1,500 x g) for 15 min at room temperature. The supernatant fluid was discarded and the packed cells were washed 3 times with 0.9% NaCl. Finally, the cells were resuspended in 199 tissue culture medium and adjusted to a final concentration of 1×10^9 cells per ml.

Collection of Guinea Pig Lung Macrophages

In order to obtain a large population of lung macrophages, guinea pigs were sacrificed and their thoracic cavities were opened. The trachea and lungs were then removed as a single complex and rinsed with 0.9% NaCl that had been warmed to room temperature. The lungs were sponged free of excess fluid with gauze pads and 0.9% NaCl was injected into the trachea

[7]Difco Laboratories, Detroit, Michigan.

until the lungs were distended. The trachea was clamped off and the lungs were massaged for 10 min. The fluid was then drained into a sterile tube and the cell suspension was centrifuged at 3,000 rpm (1,500 X g) for 10 min. A small amount of cell and tissue debris and mucous was removed with a capillary pipette. This technique was a modification of those described by Myrvik et al. (1961) and Maxwell et al. (1964).

Preparation of Leukocyte Lysate

A leukocyte suspension of 1 x 10^8 to 1 x 10^{11} cells per ml, containing primarily mononuclear cells from buffy coat or spleen preparations, was subjected to 6 cycles of freezing in an alcohol bath in the deep freeze at -70 C and thawing at 37 C in a water bath. The cellular debris was removed by centrifugation at 3,500 rpm (2,500 X g) for 10 min and the supernatant fluid was used in various experiments to be described, after absence of intact cells was assured by microscopy.

Biochemical Analysis of Serum, Cells, and Cell Lysates

Naphthol Yellow S Staining of Buffy Coat Leukocytes

Slide preparations were made of buffy coat leukocytes from both sensitized and normal guinea pigs and stained with Naphthol Yellow S in order to measure total cell protein. For this purpose, a small drop of leukocyte suspension was added to a standard size microscope slide (Gold Seal) and a thin film of cells was spread over the surface of the slide. The slides were hydrated and stained 15 min at room temperature in 1% Naphthol Yellow S in 1% acetic acid. The pH of this solution was 2.78 and the ionic strength was 0.01. After staining, the slides were

removed without being blotted and placed in 50 ml of 1% acetic acid for 15-24 hr at room temperature. The slides were then blotted and passed through tertiary butyl alcohol and xylol at 15 sec intervals and mounted in Schillabers Refractive Index oil. The slides were photographed using an Alphax 35 mm Camera and Kodak Tri-X Pan film. The intensity of the staining reaction was measured with a photo-volt densitometer.

Electrophoretic Determinations

The electrophoretic separation of proteins and glycoproteins in serum and in lysates of sensitized and normal cells was performed on filter paper strips (Whatman 3 mm) using the Durrum type electrophoresis apparatus (Spinco Model R).[8] The separations were performed at room temperature at a constant current of 1 ma per strip in pH 8.6 veronal buffer (Spinco B-2 buffer) for 16 hr. After separation, the samples were fixed by drying at 100 C for 30 min.

The strips to be analyzed for protein distribution were stained with bromphenol blue by the method of Jencks *et al.* (1955). The strips were immersed in the protein dye (Spinco B-4) for 30 minutes at room temperature. Excess dye was removed from the strips by washing them thrice in 5% acetic acid for 6 min. The strips were then placed in a closed vessel containing ammonium hydroxide vapors for at least 10 min. They were removed one at a time for scanning. The relative per cent distribution of the various components was determined by scanning the stained strips in a Spinco Analytrol (Model RA) using blue filters.

The strips to be analyzed for glycoprotein were stained according to the periodic acid Schiff method of Koiw and Gronwall (1952) and

---

[8]Beckman Instruments, 2500 Harbor Blvd., Fullerton, California.

Shetlar et al. (1956). The strips were fixed in absolute ethanol for 5 min, treated with periodic acid for 5 min, and then rinsed in 70% ethanol. A solution of sodium bisulfate and potassium iodide was used for reducing the carbohydrate moiety. After rinsing in 70% alcohol the strips were stained for 40 min at room temperature using basic fuchsin sulfite, followed by three rinses in potassium metabisulfite. The strips were dehydrated, allowed to air dry, and were scanned in a Spinco Analytrol (Model RA) using Klett 52 green filters.

Glycoprotein Determinations

The glycoprotein content of sensitized and normal cells was determined according to the method of Shetlar et al. (1948). Buffy coat lymphocytes, spleen mononuclear cells, and peritoneal exudate cells were diluted 1:2 with 0.9% NaCl. Two-tenths ml of the diluted samples was pipetted, drop by drop, into 10 ml of absolute ethanol in a glass stoppered centrifuge tube. The precipitate which formed was centrifuged at 3,000 rpm (1,500 X g) for 10 min at room temperature and washed with 10 ml of absolute ethanol. After re-centrifugation, the ethanol was decanted and the precipitate was drained by inverting the tube. To the precipitate was added 1 ml of distilled water and 7 ml of 77% sulfuric acid and the tube was allowed to stand at room temperature for 10 min to dissolve the precipitate. It was then placed in an ice bath for 15 min. One ml of 1% aqueous tryptophane solution was added to each tube without shaking. The contents were mixed and the tube was placed immediately in a boiling water bath for 20 min. It was shaken once at the end of 10 min. The tube was then removed from the water bath, cooled for 5 min in an ice bath, and then allowed to stand for 25 min at room temperature. The

optical density of the solution was read in a Model B Beckman Spectrophotometer[8] at 500 mμ and the glycoprotein content of unknowns was calculated by reference to a standard curve prepared with a solution containing equal amounts of d-galactose and d-mannose.

Protein Determinations

A modification of the method of Lowry et al. (1951) was used for the determination of total serum protein. The serum samples were diluted in 1 ml buffered (pH 8.0) 0.9% NaCl to contain 25-200 μg protein per ml. A 5 ml quantity of reagent C, consisting of 50 ml of reagent A (10 mg of sodium carbonate made up to 500 ml with 0.1 N NaOH) and 1 ml of reagent B (1 g of potassium acetate and 0.5 gm of copper sulfate made up to 100 ml with distilled water), was added to 1 ml of diluted serum and the tubes were allowed to stand for 10 min at room temperature. A 0.5 ml quantity of reagent D (equal volumes of 1 N Folin-Phenol reagent and distilled water) was added rapidly, with immediate agitation, to each tube and they were then allowed to stand at room temperature for 20 min. The optical density of the solutions was read in a Beckman Junior Spectrophotometer[8] at a wave length of 650 m after standardizing the instrument with a blank tube containing the buffered saline. A standard curve was prepared using bovine serum albumin.[9] Serial dilutions of the albumin were made in the buffered saline to give a range from 10 to 100 μg of protein nitrogen per ml and determinations were made by the method described above. The optical density of the unknown solutions was converted to

---

[8]Beckman Instruments, 2500 Harbor Blvd., Fullerton, California.

[9]Armour Pharmaceutical Co., Kankakee, Illinois.

concentration of protein by reference to the standard curve.

Preparation of Antisera

Antisera to lysates of normal and sensitized cells were prepared in rabbits. The rabbits were given intramuscular injections of 0.5 ml of lysate incorporated into 0.5 ml Freund's incomplete adjuvant (Difco)[7] at weekly intervals for a period of seven weeks. Seven days after the last injection the animals were bled by cardiac puncture and the blood was placed in sterile tubes and allowed to clot at room temperature. The tubes were then placed at 7 C and after 18 hr the clot was separated by centrifugation at 3,000 rpm (1,500 X g) for 10 min. The sera were harvested, placed in sterile tubes, and stored at -20C until ready for use.

Immunodiffusion

Immunodiffusion methods similar to those of Ouchterlony (1949), Oudin (1952), and Feinberg (1957) were adapted for this study. A 1% solution of highly purified agar (Ionagar No. 2, Oxoid)[10] was prepared in 0.85% NaCl containing 0.01% merthiolate as a preservative. The solution was autoclaved at 121 C under 15 lb pressure for 15 min. The hot agar was pipetted in 15 ml quantities into sterile, standard size, disposable, plastic Petri dishes. The plates were allowed to stand at room temperature with their lids ajar until the agar hardened. For the purpose of further drying the plates were inverted and allowed to stand for 24 hr with the covers in place before being stored in an air tight plastic bag. The agar plates were allowed to harden for approximately two weeks before

---

[7]Difco Laboratories, Detroit, Michigan.

[10]Consolidated Laboratories, Box 234, Chicago Heights, Illinois.

use.

A commercial Feinberg agar cutter[10] (Shandon, Code No. 1801) which formed either two or four small wells arranged around a large central well, was used for preparing wells in the agar plates. Various well arrangements were used for the double diffusion studies. One arrangement consisted of four outer wells and a central well. This pattern was used to test antiserum in the central cell against several antigens in the four outer wells. In addition, the effect of dilution of antigen or antiserum upon the precipitin reaction was determined by placing the dilutions in the four outer wells. Another type of well arrangement consisted of two outer adjacent wells and a central well. This pattern was used to test for cross reactivity between two antigens in the outer wells when they were diffused against antiserum in the central well.

After the wells were cut the agar plugs were removed with a Pasteur capillary pipette attached to a suction apparatus. The appropriate wells were then filled with approximately 0.1 ml of antigen or antiserum by means of a Pasteur capillary pipette. The plates were incubated at 25 C and examined after varying lengths of time for the development of bands of precipitation. The number, intensity, and patterns of the bands were photographed using a Polaroid 800 camera.

Immunoelectrophoresis

Microimmunoelectrophoresis was carried out with a commercial apparatus (LKB 6800 A)[11] according to methods described by Grabar and

---

[10]Consolidated Laboratories, Chicago Heights, Illinois.

[11]LKB-Produkter AB, Stockholm, Sweden.

Williams (1953) and Grabar (1959).

The microscope slides were cleaned in sulfuric acid-dichromate and stored in acetone or ethanol until used. A film of hot adhesive agar solution was applied to the clean slides with a small brush and allowed to dry. The adhesive agar consisted of a 1% solution of Difco Special Noble Agar[7] and 0.05% glycerine in distilled water. Ten milliters of the 1% Nobel buffered (pH 8.6) agar was then added to each slide. After the agar had hardened overnight at 4 C the wells and troughs were cut using LKB gel punch sets. For comparing two antigen samples against a single immune serum the standard Die 6810 A set was used. It forms two wells, one on each side of a single trough. For comparing two immune sera against a single antigen the Standard Die 6811 B set was used. It forms three wells, one on each side of, and one between, two troughs. The well plugs were removed with a suction needle and to each well was added the cell lysate to be analyzed. The tray containing the slides was then placed in the LKB electrophoresis apparatus, 3276 BN. After one hr at 50 ma the slides were removed, the trough plugs which had been previously cut were removed with a suction needle, and immune serum was deposited in the troughs.

The trays of slides were incubated at room temperature in a humid chamber for approximately 20 hr, after which they were placed in several changes of 1% NaCl for 20 hr to remove all remaining unprecipitated protein. They were then placed in distilled water for 1 hr. The slides were dried and stained in amido black for 5 min. They were then immersed for 10 min in a rinsing solution consisting of methyl alcohol, acetic

---

[7]Difco Laboratories, Detroit, Michigan.

acid, and distilled water in a proportion of 45:10:45. This procedure was repeated three times and after drying, the slides were stored permanently in a special slide tray. Photographs of the slides were made using a Polaroid 800 camera.

Antibody Adsorption

Antiserum to sensitive cell lysates was analyzed by adsorption techniques. The antiserum (either undiluted or diluted 1:5 or 1:10) was added to an equal volume of normal cell lysate. The normal cell lysate was either undiluted or diluted 1:10. The mixture was allowed to react for 30 min at 37 C and the precipitate was removed by centrifugation at 3,000 rpm (1,500 X g) for 10 min. The supernatant was added to the central well of an Ouchterlony plate and diffused against varying dilutions of sensitized cell lysate.

Fractionation of Cell Lysates Using Sephadex Gel

Ten g of Sephadex G-200[12] (lot No. To-5761; particle size 40-120u) and 30 g of Sephadex G-50 (lot No. To-3569; particle size 100-300u) were suspended in excess distilled water and allowed to swell for 3 days with intermittent stirring and decantation. The gel was then washed with phosphate buffered 0.9% NaCl, pH 7.2. Each column was prepared by pouring a thin slurry of gel particles in the buffered saline solution into a vertical tube, already partly filled with the saline, and at the same time allowing excess of liquid to percolate through the growing gel bed. The tube containing Sephadex G-200 was 45 cm X 2.5 cm and the addition

[12]Pharmacia Fine Chemicals Incorporated, 800 Centennial Ave., Pescataway New Market, New Jersey.

of gel was continued until a bed height of 30 cm was obtained. The tube containing Sephadex G-50 was 40 cm X 1.5 cm and the final bed height was 23 cm. The void volume of each column was checked using dextran blue or hemoglobin. The top 2 or 3 cm of the columns were replaced with fresh gel after 4 or 5 uses in order to eliminate irregularities which develop at the top of the column with repeated use. The bottom of each column had a plastic disk to support the gel layer and a thin layer of glass wool above the disk in order to prevent blockage by the gel particles.

Using the method described by Baram and Mosko (1965) 2 ml of mononuclear cell lysate prepared as described previously was passed through the Sephadex G-200 column and 2 ml aliquots were collected from the column effluent. Thirty-six aliquots were collected and the ultraviolet absorption spectrum of each sample was determined using the Cary (Model 14) Recording Spectrophotometer.[13] The last 2 ml of effluent fraction was referred to as fraction IV. It was separated further on Sephadex G-50. Lysates of cells from normal and sensitized guinea pigs and humans were fractionated using this procedure.

Paper Chromatography of Sephadex G-50 Effluent Fractions

The effluent fraction III from Sephadex G-50 was added to an equal volume of 2N HCl and subsequently digested at 100 C for 4 hr in a sand bath. The hydrolysate was spotted on S and S Orange Ribbon Chromatography Paper No. 589.[14] An unhydrolyzed sample, and a standard solution containing 10 µg each of glucose, lactose, ribose, galactose, mannose,

---

[13]Applied Physics Corporation, Monrovia, California.

[14]E. H. Sargent Co., 4647 W. Foster, Chicago, Illinois.

deoxyribose, and glucosamine were also spotted as controls. The materials were chromatographed for 24 hr at room temperature in a solvent consisting of n-butyl alcohol, pyridine, and 0.1N HCl in a ratio of 5:3:2. The staining solution was composed of 1.69 g 2-aminobiphenyl, 0.9 g of oxalic acid, 5 ml glycerol, 10 ml distilled water, and 84 ml of acetone. The chromatograms are sprayed with the stain and placed in a 120 C oven for 5 min for the development of the colored spots. The color scheme consisted of pentoses-red, hexoses-greenish brown, and uronic acids-purple. The sensitivity of the reaction is 0.01 mols/square cm and the procedure used was patterned after the methods of Block (1964) and Trevelyan et al. (1950).

Preparation of Dialyzed Serum

Serum was prepared by collecting whole blood from five sensitized guinea pigs that had been skin tested with 5 μg PPD 48 hr before bleeding. The animals were bled by cardiac puncture and the blood was placed in sterile tubes and allowed to clot at room temperature. The tubes were then placed at 7 C and after 18 hr the clot was separated by centrifugation at 3,000 rpm (1,500 X g) for 10 min. The sera were harvested, placed in sterile tubes, and stored at -20 C until ready for use. Using the method of Tsuji et al. (1964) 2 ml of serum was placed in Visking tubing[6] having a diameter of 21 mm. The sample was dialyzed against 100 volumes of demineralized water for 12 to 24 hr at 4 C with constant stirring and the dialysate was used for passive transfer.

Preparation of Microsome Fractions

Microsomes from peripheral blood lymphocytes, lung macrophages,

[6]Scientific Products, 2505 Butler, Dallas, Texas.

spleen lymphocytes, and peritoneal exudate cells were prepared according to the procedure described by Fishman and Silverman (1957). 1 X 10^9 to 1.6 X 10^9 cells were added to 10 ml of .25M sucrose solution that had been previously chilled to 4 C. The cell suspension was homogenized for one min at 4 C in a coaxial homogenizer (Potter-Elvejehm type)[15] 6,000 X g. The various subcellular fractions were then separated by differential centrifugation at the following speeds, using a Serval Refrigerated Centrifuge:[16] Fraction I (nuclei), 600 X g for 10 min; Fraction II (mitochondria), 8,500 X g for 10 min; Fraction III (microsomes), 20,000 X g for 30 min; and Fraction IV, the remaining supernatant. Fraction I was subjected to 6 cycles of freezing at -70 C in an alcohol bath in a deep freeze and thawing at 37 C in a water bath to insure absence of whole cells, although none was observed by microscopy.

Passive Transfer Experiments

In all the passive transfer experiments, normal guinea pigs that had not been previously skin tested, were used as recipients. The recipient animals were injected intraperitoneally with a suspension of 1 x 10^{11} cells or the equivalent of cell lysate or extract. In a preliminary study the materials injected consisted of the following: cell free extract from normal guinea pigs, cell free extract from sensitized guinea pigs, viable normal cells, and viable sensitized cells. In a second experiment donor materials consisted of: cell free extract from normal guinea pigs, cell free extract from sensitized guinea pigs, Sephadex G-200 Fraction II

---

[15]W. H. Curtin Company, 514 E. 2nd, Tulsa, Oklahoma

[16]Ivan Sorvall, Inc., Norwalk, Connecticut.

from normal or sensitized cells, Sephadex G-200 Fraction IV from normal or sensitized cells, Sephadex G-50 Fraction I from normal or sensitized cells. All donor guinea pigs were skin tested with 5 µg PPD 48 hr before use in the transfer experiments and only those showing skin reactions with an induration of 15-18 mm were used. The recipient animals were skin tested with 5 µg PPD 1, 3, 7, and 14 days after transfer.

Histological Studies

Skin test sites were obtained by biopsy from various experimental and control guinea pigs used in passive transfer experiments. The skin test sites were collected from the following animals: normal, tuberculin negative; sensitized, tuberculin positive; recipient of sensitized cells; recipient of normal cells; and recipients of lysates of sensitized or normal cells. The tissues were subjected to standard histological procedures involving: fixation in 40% formaldehyde; dehydrating in 70, 80, 90, 95%, and absolute ethanol; clearing in absolute ethanol-xylol in a ratio of 1:1, followed by pure xylol; and imbedding in paraffin using an Autotechnicon[17] and Tissue-Teck.[18] The paraffin sections were cut using an 820 Spencer Microtome[3] and were affixed and mounted on standard Gold Seal slides using Mayer's Albumin Fixative.

After the sections were fixed to the slides the paraffin was removed with absolute xylol. The slides were then transferred to absolute alcohol to remove the xylol and were hydrated in an alcohol series

---

[17]The Technicon Company, Chauncey, New York.

[18]Lab-Tek Instruments Company, Westmont, Illinois.

[3]Fisher Scientific Company, Fairlawn, N. J.

proceeding from absolute ethanol to 95, 90, 80, and 70%. The sections were stained according to the Harris Hematoxylin Eosin method (1957). Each slide was placed in hematoxylin for 1 min until the section had a pronounced blue color. They were then transferred into a series of washes consisting of distilled water for 5 min, 70% ethanol-acid for 5 min, and 70% ethanol-alkaline or alkaline tapwater for 10 sec until the blue color was restored to the section. The sections were then stained for 30 sec in eosin, followed by complete dehydration in the alcohol series (70, 80, 90, 95%, and absolute ethanol). The sections were cleared in xylol and preserved and permanently mounted with commercial permount.

Measurement of Sensitivity of Leukocytes to PPD

Capillary Tube Assay

The degree of cellular sensitivity of leukocytes to PPD was measured according to a capillary tube method devised by Wasserman and Packalen (1959). Purified protein derivative (PPD) in concentrations of 5 to 10 µg was added to 0.4 ml whole blood from various test animals. The controls consisted of the whole blood with no PPD present. Thirteen to twenty capillary tubes (75 mm x 0.5-0.9 mm) were filled with the mixture and one end of each tube was closed with plasticene. The tubes were centrifuged at 3,000 rpm (1,500 X g) for 15 min and the length of the buffy coat in each tube was measured in mm using a standard ocular micrometer. The tubes were then incubated for 18 hr at 37 C and the length of each buffy coat was measured again to determine the distance of migration. The cytotoxic index was computed according to the method of Heilman et al. (1944): Mig. = migration

$$\text{Cytotoxic Index} = \frac{\text{Ave.Mig.Normal cells}}{\text{Ave.Mig.Normal cells + PPD}} \times \frac{\text{Ave.Mig.Sen.cells + PPD}}{\text{Ave.Mig.Sen.cells}}$$

A value less than 1.00 is indicative of significant inhibition.

In Vivo Assay

The degree of cellular sensitivity of leukocytes to PPD was also measured by a modification of the *in vivo* method described by George and Vaughn (1962). 1×10^5 lung macrophages, 1×10^7 peripheral blood lymphocytes, or 1×10^8 spleen lymphocytes were mixed with 5 μg PPD in a total volume of 0.1 ml and immediately injected intradermally into normal guinea pigs. The skin reactions were observed for the next 48 hr and the 24 hr reading was recorded. The mononuclear cells were prepared according to methods described previously.

Passive Sensitization of Normal Cells with Cell Lysates, Dialyzed Serum, Microsomes, and Sephadex Fractions

Using methods similar to those described by Oprescu (1962), 1 ml of the dialyzed serum, microsomes, Sephadex fractions, or cell lysates prepared from sensitized animals according to methods described previously, were incubated with normal spleen lymphocytes (1×10^8), peripheral blood lymphocytes (1×10^7), or lung macrophages (1×10^5) for 2 hr at 37 C. The mixtures were then centrifuged at 3,000 rpm (1,500 X g) for 15 min and the supernatant was discarded. The packed cells were washed and resuspended in 0.9% NaCl. This procedure was repeated three times. The cells were then mixed with 5 μg PPD in a total volume of 0.1 ml and immediately injected intradermally into normal guinea pigs. The skin reactions were observed for the next 48 hr and the 24 hr reading was recorded. On occasion the cells were injected intradermally into one skin site and the

5 μg of PPD was injected into an adjacent site.

The dialyzed serum and sensitized cell lysates were also incubated with buffy coat leukocytes from normal guinea pigs. In addition, Sephadex fractions and microsomes from both guinea pigs and humans were incubated with buffy coat leukocytes of the respective species. For this purpose, 5 ml of whole heparinized blood was centrifuged at 3,000 rpm (1,500 X g) for 15 min. The buffy coat was removed with a Pasteur capillary pipette and added to 1 ml of dialyzed serum, microsomes, Sephadex fractions, or cell lysates. After 2 hr at 37 C the mixture was centrifuged at 3,000 rpm (1,500 X g) for 15 min and the supernatant was discarded. The packed cells were washed and resuspended in 0.9% NaCl. This washing procedure was repeated three times and the cells were added back to the plasma from which they were removed. The degree of cellular sensitivity was then assayed according to the capillary tube method described previously. The cellular sensitivity of actively and passively sensitized guinea pigs was also examined by these techniques.

Interspecies Transfer of Tuberculin Sensitivity

Cell lysates, microsomes, and Sephadex fractions from sensitized guinea pigs were used in attempts to sensitize human buffy coat leukocytes to PPD according to techniques described previously. The same experimental design was used to determine whether extracts of human leukocytes are capable of sensitizing guinea pig cells.

CHAPTER III

RESULTS

The early phase of this investigation involved the measurement of total cell protein in sensitized and normal buffy coat leukocytes using the Naphthol Yellow S procedure. It was found from the densitometric calculations that the mononuclear leukocytes from 10 tuberculin sensitive guinea pigs had at least 25% greater dye binding capacity than the mononuclear leukocytes from 10 normal guinea pigs. The results of these experiments are shown in Table 1.

These results correlated well with the results of the electrophoretic analysis of lysates of normal and sensitized peripheral blood leukocytes prepared by the freeze-thaw technique. The analysis of cell lysates derived from 1×10^8 leukocytes/ml from 25 normal and 25 sensitized guinea pigs indicated that the sensitized cell lysates contained approximately twice as much material that migrated as an alpha globulin as compared to the normal cell extract. These results are presented in Table 2.

Since the alpha globulins contain a number of glycoprotein compounds the extracts from buffy coats of 34 sensitized and 34 normal guinea pigs were analyzed biochemically. These cells were obtained by the glass bead filtration technique described previously and consisted of 90% buffy

TABLE 1

RELATIVE PROTEIN CONTENT OF BUFFY COAT MONONUCLEAR CELLS

Source of Cells	Total Number Samples/Animal	Total Number Cells/Sample	Densitometric Units Mean (Range)
Normal Guinea Pigs (10)[a]	10	100	2.6(2.2-2.8)
Sensitized Guinea Pigs (10)	10	100	3.4(3.1-3.9)[b]

[a]Number of animals

[b]$P = 0.05$

TABLE 2

RELATIVE ALPHA GLOBULIN CONTENT OF PERIPHERAL BLOOD LEUKOCYTES

Source of Cells	Total Number Samples/Animal	Globulin Units Mean (Range)
Normal Guinea Pigs (25)[a]	12	21.5(15-27)
Sensitized Guinea Pigs (25)	12	46.0(35-108)

[a]Number of animals

coat lymphocytes and less than 10% neutrophils. The cell suspension obtained from spleens using this technique yielded a suspension containing 98% mononuclear cells and only 2% granulocytes. From the results presented in Table 3 it will be seen that the extracts from 1 x 10^8 sensitized mononuclear cells/ml contained an average of 1.71 mg/ml glycoprotein while the extracts from normal leukocytes contained only 1.20 mg/ml. This would represent at least a 25% higher level of glycoprotein in the sensitized cell extract as compared to the normal cell extract.

It was found by electrophoresis of the lysates of leukocytes of 10 sensitized and 10 normal guinea pigs, and staining with PAS, that the alpha globulin component was probably a glycoprotein because of its dye binding capacity. The extract contained 1 x 10^8 cells/ml and 3 samples from each guinea pig were examined. These results are presented in Table 4. The content of the strong PAS positive component was again at least 25% higher in the sensitized leukocyte extracts than the normal cell extracts.

Total serum protein determinations and electrophoretic distribution of the serum proteins were done using 10 normal and 10 sensitized guinea pigs. Serum was collected both before and 48 hr after application of a skin test with 5 μg PPD. The results are presented in Table 5. It will be seen that serum from tuberculin sensitive guinea pigs contained significantly more alpha 1 globulin that did that from normal animals. Forty-eight hours after a PPD skin test, sensitized guinea pigs responded with a further increase in serum alpha 1 globulin, plus an increase in alpha 2 globulin and a decrease in albumin.

The antigenic properties of "transfer factor" were studied by

TABLE 3

GLYCOPROTEIN CONTENT OF CELL LYSATES

Source of Cells	Total Number of Samples	Glycoprotein Mean (Range) mg/ml
Normal Guinea Pigs (34)[a]	102	1.20(1.16-1.34)
Sensitized Guinea Pigs (34)	102	1.71(1.60-1.86)[b]

[a] Number of animals

[b] P = .005

TABLE 4

ELECTROPHORETIC ANALYSIS OF GLYCOPROTEIN CONTENT OF CELL LYSATES

Source of Cells	Total Number of Samples	Globulin Units Mean (Range)
Normal Guinea Pigs (10)[a]	30	16.5(15-17)
Sensitized Guinea Pigs (10)	30	23.5(22-35)

[a]Number of animals

TABLE 5

EFFECT OF PPD SKIN TEST ON ELECTROPHORETIC DISTRIBUTION OF SERUM PROTEINS IN GUINEA PIGS

Source of Serum	Gm%					
	Albumin	Alpha 1 globulin	Alpha 2 globulin	Beta globulin	Gamma globulin	Total Protein
Normal Guinea Pigs:						
Pre-Test	4.20	0.30	0.48	0.80	1.31	7.09
Post-Test	4.00	0.30	0.50	0.80	1.30	6.90
Sensitized Guinea Pigs:						
Pre-Test	4.00	0.50[a]	0.51	0.80	1.27	7.08
Post-Test	1.75[a]	2.00[a]	0.90[a]	0.81	1.25	6.71

[a] $P = .05-.01$

producing antisera in rabbits to lysates of normal and sensitized guinea pig leukocytes. The rabbit anti-leukocytic serum was tested for the presence of precipitating antibodies using immuno-diffusion plates and immunoelectrophoresis. Antiserum (either undiluted or diluted 1:10 or 1:100) against normal or sensitized cell lysates was tested against undiluted cell lysates. The antiserum was placed in the center well of the Ouchterlony plate. In two outer wells was placed normal cell lysate; in the other two outer wells was placed sensitized cell lysate. The results of a typical experiment are illustrated in Figure 1. After 24-48 hr incubation at least 6 intense precipitin bands had developed in all systems used. From these results, however, it appeared that the sensitized cells did not have a specific antigen that was not present in normal cells. The effect of dilution of the antigen is illustrated in Figure 2. It was found that diluting the antigen reduced the number of precipitin bands from 6 to 3 when diffused against appropriate antiserum. Again, however, the same antigenic materials was detected in both the sensitized and normal cell lysates. The sera against normal cell lysates reacted equally well with diluted sensitized cell lysates and the sera against sensitized cell lysates formed the same number and type of bands with diluted sensitized or normal cell lysates. A maximum of 6 precipitin bands was demonstrable using the plate techniques.

Microimmunoelectrophoresis was carried out on the same materials. Antiserum (either undiluted or diluted 1:10 or 1:100) against normal or sensitized cell lysates was tested against undiluted cell lysates. The antiserum was placed in the center trough following the electrophoretic run with sensitized cell lysates in one well and normal cell lysate in

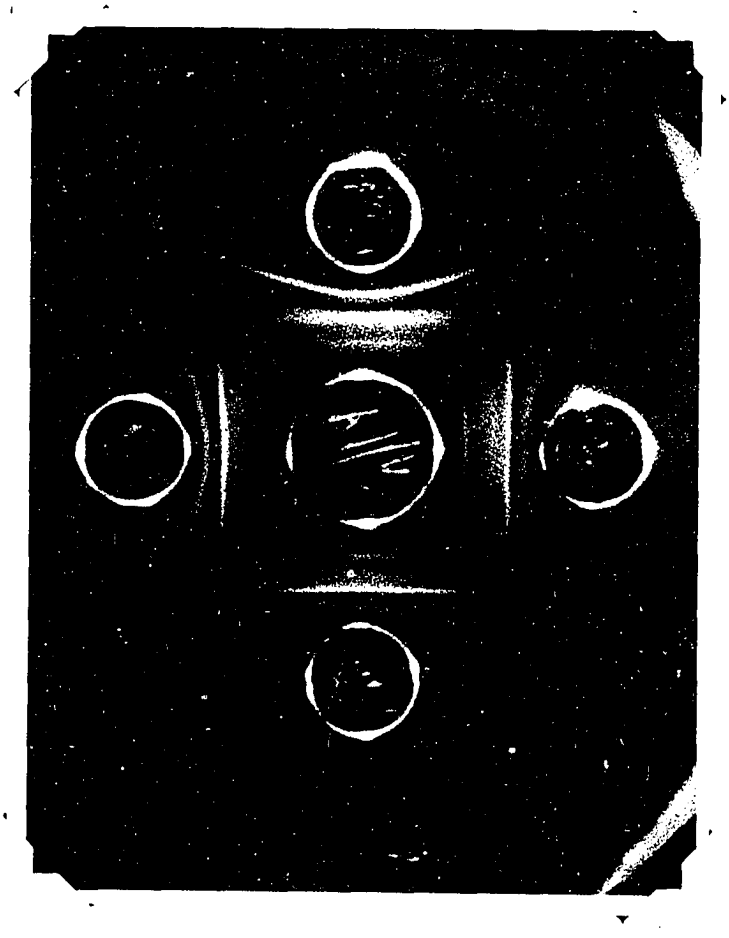

Figure 1. Photograph of Reaction Between Antiserum Against Sensitized Cell Lysates (Center Well) and Normal Cell Lysates (Upper and Lower Outer Wells) and Sensitized Cell Lysates (Lateral Wells). Magnification X 4.

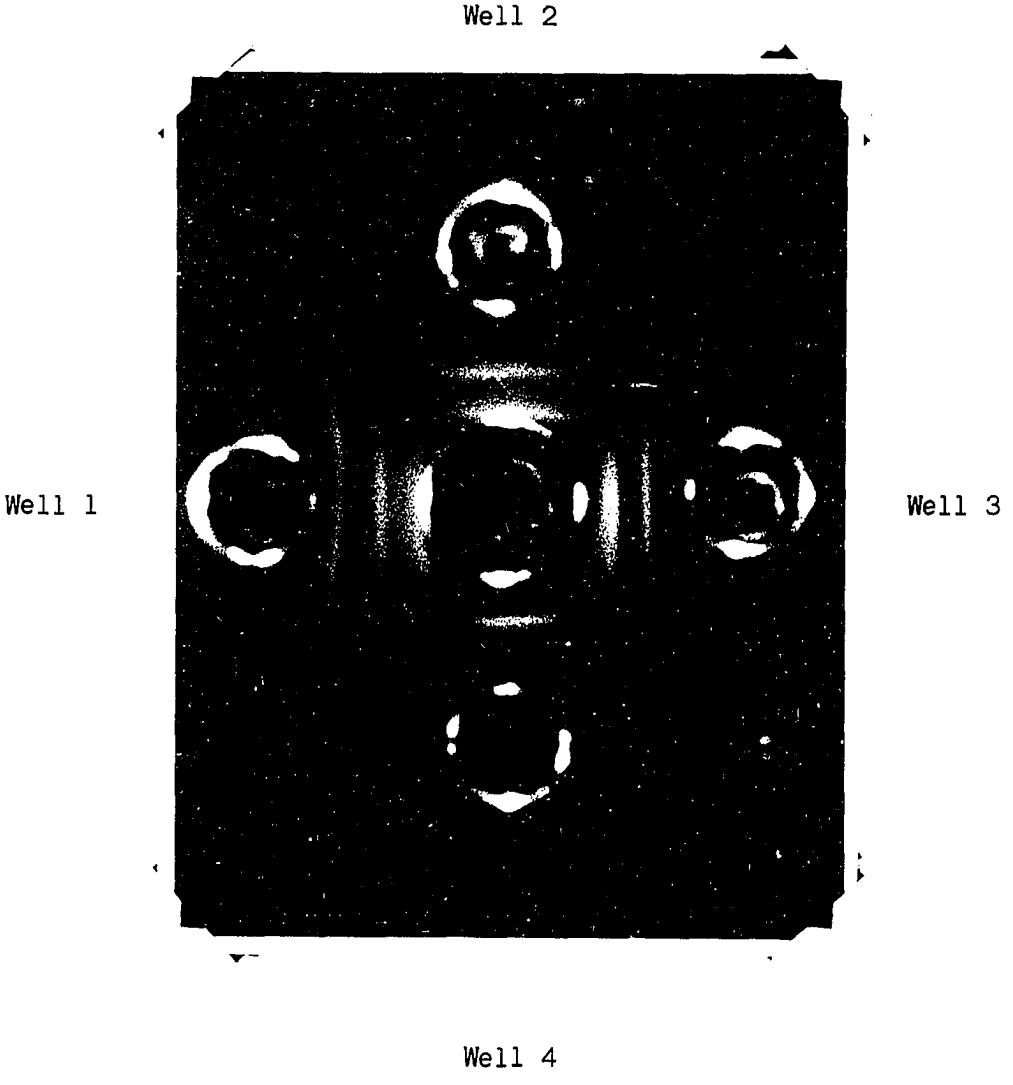

Figure 2. Photograph of Reaction Between Antiserum Against Sensitized Cell Lysates (Center Well) and Dilutions of Sensitized Cell Lysates; Undiluted Lysates in Well 1 and 2, 1:10 Dilution in Well 3, and 1:100 Dilution in Well 4. Magnification X 4.

the other well. The results of these experiments are presented in Figures 3 and 4. Figure 3 illustrates the reaction of anti-normal leukocytic serum versus cell lysates. Figure 4 illustrates the reaction of anti-sensitized leukocytic serum versus cell lysates. From these results it appeared that the sensitized cells did not have a specific antigen that was not present in normal cells.

The antiserum to the sensitized cell lysates was analyzed further by adsorption techniques. The results revealed that normal cell lysates adsorbed all antibody contained in the antiserum prepared against sensitized cell lysate. Following adsorption, the antiserum was added to the center well of an Ouchterlony plate and diffused against varying dilutions of sensitized cell lysates. No precipitin bands were observed.

Following separation of peripheral blood mononuclear cells and spleen lymphocyte lysates using Sephadex gel filtration, the Fraction IV from Sephadex-200 was found to have 2 major peaks with ultraviolet absorption maxima at 260 mμ and 280 mμ. This is illustrated in Figure 5. The Sephadex G-200 Fraction IV was then separated using Sephadex G-50. Fraction I contained a major peak with maximum absorption at 280 mμ while Fraction III contained a major peak at 260 mμ. This is illustrated in Figure 6.

Hydrolysis and paper chromatography of Sephadex G-50 Fraction III revealed the presence of ribose sugar. This sugar could not be detected before hydrolysis, nor was any other sugar detected in the sample. In addition to ribose, the sample was analyzed for glucose, lactose, galactose, mannose, deoxyribose, and glucosamine.

1×10^8 normal mononuclear leukocytes incubated with 1 ml of

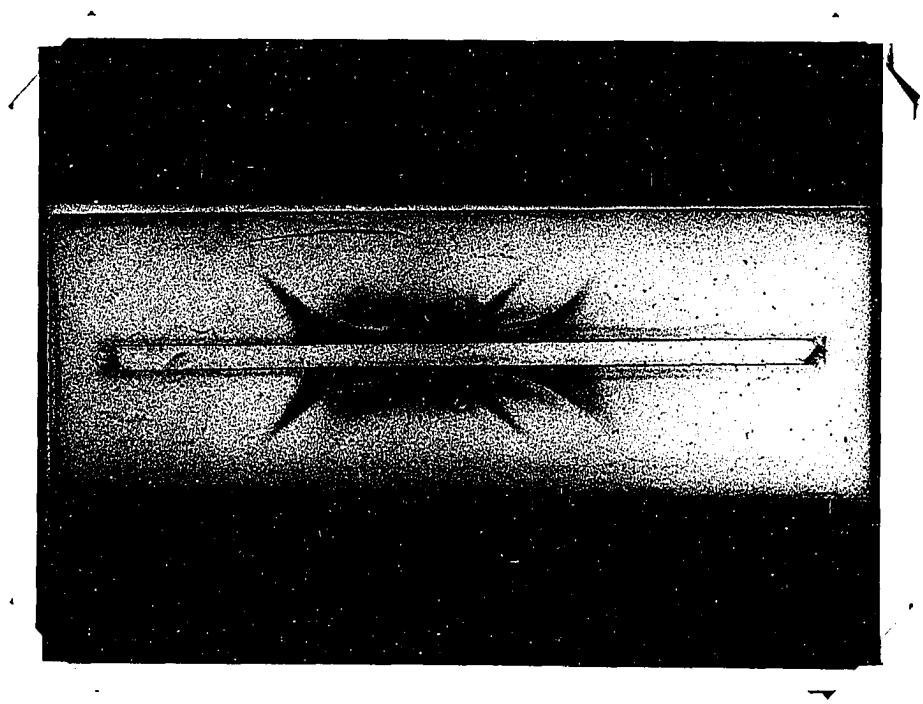

Figure 3. Photograph of Reaction Between Anti-Normal Leukocyte Serum (Center Trough) and Normal Cell Lysate (Upper Well) and Sensitized Cell Lysate (Lower Well). Magnification X 2.

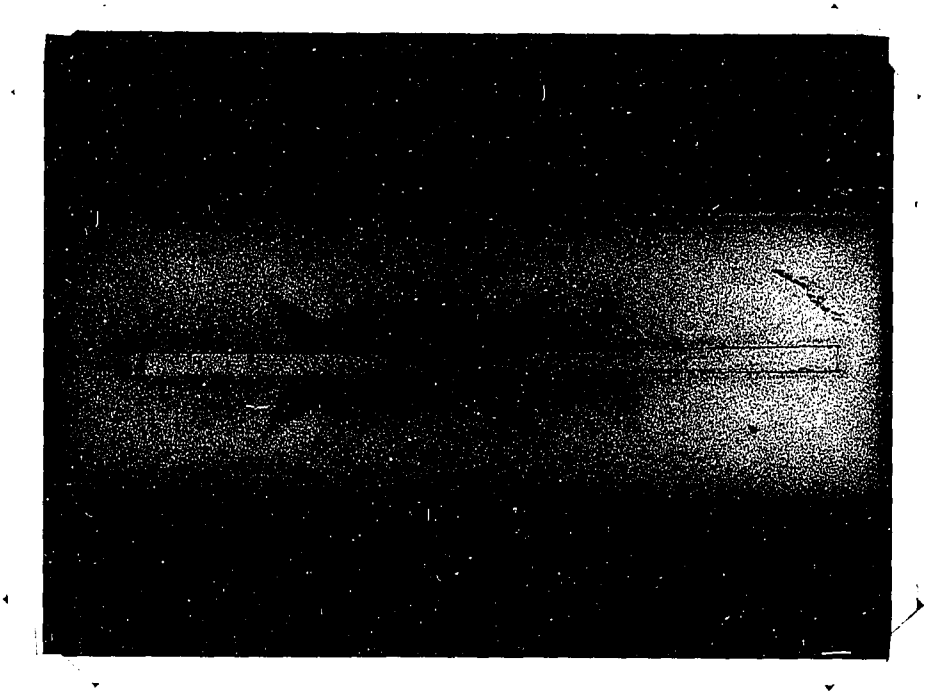

Figure 4. Photograph of Reaction Between Anti-Sensitized Leukocyte Serum (Center Trough) and Normal Cell Lysate (Upper Well) and Sensitized Cell Lysate (Lower Well). Magnification X 2.

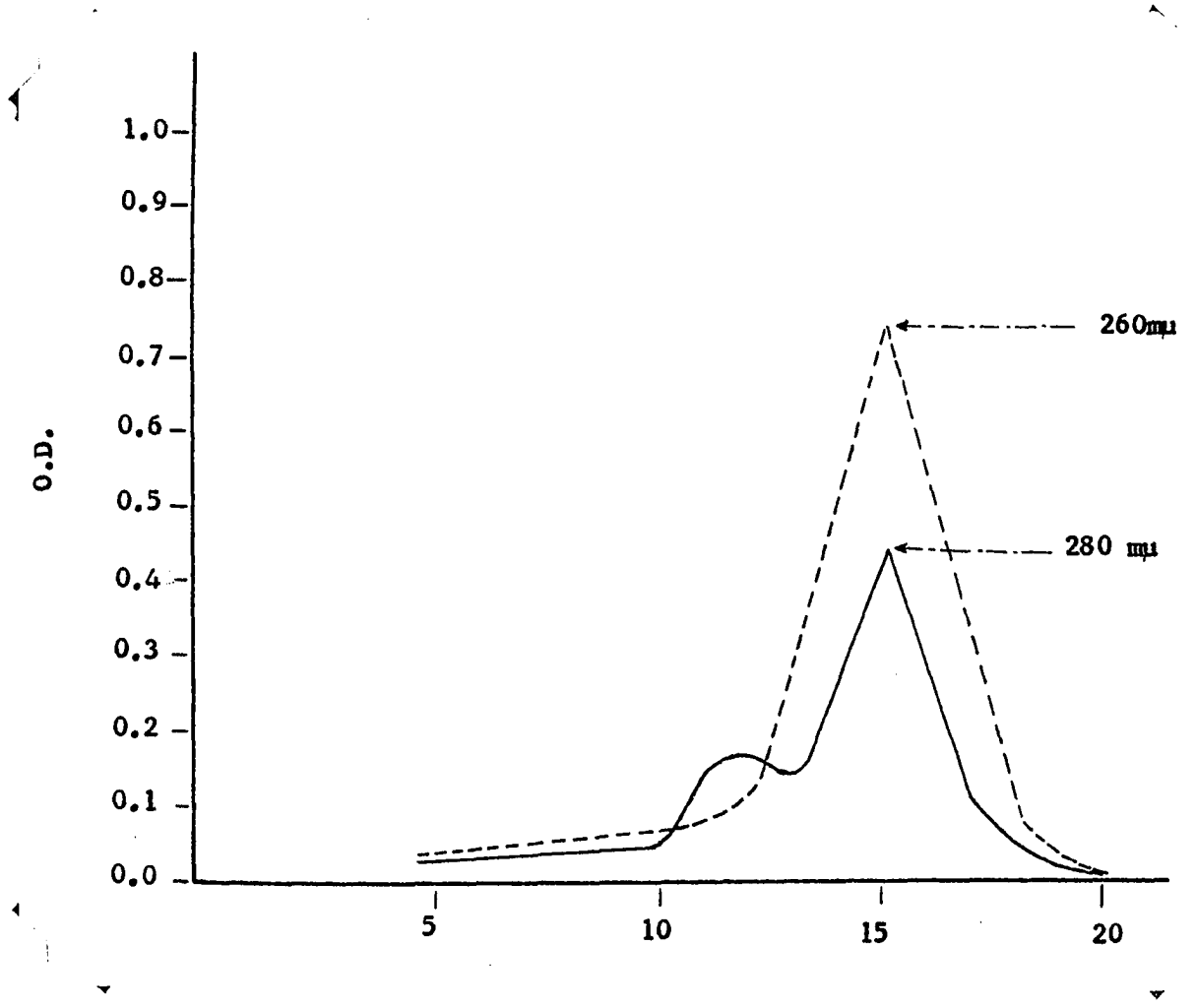

Figure 5. Ultraviolet Absorption Spectrum of Fraction IV Obtained by Sephadex G-200 Filtration.

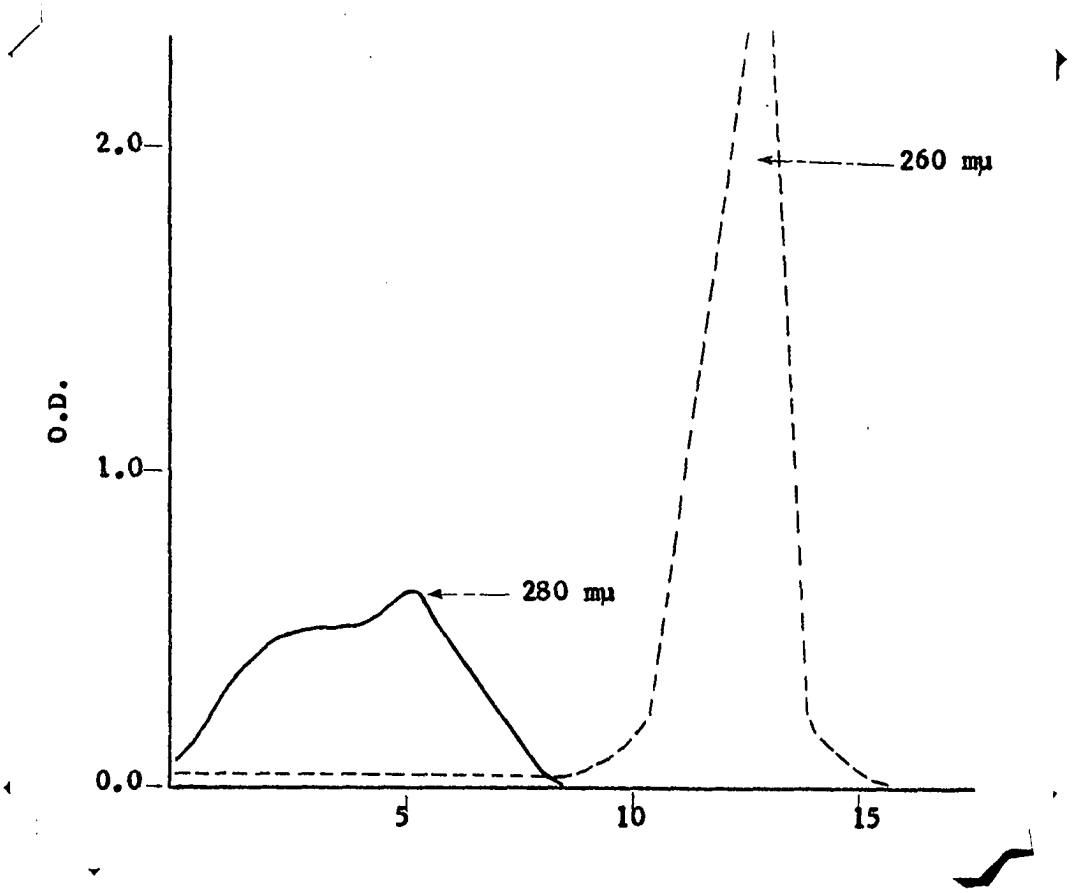

Figure 6. Ultraviolet Absorption Spectrum of Fraction IV Separated by Sephadex G-50 Filtration.

sensitized cell lysate or Sephadex G-50 Fraction III from peripheral blood mononuclear cells and spleen lymphocytes were analyzed for their content of glycoprotein after 10 min and 2 hr. The results of this study are shown in Table 6. It will be seen that, with both materials, 2 hr of incubation resulted in glycoprotein levels essentially equal to those seen in actively sensitized cells (Table 3).

The results of the preliminary passive transfer study will be seen in Table 7. Both viable sensitized cells and lysates thereof were capable of passive transfer. The skin reactions in the recipient animals did not appear until after 12 hr and were maximal in 24 hr, with characteristic erythema and induration. Skin reactions in animals that received lysates of sensitized cells and viable, sensitized cells were much weaker than those seen in actively sensitized donors. Transfer of cell lysates or viable cells from normal donors into normal recipients never resulted in production of skin sensitivity. Normal controls were repeatedly skin tested with PPD to be certain such treatment did not induce tuberculin sensitivity.

The histology of positive skin test sites in guinea pigs sensitized passively appeared to be similar to that seen in actively sensitized animals. Both sites had large infiltrations of mononuclear cells, few or no polymorphonuclear leukocytes, and no observable vascular damage. The skin test sites of normal controls and of normal guinea pigs which received whole cells or lysates from a normal donor were histologically normal. Figure 7 illustrates the PPD skin test site in a recipient animal that received normal cells in the passive transfer. Figure 8 illustrates the PPD skin test site in a recipient animal that received whole sensitized

TABLE 6

GLYCOPROTEIN CONTENT OF NORMAL LEUKOCYTES INCUBATED IN PRESENCE OF SENSITIZED CELL LYSATE OR SEPHADEX G-50 FRACTION III

Cell System	Incubation Period	Glycoprotein Mean (Range) (mg/ml)
Normal cells (12)[a] + cell lysate (6)[b]	10 min	1.22(1.20-1.23)
	2 hr	1.71(1.70-1.76)[c]
Normal cells (12) + Sephadex G-50 Fraction III (6)	10 min	1.21(1.20-1.26)
	2 hr	1.70(1.68-1.76)[c]

[a] Number of recipient animals.

[b] Number of donor animals.

[c] $P = .005$

TABLE 7

PASSIVE TRANSFER OF TUBERCULIN SENSITIVITY

Material Transferred (Number of Animals)	PPD Skin Test Results (mean mm. induration) Days after Transfer			
	1	3	7	14
Viable normal cells (2)	0	0	0	0
Normal cell lysates (3)	0	0	0	0
Viable sensitized cells (2)	5	8	8	10
Sensitized cell lysates (3)	3	7	8	9



Figure 7. Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received Cells from a Normal Guinea Pig. Magnification X 430.

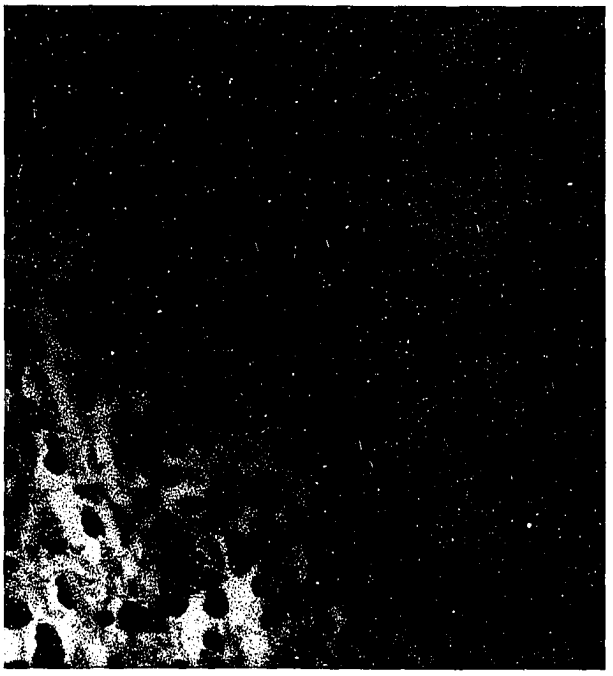

Figure 8. Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received Cells from a Sensitized Guinea Pig. Magnification X 430.

cells in passive transfer. Figure 9 illustrates the PPD skin test site in a recipient animal that received sensitized cell lysate in passive transfer.

Further passive transfer studies involved the use of cell lysates and Sephadex fractions. The results of these experiments are illustrated in Table 8. It will be seen that Sephadex G-200 Fraction IV and G-50 Fractions I and III from sensitized cells were, like crude cell lysate, capable of passive transfer of tuberculin sensitivity. It is of interest to note that animals that received Fraction III were somewhat slower in developing significant skin reactivity than those that received either Fraction I, Fraction IV, or crude cell lysate. Again, the histology of positive skin test sites in guinea pigs sensitized passively appeared to be similar to that seen in actively sensitized animals. Both sites had large infiltrations of mononuclear cells, few or no polymorphonuclear leukocytes, and no observable vascular damage. The skin test sites of normal controls and of normal guinea pigs which received lysate or fractions from normal donors were histologically normal. Figure 10 illustrates a PPD skin test site in a recipient animal that received G-50 Fraction III from sensitized cells.

The results of preliminary studies on the use of the capillary tube assay technique for measuring cellular sensitivity are illustrated in Table 9. The results indicated that PPD has a significant effect on the migration of leukocytes from sensitized animals. There appeared to be little or no effect of PPD on migration of normal blood cells except at the higher concentration (10 μg).

Preliminary observations on the use of the *in vivo* assay

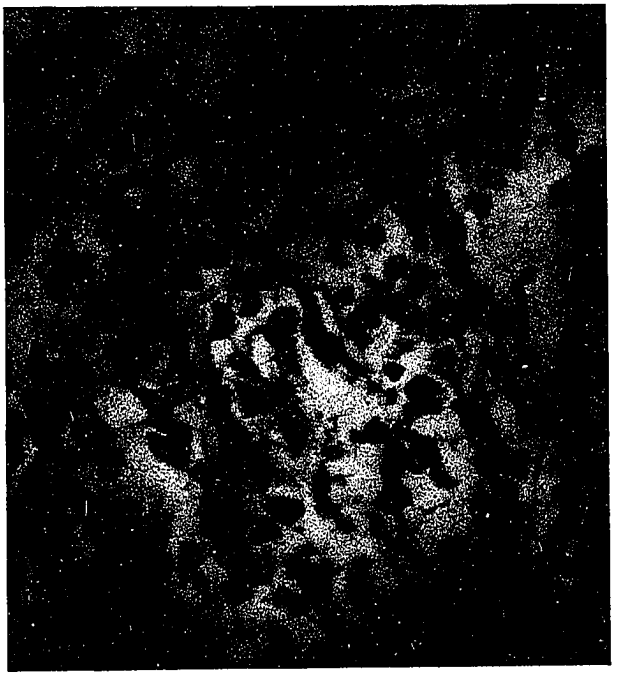

Figure 9. Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received Cell Lysates from a Sensitized Guinea Pig. Magnification X 430.

TABLE 8

PASSIVE TRANSFER OF TUBERCULIN SENSITIVITY
WITH SENSITIZED CELL FRACTIONS

Material Transferred (Number of Animals)	PPD Skin Test Results (mean mm. induration) Days after Transfer			
	1	3	7	14
Normal cell lysates (2)	0	0	0	0
Sensitized cell lysates (3)	3	5	8	10
Fraction IV, G-200, normal cells (2)	0	0	0	0
Fraction IV, G-200, sensitized cells (3)	5	7	10	9
Fraction III, G-50, normal cells (2)	0	0	0	0
Fraction III, G-50, sensitized cells (3)	0	3	8	10
Fraction I, G-50, normal cells (2)	0	0	0	0
Fraction I, G-50, sensitized cells (3)	3	7	9	0
Fraction II, G-200, sensitized cells (2)	0	0	0	0
Fraction II, G-200, normal cells (2)	0	0	0	0

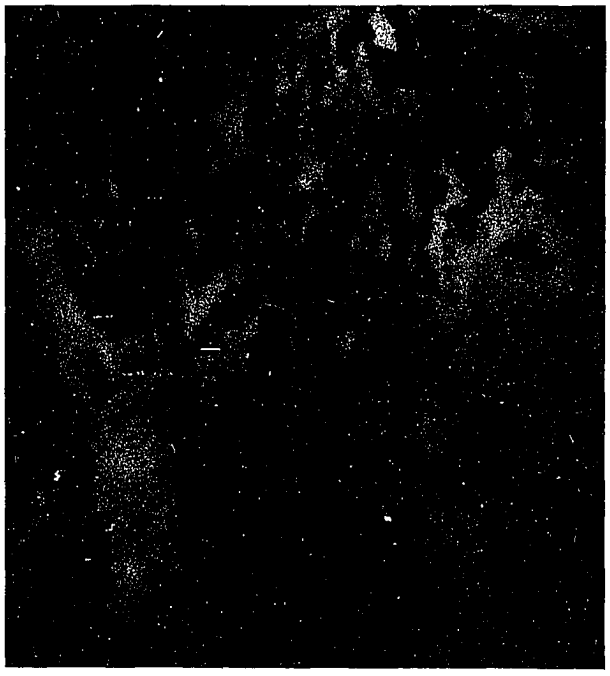

Figure 10. Section of PPD Skin Test Site in a Normal Guinea Pig Which Had Received G-50 Fraction III of Cells from a Sensitized Guinea Pig. Magnification X 430.

TABLE 9

IN VITRO CAPILLARY TUBE MIGRATION OF BUFFY COAT LEUKOCYTES FROM TUBERCULIN SENSITIVE AND NORMAL ANIMALS

	Cytotoxic Index[a]				Average Cytotoxic Index
Sensitized Cells +					
No Antigen	.98	1.01	.97	.98	1.01
	1.05	.96	1.07	1.08	
	.98	1.12	.98	1.12	
	1.02	.95	.95	.97	
PPD, 5 µg	.23	.15	.29	.26	.26[b]
	.23	.21	.30	.31	
	.28	.35	.28	.28	
	.18	.26	.27	.28	
PPD, 10 µg	-.02	-.05	-.07	-.05	-.06[b]
	-.05	-.02	-.05	-.07	
	-.10	-.15	-.10	-.08	
	-.21	-.02	-.12	-.04	
Normal Cells +					
No Antigen	.98	1.01	1.05	.99	1.03
	.98	1.21	1.20	1.09	
	1.02	.97	.98	1.10	
	.97	.95	1.07	.97	
PPD, 5 µg	1.02	.98	1.00	.98	.97
	.95	.95	.99	1.00	
	.97	.97	.97	1.02	
	.87	.98	.95	.98	
PPD, 10 µg	.76	.69	.78	.68	.73
	.79	.75	.79	.67	
	.68	.65	.78	.75	
	.72	.75	.78	.67	

[a] Each value represents a cytotoxic index derived from cells obtained from a single guinea pig.

[b] $P = 0.005$

technique to measure cellular sensitivity are shown in Table 10. It will be seen that, by this method, peripheral blood lymphocytes, lung macrophages, and to a lesser degree, spleen lymphocytes, were all capable of passive transfer. There appeared to be little or no effect of PPD on normal cells when injected into normal guinea pigs except a small inflammatory reaction. Injection of PPD alone into the intradermal site in normal guinea pigs resulted in no visable reactions. Injection of normal or sensitized cells resulted in only a small inflammatory reaction.

Passive sensitization of normal guinea pigs with 1×10^7 peripheral blood lymphocytes from sensitized donor animals could be inhibited when .1 ml of undiluted antiserum against sensitized cell lysates was mixed with the sensitized cell-PPD mixture described previously. These results are shown in Table 11

The next experiments were designed to determine the ability of normal cells to be sensitized to PPD following incubation with cell lysates from sensitized animals. In these experiments 1×10^7 normal guinea pig peripheral blood mononuclear leukocytes were incubated with cell free extracts from 1×10^{11} disrupted spleen cells or buffy coat leukocytes. The results of these experiments are presented in Table 12, where it will be observed that normal peripheral blood mononuclear cells were capable of being sensitized to PPD following incubation with cell extracts from spleen lymphocytes or buffy coat leukocytes. Each value represents the result seen with a single recipient. The degree of sensitivity was not as great as that seen with cells collected from actively sensitized animals but the results illustrated the ability of normal cells to be sensitized using cell free extracts. Table 13 illustrates the

TABLE 10

SKIN TEST RESULTS IN GUINEA PIGS INJECTED WITH 5 μg PPD MIXED WITH VIABLE CELLS FROM SENSITIZED OR NORMAL ANIMALS

mm. induration (24 hr)	
Cells from Sensitized Donors	Cells from Normal Donors
Peripheral Blood Lymphocytes (1×10^7)	
10.0	1.6
9.5	1.4
11.0	1.2
12.0	1.0
9.0	1.2
10.0	1.5
12.0	1.2
12.0	1.2
Avg. = 10.7[a]	Avg. = 1.3
Lung Macrophages (1×10^5)	
12.5	1.0
13.0	1.5
12.5	1.2
12.0	1.6
12.0	1.0
15.0	1.2
12.0	1.4
14.0	1.2
Avg. = 12.8[a]	Avg. = 1.3
Spleen Lymphocytes (1×10^8)	
2.5	1.2
2.8	1.6
3.0	1.5
2.5	1.5
3.5	1.4
4.0	1.2
3.5	1.5
3.5	1.4
Avg. = 3.1[a]	Avg. = 1.4

[a] $p = .05$

TABLE 11

THE EFFECT OF ANTISERA ON THE IN VIVO ASSAY OF CELL SENSITIVITY

Cells from Sensitized Donors[a] +	mm. induration (24 hr)
PPD	10.0 9.0 10.5
PPD + Antiserum against sensitized cells	2.0 1.5 2.0
Antiserum against sensitized cells	< 1.0 < 1.0 < 1.0
PPD + antiserum against normal cells	9.0 7.0 10.0

[a] Each value represents a single donor of sensitized cells.

TABLE 12

SKIN TEST RESULTS IN GUINEA PIGS INJECTED WITH NORMAL CELLS INCUBATED WITH NORMAL AND SENSITIZED CELL LYSATES

Cell System	mm. induration (24 hr)
Normal cells + No Antigen	< 1
	< 1
	< 1
	< 1
	< 1
Normal cells + PPD	1.4
	1.2
	1.2
	1.4
	1.4
Normal cells + normal cell lysates + PPD	1.2
	1.4
	1.4
	1.2
	1.6
Normal cells + sensitized cell lysates + PPD	7.6[a]
	8.0[a]
	8.5[a]
	9.0[a]
	7.5[a]
Sensitized cells + No Antigen	< 1
	< 1
	< 1
	< 1
	< 1
Sensitized cells + PPD	12.2[a]
	10.0[a]
	10.0[a]
	10.5[a]
	10.2[a]

[a] $P = .05$

TABLE 13

IN VITRO CAPILLARY TUBE MIGRATION OF BUFFY COAT LEUKOCYTES INCUBATED WITH NORMAL AND SENSITIZED CELL LYSATES

Cell System	Cytotoxic Index[a]		Average Cytotoxic Index
Normal cells + No Antigen	1.02	.99	1.01
	1.05	.98	
	1.04	1.01	
	1.10	1.02	
	1.05	.98	
	.95	.98	
Normal cells + PPD	.92	.96	.94
	.90	.98	
	1.00	.94	
	.97	.96	
	.95	.92	
	.90	.94	
Normal cells + Normal cell lysates + PPD	.92	.95	.93
	.90	.95	
	.92	.92	
	.96	.98	
	.94	.90	
	.97	.90	
Normal cells + Sensitized	.20	.32	.28[a]
	.32	.30	
	.30	.29	
	.23	.34	
	.27	.30	
	.28	.31	
Sensitized cells + No Antigen	.98	.96	.97
	.96	.97	
	.98	.96	
	.98	.96	
	1.00	.97	
	.98	.97	
Sensitized cells + PPD	.21	.27	.26[a]
	.23	.28	
	.25	.26	
	.26	.28	
	.18	.27	
	.27	.26	

[a]Each value represents a cytotoxic index derived from cells obtained from a single guinea pig.

[b]P = .005

results obtained when the same cell extracts were incubated with normal buffy coat leukocytes in order to determine the ability of the cells to be sensitized to PPD as measured by the in vitro capillary tube assay. It was found that, following incubation with lysates of sensitized cells, normal cells were inhibited in their migration in capillary tubes to the same degree as cells from an actively sensitized animal.

Dialyzed serum was successful in sensitizing normal alveolar macrophages on 2 of 5 occasions as measured by the in vivo technique. 1×10^5 lung macrophages were incubated with 1 ml of dialyzed serum from 5 normal and 5 sensitized guinea pigs. Following incubation, the cells and PPD were inoculated into intradermal sites in normal guinea pigs. The results of these experiments are presented in Table 14. As seen in Table 15, dialyzed serum was unsuccessful in sensitizing normal buffy coat leukocytes to inhibition of capillary tube migration in the presence of PPD.

The results of the experiments performed to determine the ability of normal cells to be sensitized to PPD following incubation with subcellular particles or Sephadex fractions from various mononuclear cells are presented in Tables 16 and 17. The normal cell types used in these experiments consisted of 1×10^5 lung macrophages or 1×10^7 peripheral blood lymphocytes prepared according to methods described previously. These cells types were incubated with subcellular particles or Sephadex fractions from peripheral blood lymphocytes, lung macrophages, spleen lymphocytes or peritoneal exudates. The results of the in vivo assay studies (Table 16) indicated that normal cell can be sensitized to PPD following incubation with microsomes, Sephadex G-200 Fraction IV,

TABLE 14

SKIN TEST RESULTS IN GUINEA PIGS INJECTED WITH NORMAL CELLS INCUBATED WITH DIALYZED SERUM FROM NORMAL OR SENSITIZED ANIMALS

Cell System	mm. induration (24 hr)
Normal cells + normal serum	< 1
	< 1
	< 1
	< 1
	< 1
Normal cells + sensitized serum	< 1
	< 1
	< 1
	< 1
	< 1
Normal cells + normal serum + PPD	2.5
	1.5
	2.5
	2.0
	2.0
Normal cells + sensitized serum + PPD	5.5
	2.1
	2.0
	6.5
	2.0

TABLE 15

IN VITRO CAPILLARY TUBE MIGRATION OF BUFFY COAT LEUKOCYTES INCUBATED WITH DIALYZED SERUM FROM NORMAL OR SENSITIZED ANIMALS

Cell System	Cytotoxic Index[a]	Average Cytotoxic Index
Normal cells + normal serum	.98 1.02 .98 .99 1.04	1.00
Normal cells + sensitized serum	.98 .95 1.02 .96 .98	.98
Normal cells + normal serum + PPD	.98 .98 1.05 .98 1.00	1.00
Normal cells + sensitized serum + PPD	.98 .95 .96 .98 .96	.97

[a]Each value represents results from a single animal.

TABLE 16

SKIN TEST RESULTS IN GUINEA PIGS INJECTED WITH NORMAL CELLS INCUBATED WITH SUBCELLULAR FRACTIONS AND SEPHADEX FRACTIONS FROM SENSITIVE CELLS

Cell System	Donor Cell Type[a]	mm. induration (24 hr)
Normal cells + No antigen	1	< 1
	2	< 1
	2	< 1
Sensitized cells + No antigen	1	1
	2	1
	2	1
Normal cells + PPD	1	1.2
	2	1.2
	2	1.5
Sensitized cells + PPD	1	12.0[b]
	2	10.5[b]
	2	10.0[b]
Normal cells + Fraction IV G-200 + PPD	1	6.0[b]
	2	6.0[b]
	2	6.5[b]
Normal cells + Fraction I G-50 + PPD	1	9.0[b]
	2	7.5[b]
	2	7.0[b]

TABLE 16 Continued on Page 78

TABLE 16--Continued

Cell System	Donor Cell Type[a]	mm. induration (24 hr)
Normal cells + Fraction III G-50 + PPD	1	8.0[b]
	2	7.0[b]
	2	7.5[b]
Normal cells + Microsomes + PPD	1	7.5[b]
	2	7.0[b]
	2	5.5[b]
Normal cells + Nuclear and cell membranes + PPD	1	1.5
	2	1.8
	2	1.8
Normal cells + G-200 Fraction I + PPD	1	< 1
II + PPD	2	< 1
	2	< 1
Normal cells + G-50 Fraction II + PPD	1	< 1
II + PPD	2	< 1
IV + PPD	2	< 1

[a] 1 = lung macrophages; 2 = peripheral blood lymphocytes

[b] P = .05

TABLE 17

IN VITRO CAPILLARY TUBE MIGRATION OF BUFFY COAT LEUKOCYTES INCUBATED WITH SUBCELLULAR FRACTIONS AND SEPHADEX FRACTIONS FROM SENSITIVE CELLS

Cell System	Cytotoxic Index[a]	Donor Cell Type[b]	Average Cytotoxic Index
Normal cells + No Antigen	.98		1.01
	.98		
	1.05		
	1.02		
	1.05		
Sensitized cells + No Antigen	.98		.98
	.95		
	1.02		
	.96		
	.98		
Normal cells + PPD	.98		.97
	.95		
	.96		
	.98		
	.96		
Sensitized cells + PPD	.23		.24[c]
	.28		
	.24		
	.28		
	.20		
Normal cells + Fraction IV G-200 + PPD	.34	1	.38[c]
	.40	1	
	.39	2	
	.37	2	
	.40	2	
Normal cells + Fraction I G-50 + PPD	.25	1	.24[c]
	.23	1	
	.23	2	
	.25	2	
	.25	2	

TABLE 17 Continued on Page 80

TABLE 17--Continued

Cell System	Cytotoxic Index[a]	Donor Cell Type[b]	Average Cytotoxic Index
Normal cells + Fraction III G-50 + PPD	.28	1	.29[c]
	.30	1	
	.27	2	
	.30	2	
	.32	2	
Normal cells + Microsomes + PPD	.32	1	.33[c]
	.32	1	
	.30	2	
	.38	2	
	.35	2	
Normal cells + Nuclear and cell membranes + PPD	.95	1	.97
	.98	1	
	.96	2	
	.98	2	
	.99	2	
Normal cells + G-200 Fraction I + PPD	.98	1	.96
II + PPD	.98	1	
I + PPD	.96	2	
II + PPD	.94	2	
III + PPD	.95	2	
Normal cells + G-50 Fraction II + PPD	.98	1	.98
II + PPD	1.00	2	
IV + PPD	.98	1	
IV + PPD	.96	2	
IV + PPD	.98	2	

[a]Each value represents a cytotoxic index derived from cells obtained from a single guinea pig.

[b]1 = From peritoneal exudates or lung macrophages; 2 = from peritoneal exudates, lung macrophages or peripheral blood mononuclear cells.

[c]$P = .005$

or Sephadex G-50 Fractions I and III. Nuclear membranes, cell membranes, and other Sephadex fractions were unsuccessful in passive sensitization. Injection of PPD alone into normal guinea pigs resulted in no visible reactions. Injection of normal or sensitized cells resulted in a small inflammatory reaction. Table 17 illustrates the results of experiments in which buffy coat leukocytes were incubated with subcellular fractions and Sephadex fractions and assayed for sensitivity by the in vitro capillary tube technique. The normal cells from 2 of the animals were incubated with a mixture from peritoneal exudates and lung macrophages and the normal cells from the other three animals were incubated with a mixture from all cell lines, as described in the previous experiment. The results indicated that normal buffy coat leukocytes can be sensitized to PPD following incubation with microsomes, Sephadex G-200 Fraction IV, or Sephadex G-50 Fractions I and III from various cell lines. Nuclear membranes, cell membranes, and other Sephadex Fractions were unsuccessful in passive sensitization. From the results of these studies it appeared that the inhibition of migration in the capillary tubes was of the same magnitude as that seen with cells from actively sensitized animals.

The degree of cellular sensitivity to PPD in the guinea pigs that were passively sensitized with Sephadex G-50 Fraction III or G-200 Fraction IV was measured according to the in vivo assay system and the in vitro capillary tube method. Using methods described previously, 1×10^7 peripheral blood mononuclear cells were harvested from six guinea pigs that had been sensitized passively (Table 8). The results of these experiments are presented in Tables 18 and 19. The degree of cellular sensitivity was not as great as that seen with cells collected from

TABLE 18

SKIN TEST RESULTS IN NORMAL GUINEA PIGS INJECTED WITH 5 μg PPD MIXED WITH PERIPHERAL BLOOD LEUKOCYTES FROM PASSIVELY SENSITIZED OR NORMAL GUINEA PIGS

Test System	mm. induration[a] (24 hr)		
Sensitized cells + PPD	8.0[b]	8.5[b]	8.0[b]
Sensitized cells	1	1	1
Normal cells + PPD	1.5	1.2	1.2
Normal cells	1	1	1

[a]Each value represents cells from a normal guinea pig sensitized passively with Sephadex G-50 Fraction III.

[b]P = .05

TABLE 19

IN VITRO CAPILLARY TUBE MIGRATION OF BUFFY COAT LEUKOCYTES FROM PASSIVELY SENSITIZED OR NORMAL GUINEA PIGS

	Cytotoxic Index[a]	
Sensitized cells +		
No Antigen	.95	(1)
	.98	(1)
	.98	(2)
	.97	(2)
PPD, 5 µg	.26[b]	(1)
	.30[b]	(1)
	.27[b]	(2)
	.32[b]	(2)
Normal cells +		
No Antigen	1.02	(1)
	.98	(1)
	.97	(2)
	1.06	(2)
PPD, 5 µg	.87	(1)
	.95	(1)
	.96	(2)
	.86	(2)

[a]Each value represents a cytotoxic index derived from cells obtained from a single normal guinea pig sensitized with Sephadex G-50 Fraction III or G-200 Fraction IV.

(1) = Passively sensitized with G-200 Fraction IV.

(2) = Passively sensitized with G-50 Fraction III.

[b]P = .005

actively sensitized animals but the results showed that the leukocytes in these guinea pigs sensitized passively were sensitive to PPD (Table 18). In Table 19 are presented the results of the in vitro capillary tube assay of the buffy coat leukocytes from the guinea pigs that were passively sensitized with either G-200 Fraction IV or G-50 Fraction III. It was found in these experiments that the cells from guinea pigs that had been passively sensitized with fractions prepared from sensitized animals were inhibited in their migration in capillary tubes to the same degree as cells from an actively sensitized animals.

The in vitro capillary tube assay technique was used to determine the effect of PPD on the migration of buffy coat leukocytes from tuberculin positive and negative human donors. The results, presented in Table 20, indicated that cellular sensitivity in the human system, as measured by inhibition of buffy coat migration, was similar to that seen in the guinea pig.

The buffy coat leukocytes from 9 normal and 18 tuberculin sensitive human donors were examined for their glycoprotein content. The results of these experiments, shown in Table 21, revealed no significant difference between normal and sensitized human cells.

The results of experiments designed to determine the ability of normal human leukocytes to be sensitized to PPD following incubation with subcellular particles and Sephadex fractions from sensitized human peripheral blood mononuclear cells appear in Table 22. In these experiments the results seen were similar to those found in the guinea pig system.

Interspecies (guinea pig to human and human to guinea pig) transfer of tuberculin sensitivity using cell lysates, microsomes, and Sephadex

TABLE 20

IN VITRO CAPILLARY TUBE MIGRATION OF BUFFY COAT LEUKOCYTES FROM TUBERCULIN SENSITIVE AND NORMAL HUMANS

	Cytotoxic Index[a]
Sensitized cells +	
No Antigen	1.20
	1.28
	1.35
	1.28
	1.34
	1.35
PPD, 5 µg	.35[b]
	.40[b]
	.42[b]
	.35[b]
	.37[b]
	.40[b]
PPD, 10 µg	.18[b]
	.20[b]
	.20[b]
	.18[b]
	.22[b]
	.23[b]
Normal cells +	
No Antigen	1.30
	1.36
	1.27
PPD, 5 µg	1.26
	1.20
	1.22
PPD, 10 µg	1.02
	1.00
	1.02

[a]Each value represents a cytotoxic index derived from cells obtained from a single donor.

[b]P = .05-.01

TABLE 21

GLYCOPROTEIN CONTENT OF CELL LYSATES

Source of Cells	Total Number of Samples	Glycoprotein Mean (Range) mg/ml
Normal humans (9)[a]	27	1.27(1.18-1.32)
Tuberculin positive humans (18)	54	1.25(1.18-1.36)

[a]Number of donors

TABLE 22

IN VITRO CAPILLARY TUBE MIGRATION OF NORMAL HUMAN BUFFY COAT LEUKOCYTES INCUBATED WITH SUBCELLULAR PARTICLES AND SEPHADEX FRACTIONS FROM SENSITIZED HUMAN LEUKOCYTES

Cell System	Cytotoxic Index[a]
Normal cells + No Antigen	1.30 1.34 1.30
Sensitized cells + No Antigen	1.28 1.34 1.28
Normal cells + PPD	1.28 1.28 1.30
Sensitized cells + PPD	.40[b] .38[b] .45[b]
Normal cells + G-200 Fraction IV + PPD	.50[b] .45[b] .48[b]
Normal cells + G-50 Fraction I + PPD	.40[b] .38[b] .38[b]

TABLE 22 Continued on Page 88

TABLE 22--Continued

Cell System	Cytotoxic Index[a]
Normal cells + G-50 Fraction III + PPD	.39[b]
	.40[b]
	.42[b]
Normal cells + Microsomes + PPD	.40[b]
	.42[b]
	.47[b]
Normal cells + Nuclear and cell membranes + PPD	1.30
	1.28
	1.30
Normal cells + G-200 Fraction I + PPD	1.30
II + PPD	1.32
III + PPD	1.32
Normal cells + G-50 Fraction II + PPD	1.29
	1.32

[a]Each value represents a cytotoxic index derived from cells obtained from a single donor.

[b]P = .05

fractions prepared from peripheral blood mononuclear cells appeared to be unsuccessful as measured by the in vitro capillary tube migration assay (Tables 23 and 24).

TABLE 23

IN VITRO CAPILLARY TUBE MIGRATION OF NORMAL HUMAN BUFFY COAT LEUKOCYTES INCUBATED WITH VARIOUS SENSITIZED GUINEA PIG LEUKOCYTE EXTRACTS

Cell System	Cytotoxic Index[a]
Normal cells + No Antigen	1.32 1.30 1.38
Sensitized cells + No Antigen	1.28 1.32 1.37
Normal cells + PPD	1.28 1.30 1.25
Sensitized cells + PPD	.40[b] .45[b] .42[b]
Normal cells + Sensitized cell lysates + PPD	1.32 1.36 1.28
Normal cells + G-200 Fraction IV + PPD	1.37 1.37 1.35

TABLE 23 Continued on Page 91

TABLE 23--Continued

Cell System	Cytotoxic Index[a]
Normal cells + G-50 Fraction I + PPD	1.32
	1.29
	1.29
Normal cells + G-50 Fraction III + PPD	1.30
	1.28
	1.31
Normal cells + Microsomes + PPD	1.27
	1.25
	1.27
Normal cells + G-200 Fraction I + PPD	1.31
II + PPD	1.33
III + PPD	1.28
Normal cells + G-50 Fraction II + PPD	1.29
IV + PPD	1.31

[a]Each value represents a cytotoxic index derived from cells obtained from a single guinea pig or human.

[b]$P = .05$

TABLE 24

IN VITRO CAPILLARY TUBE MIGRATION OF NORMAL GUINEA PIG BUFFY COAT LEUKOCYTES INCUBATED WITH VARIOUS SENSITIZED HUMAN LEUKOCYTE EXTRACTS

Cell System	Cytotoxic Index[a]
Normal cells + No Antigen	1.02
	.98
	.95
Sensitized cells + No Antigen	.98
	.97
	.95
Normal cells + PPD	.98
	.98
	1.01
Sensitized cells + PPD	.23[b]
	.28[b]
	.28[b]
Normal cells + Sensitized cell lysates + PPD	.98
	.97
	1.01
Normal cells + G-200 Fraction IV + PPD	.97
	.92
	.98

TABLE 24 Continued on Page 93

TABLE 24--Continued

Cell System	Cytotoxic Index[a]
Normal cells + G-50 Fraction I + PPD	.97
	.96
	.98
Normal cells + G-50 Fraction III + PPD	.97
	.95
	.95
Normal cells + Microsomes + PPD	.98
	1.02
	.98
Normal cells + G-200 Fraction I + PPD	.98
II + PPD	.97
III + PPD	.97
Normal cells + G-50 Fraction II + PPD	.97
IV + PPD	.96

[a]Each value represents a cytotoxic index derived from cells obtained from a single guinea pig or human.

[b]$P = .005$

CHAPTER IV

DISCUSSION

The belief that cellular antibody is involved in tuberculin hypersensitivity has received a great deal of support from the many passive transfer studies that have been reported in the literature. It is currently believed that the antibody responsible for this form of hypersensitivity is closely associated with the leukocyte. The early phases of the present studies were designed to reveal possible biochemical differences in the leukocytes of normal and sensitized guinea pigs. If sensitized leukocytes possess a cell-bound factor that is capable of reacting with antigen *in vivo* and *in vitro* it would appear likely that the factor would be protein or protein-like. The total cell protein of mononuclear leukocytes was measured using Naphthol Yellow S and it was found that the cells from tuberculin sensitive guinea pigs had a 25% or greater dye binding capacity than cells from normal animals. This would suggest that sensitized cells have an abnormally high content of protein or protein-like material. David *et al.* (1964) reported that the *in vitro* migration of peritoneal cells from animals exhibiting delayed hypersensitivity was consistently and markedly inhibited by specific antigen. The inhibition was completely abolished by incubating the sensitive cells in trypsin or chymotrypsin, suggesting that the proteolytic enzymes digest a material from the surfaces of the sensitive cells which may temporarily

desensitize them. Trypsinized sensitive cells were capable of transferring passively delayed hypersensitivity and peritoneal exudates taken from the recipient animals were inhibited from migrating in vitro by specific antigen. These results suggested that the cells rapidly resynthesize the material removed by trypsin. One of the first suggestions that an antibody-like material may be involved in the tuberculin reaction was presented by Cole and Favour (1955) when they showed that plasma collected from tuberculin positive guinea pigs within 48 hr following a PPD skin test could be fractionated to yield an active transfer factor in a subfraction of alpha globulin. They felt that the active material is normally not present in the circulation but is released from leukocytes during development of the cutaneous reaction.

On the basis of the results of protein determinations on sensitized and normal leukocytes it was felt that the type of protein involved might better be elucidated using paper electrophoresis. Normal and sensitized cell extracts were analyzed electrophoretically and it was found that the extracts from sensitized cells contained from approximately twice as much material that migrated as an alpha globulin. Jeter, Laurence, and Seebohm (1957) analyzed extracts of leukocytes from normal guinea pigs and guinea pigs hypersensitive to tuberculin and 2-4 dinitrochlorobenzene. They showed that an electrophoretic component resembling an alpha globulin was present in high concentration in cells from sensitive, but not normal animals. In addition, Lawrence and Pappenheimer (1957) showed that the factor in leukocytes responsible for passive transfer can be removed in vitro from the donor cells during incubation in normal plasma or serum in the presence of PPD. Lawrence and Pappenheimer

(1957) had shown previously that donor leukocytes remain morphologically intact during the release of the factor in the presence of PPD and the cell-free supernatant fluid would transfer tuberculin sensitivity passively to normal recipients.

Since the alpha globulins contain a number of glycoprotein components the extracts of sensitized and normal guinea pig cells were analyzed for glycoprotein. These investigations revealed that sensitized cells contained significantly more glycoprotein than did normal cells. Electrophoretic analysis of the serum proteins of sensitized and normal guinea pigs revealed a higher concentration of alpha-1 globulin in the former, and a further increase on response to a positive skin test. The possibility of a similarity between the alpha globulin component of the sensitized cells and serum and the IV-10 fraction of Cole and Favour (1955) seems rather likely.

The antigenic properties of "transfer factor" were studied by producing antisera in rabbits to lysates of normal and sensitized guinea pig leukocytes. Multiple precipitin bands were produced on immunodiffusion plates and immunoelectrophoresis, however no antigen specific for sensitized cells could be detected. Baram and Mosko (1962) chromatographed extracts of sonicated leukocytes from PPD-hypersensitive and PPD-nonhypersensitive humans on DEAE cellulose. They detected no difference in the number and position of the components from the cells of PPD-hypersensitive and PPD-nonhypersensitive donors by immuno- and cellulose acetate electrophoresis. From the results of the gel diffusion and immunoelectrophoretic studies reported here it seemed possible that "transfer factor" might be either non-antigenic (or weakly so) or does

not differ antigenically from normal cell constituents. This concept was further confirmed by the adsorption studies in which it was found that normal cell lysates adsorbed all of the detectable antibody contained in antiserum prepared against sensitized cell lysates. However, it was also observed that when the antiserum against sensitized cells was mixed with sensitized cells and PPD the ability of the cells to passively transfer sensitivity by intradermal injection into normal recipients was destroyed.

The exact nature of the material or materials responsible for passive transfer of delayed hypersensitivity has become one of the extremely challenging problems in immunology at the present time. Some of the most detailed observations on the characteristics of human "transfer factor" have come from the work of Lawrence et al. (1963). They reported that dialysates of leukocyte extracts were effective in passive transfer and, based on its dialysis characteristics, "transfer factor" has a molecular weight of less than 40,000. Moreover, fractionation of the dialysate using Sephadex G-25 suggested a molecular weight of less than 10,000. The dialysate did not react on agar diffusion with antibody to human gamma globulin, gamma 2 globulin, or albumin, nor was protein detected by precipitation in 5 per cent trichloroacetic acid. The material was resistant to DNAse, RNAse, and trypsin.

Various possibilities come to mind which might explain successful passive transfer with cell extractives. The first of these would be the possibility of transferring antigen. This seems unlikely because an intense inflammation can develop at previously tested skin sites in the recipient within 6 hr after transfer. In addition, antigen incubated

with normal cells does not normally transfer sensitivity. Further, Schwerger et al. (1962) found that a positive tuberculin reaction can be elicited as early as 24 hr after passive transfer.

The next possibility would be the transfer of an antibody-like material of low molecular weight. The apparent low molecular weight of "transfer factor" suggests a relatively small polypeptide chain. Gell and Benacerraf (1961) reported that it could not be an antibody of the conventional type. The implication of this possibility is that delayed type hypersensitivity is not due to antibody activity of the recognized intact immunoglobulins but is due to a smaller molecule with stereospecificity for antigen. This would be illustrated by the fact that a reaction with the antigen is not demonstrated by mixing isolated factor together with antigen in vitro, and by the fact that, after transfer, it is not readily catabolized in the host.

The third possible mechanism is that the transfer material contains information for the formation of an antibody-like material. If "transfer factor" is an informational molecule, then presumably it is a messenger RNA since the failure of DNAse to affect transfer appears to exclude DNA. The messenger RNA must have the following characteristics: resistant to RNAse, capacity for self-replication, and a very small molecular weight molecule. Turk (1961) and Turk and Asherson (1962) were able to transfer contact sensitivity in guinea pigs using subcellular fractions. Fong et al. (1963a and 1963b) were able to passively transfer cellular resistance in tuberculosis using microsomes and ribsome fractions, but not nuclear membranes and mitochondrial fractions. Mannick and Egdahl (1962) incubated ribonucleic acid from lymph nodes or rabbits

immunized with skin homografts with neutral lymph node cells from non-grafted rabbits and found that the latter were apparently altered to a state of transplantation immunity as manifested by a positive skin test upon injection of the cells into the skin of the donor of the skin homografts. Clark and Wilson (1963) and Michelazzi et al. (1964) also reported localization of RNA responsible for direct transfer activity. Jankovic and Dvorak (1962) found that RNAse abolished the ability to produce transfer reactions in homograft passive transfer. Some of the most convincing evidence for the involvement of RNA and/or protein in the "transfer factor" is that reported by David (1965). He found that puromycin suppressed delayed hypersensitivity in vitro and suggested that the mode of action of puromycin might involve the premature release of partly formed peptides from the ribosomes. Bloom et al. (1964), reported that cells treated with mitomycin C remain viable although their RNA synthesis is essentially completely inhibited. Such cells did not transfer hypersensitivity, suggesting that continuing synthesis of RNA and/or protein by cells is required for the development of hypersensitivity. Cells treated with low concentrations of the antibiotic, in which RNA synthesis has been diminished to 30% of that of untreated cells, still transfer substantial levels of hypersensitivity. Since the antibiotic acts on the DNA to the extent that a detectable diminution of RNA synthesis is observed, it appears unlikely that cells could divide. This suggested that the transferred cells are themselves directly involved in inducing the reactivity in the recipients, and that cell division is not required.

In the present studies passive transfer of tuberculin sensitivity to normal recipients was accomplished using several different materials.

In the preliminary work, both intact and disrupted leukocytes were capable of passive transfer. When the lysates of sensitized cells were passed through Sephadex G-200, the last fraction, termed Fraction IV and which would contain materials of molecular weight of 40,000 or less, was capable of transferring sensitivity to normal intact animals or leukocytes thereof. On further fractionation of this material using Sephadex G-50, Fraction I, which was also capable of passive transfer, contained one major peak with an ultraviolet absorption maximum at 280 mμ, suggesting the presence of a protein component. Fraction III, which was also capable of passive transfer, contained one major peak with an ultraviolet absorption maximum of 260 mμ. This material would have a molecular weight of less than 10,000. Acid hydrolysis and paper chromatography of this fraction revealed only ribose sugar and no deoxyribose. It should be emphasized that cell lysates, microsomes, and Sephadex Fraction IV transferred sensitivity that was detectable throughout the testing period. Fraction I contained only the protein component and transferred sensitivity that was detectable during the first week after transfer. Fraction III, possibly containing a messenger substance that could be an RNA-like material, did not induce tuberculin sensitivity in recipient animals until 3 days after transfer. This could be considered suggestive evidence that Fraction III contains a messenger compound for the active production of cell-bound antibody, Fraction I. Fraction III was also capable of stimulating the production of glycoprotein in normal leukocytes equal to that seen in cells of actively sensitized animals. The glycoprotein could possibly be a specific cell-bound antibody involved in the delayed hypersensitivity reaction. The results of the Sephadex studies are similar to

those of Baram and Mosko (1965) using the human system in which they were able to separate sensitized cell lysates into 280 mμ and 260 mμ components that were capable of passive transfer.

The possibility that an antibody-like material is responsible for passive transfer has received support from several sides. Cole et al. (1959) accomplished passive transfer with a plasma fraction from which fibrinogen, gamma globulin, and beta globulin had been removed. As mentioned previously, electrophoretic distribution of serum proteins in tuberculosis patients indicated that "transfer factor" might be an alpha globulin. Rauch and Favour (1960) reported that a serum alpha globulin was capable of transferring passively sensitivity in guinea pigs.

Successful passive transfer of delayed type hypersensitivity probably depends on a number of factors. One of the most important of these, perhaps, is the number of cells or amount of extract transferred. Skog (1955), in his studies on passive transfer of chemical and tuberculin sensitivity, found that at least 224 million cells were necessary in a guinea pig and 170 million cells were required in man. Segre and Sharp (1965) did quantitative delayed hypersensitivity studies in guinea pigs and found that peritoneal exudate cells were the most effective in passive transfer and that a dose of 2×10^7 cells was required. They also determined that the local in vivo reaction could be elicited with as few as 1.6×10^5 cells. Salvin and Smith (1959) suggested that passive transfer cannot be accomplished with fewer than 100 cells. In the present study passive transfer was accomplished using lysates from 1×10^{11} cells. Local passive transfer was accomplished successfully with 1×10^5 lung macrophages, 1×10^7 peripheral blood lymphocytes, or 1×10^8 spleen

lymphocytes. Cells were collected only from those donors that were exquisitely skin sensitive, with an induration of at least 15-18 mm in diameter. According to Epstein and Kligman (1957) the recipient of the cells was most important. They found that local passive transfer to contact sensitivity from the same leukocyte pool in man gave varying results in recipients.

Bauer and Stone (1961) compared the immunologic function of lymph node transplants between guinea pigs of a highly inbred, histocompatible strain (isologous transfer between strain 13) with those between strains (homologous transfer between strain 13 and Hartley). They found that the transfer of immune cells in quantities inadequate to confer significant cutaneous hypersensitivity during the first post-transfer week resulted in a high degree of tuberculin sensitivity in isologous recipients during the second and third weeks. In contrast, homologous recipients manifested no significant cutaneous reactivity during the 4 week period following identical cell transfers. Thus it appears that the strain of animal may be most important in successful transfer. It also appears that the time of cell harvest from the donors is extremely important. Guthrie et al. (1966) found that lymph node cells sensitive to 1-floro-2-4 dinitrobenzene were more effective if collected during the initial 6 day period following sensitization and that peritoneal exudate cells were effective at all periods, but most effective if collected 17 days following sensitization. Thus it appears that successful passive transfer depends on many factors. In all of the present studies, Hartley strain guinea pigs that were exquisitely sensitive to tuberculin were used as donors of hypersensitive cells and cell extracts. Large numbers

of mononuclear cells were collected early following sensitization and the successes reported in this work might be accounted for by a combination of these various factors.

The degree of sensitivity to tuberculin in recipient animals was determined by skin testing and examination of histological sections. The results of chronological studies on the histological aspects of delayed reactions reported by Dienes and Mallory (1932), Follis (1940), Sandage and Birkeland (1950), and Gell and Hinde (1951) revealed that after intradermal injection of specific antigen into sensitized individuals, no macroscopic inflammation was visible for 6-24 hr, although pain might be present at the injection site. After this interval, induration and erythema appear and reach peak intensity at 16-72 hr before gradually declining. If the reaction was severe, peak intensity occurred and the local reactions may be accompanied by regional lymphadenopathy and fever. Necrosis and scarification may occur at the injection site. Erythema was variable, but firm induration, indicative of cellular infiltration, was the hallmark of the positive delayed reaction. There were dilatation of capillaries and exudation of fluid and cells, with a predominance of polymorphonuclear leukocytes. After several hours the proportion of mononuclear cells increased and by twenty-four hours the inflammatory response was almost exclusively mononuclear. The cells were distributed throughout the dermis and subcutaneous tissue, frequently concentrated around small venules. Hemorrhage and vascular damage were not seen in an uncomplicated delayed reaction, while in the Arthus reaction antigen injection leads to appreciable necrosis which was prominent in a few hours and macroscopically would show early petechial hemorrhage due to

vascular damage. In histological sections a preponderance of polymorphonuclear leukocytes was observed. In the passive transfer studies described in the present investigation, involving either whole cells, cell lysates, microsomes, or Sephadex fractions, the skin reactions in recipients did not appear until after 12 hr and were maximal in 24 hr, with characteristic erythema and induration. The histology of the positive skin test sites in passively sensitized animals had all of the characteristics of classical delayed-type reactions, as described above. Wesslen (1952b) reported that, based on their physical appearance on histological examination, the mononuclear cells appeared the same in the passively sensitized animal as in the actively sensitized animal.

As already discussed, studies involving the passive transfer of delayed hypersensitivity in guinea pigs by means of lymphocytes labelled with tritiated thymidine have produced varying results. The results reported by McCluskey et al. (1963), Turk (1962), Turk and Cort (1963), and Najarian and Feldman (1963a) suggested that the vast majority of cells that participate in delayed hypersensitivity reactions were not specifically sensitized cells. Najarian and Feldman (1963b) observed that when normal guinea pigs were injected intravenously with lymphoidal cells sensitive to PPD or 2-4 dinitroflorobenzene and labelled with tritiated thymidine only a small number of transferred cells reached the site of a subsequent skin test with homologous antigen. The fact that the lymphocytes at the test site were not all labelled does not necessarily mean that only a few of the cells were sensitized and the rest were not. David (1964) was able to inhibit the migration of guinea pig peritoneal exudate cells with antigen when only 2.5% of the cells were specifically sensitive.

When the per cent of sensitive cells fell below this number there appeared to be no inhibition of migration.

Interspecies cellular transfer of tuberculin sensitivity has been reported with varying degrees of success. Rapaport and Miller (1959) failed to transfer delayed allergy to tuberculin from man to monkey or rabbits. Fong et al. (1963b) were successful in transferring cellular resistance in homologous mice, from mice to rabbits, and from rabbits to mice and guinea pigs using microsome fractions. They were also unable to transfer cellular resistance from guinea pigs to mice or rabbits. Bacon et al. (1961) were unable to transfer tuberculin sensitivity from rats to guinea pigs. Turk and Leibowitz (1964) and Chase (1960) were able to transfer sensitivity only within, and not between inbred strains of guinea pigs. They were able to passively transfer sensitivity with ease between members of the Hartley strain or between members of Strain III. As mentioned previously, Bauer and Stone (1961) were also able to transfer sensitivity in guinea pigs only between isologous and not homologous animals. In the present study, interspecies transfer of tuberculin sensitivity in humans and guinea pigs was attempted using cell lysates, microsomes, and Sephadex fractions from sensitized donors. The results indicated that these materials were not capable of sensitizing passively cells of another species.

CHAPTER V

SUMMARY

The present study was designed to examine the biochemical and antigenic characteristics of guinea pig leukocytic "transfer factor", and to examine various constituents of guinea pig leukocytes for their capacity to transfer passively tuberculin sensitivity to normal recipients. Guinea pigs were rendered tuberculin sensitive by the intramuscular injection of heat-killed human type tubercle bacilli in paraffin oil. Both intact and disrupted leukocytes derived from sensitive animals were capable of passively transferring tuberculin sensitivity to normal recipients. The degree of sensitivity to tuberculin in recipient animals was determined by skin testing and examination of histological sections, and by *in vitro* and *in vivo* assay techniques.

Biochemical analysis of intact cells or cell lysates was accomplished through quantitative histochemical assay for protein using Naphthol Yellow S, paper electrophoresis and subsequent analysis for protein and glycoprotein, and quantitative glycoprotein assay. The results revealed that leukocytes from tuberculin sensitive guinea pigs have an abnormally high content of protein. The paper electrophoresis data suggested that the increase was accounted for as alpha globulin and this was born out by results of subsequent studies showing sensitized leukocytes had at least 25% more glycoprotein than normal cells.

For antigenic analysis, rabbits were immunized with normal or sensitized leukocytes and their sera were examined by gel diffusion and immunoelectrophoresis. The sera from rabbits immunized with sensitized cells were examined either directly or following adsorption with normal cell lysates. The gel diffusion and immunoelectrophoretic analyses revealed that guinea pig leukocytic "transfer factor" is either non-antigenic (or weakly so) or does not differ antigenically from normal cell constituents.

Nuclei, mitochondria, and microsomes were prepared from sensitized and normal leukocytes by successive differential centrifugation. In addition, sensitized and normal leukocyte lysates were separated into various fractions using Sephadex G-200 and G-50. The last fraction from the G-200 separation, termed Fraction IV, was analyzed and found to have two major peaks with ultraviolet absorption maxima at 260 m and 280 m . This fraction was separated further using Sephadex G-50, yielding Fraction I which contained a major peak with maximum absorption at 280 m and Fraction III which had an ultraviolet absorption maximum at 260 m . The latter contained ribose but no deoxyribose. The microsomes and the three Sephadex fractions from leukocytes of sensitized animals were all capable of passive transfer of tuberculin sensitivity to normal, intact guinea pigs or to the leukocytes thereof. Fraction III was also capable of stimulating the production of glycoprotein in normal leukocytes equal to the levels seen in leukocytes of actively sensitized animals.

Interspecies transfer of tuberculin sensitivity in humans and guinea pigs was attempted using cell lysates, microsomes, and Sephadex fractions from sensitized leukocytes. The results indicated that these materials were not capable of passively sensitizing cells of another species.

BIBLIOGRAPHY

ALLISON, M. J., GERSZTEN, E., BRUMMER, D. J., and CARTER, S. 1965. Enzymatic activity of leukocytes from normal and from tuberculous subjects. Am. Rev. Resp. Dis. 91:713-718.

ARNASON, B. G., JANKOVIC, B. D., WAKSMAN, B. H., and WENNERSTEIN, C. 1962. Role of thymus in the immune reactions in rats. II. Suppressive effect of thymectomy at birth on reactions of delayed (cellular) hypersensitivity and the circulating small lymphocyte. J. Expt. Med. 116:177-186.

ARTHUS, M. 1903. Injections repetees de serum de chaval chez le lapin. Compt. Rend. Soc. Biol. 55:817-820.

AZAR, M., and SPRUNT, D. H. 1965. Studies in delayed hypersensitivity transfer of tuberculin sensitivity in CF #1 mice. Fed. Proc. 24:621.

BACON, W. L., DABNEY, G. E., and WALLACE, J. H. 1961. Interspecies transfer of tuberculin sensitivity. Bact. Proc. 107.

BAER, R. L., SERRI, F., and KIRMAN, D. 1952. Attempts at passive transfer of allergic eczematous sensitivity in man by means of white cell suspension. J. Inv. Derm. 19:217-255.

BAER, R. L., and SULZBERGER, M. B. 1952. Attempts at passive transfer of allergic eczematous sensitivity in man. J. Inv. Derm. 18: 53-59.

BAIL, O. 1909. Uebertragung der Tuberkulinempfindlichkeit. Z. Immunitätsforsch. 4:470-485.

BALDRIDGE, G. D., and KLIGMAN, A. M. 1951. The nature of the tuberculin reaction: Failure to demonstrate in vitro cytotoxicity of tuberculin for the cells of sensitized animals. Am. Rev. Tuber. 63:674-678.

BALDWIN, R. W., and ILAND, C. N. 1953. Electrophoretic studies of the serum proteins in tuberculosis. Am. Rev. Tuber. 68:372-381.

BARAM, P., and MOSKO, M. M. 1962. Chromatography of the human tuberculin delayed type hypersensitivity transfer factor. J. Allergy 33:498-506.

BARAM, P., and MOSKO, M. 1965. A dialysable fraction from tuberculin-sensitive human white blood cells capable of inducing tuberculin delayed hypersensitivity in negative recipients. Immunology 8:461-474.

BARKER, M., HUMPHRIES, J. C., and SCHERAGO, M. 1963. Uptake of fluorescein labeled PPD by leukocytes from normal and tuberculous guinea pigs. Bact. Proc. p. 59.

BAUER, J. A., JR., and STONE, S. H. 1961. Isologous and homologous lymphoid transplants. I. The transfer of tuberculin hypersensitivity in inbred guinea pigs. J. Imm. 86:177-189.

BENACERRAF, B., and GELL, P. G. H. 1959a. Studies on hypersensitivity. I. Delayed and Arthus type skin reactivity to protein conjugates in guinea pigs. Immunology 2:53-63.

BENACERRAF, B., and GELL, P. G. H. 1959b. Studies on hypersensitivity. The relation between delayed reactivity to the picryl group of conjugates and contrast sensitivity. Immunology 2:219-229.

BENACERRAF, B., and LEVINE, B. B. 1962. Immunological specificity of delayed and immediate hypersensitivity reactions. J. Expt. Med. 115:1023-1035.

BENTZON, J. W. 1953. The effect of certain infectious diseases on tuberculin allergy. Tubercle. 34:34-41.

BLOCH, H., and NORDIN, A. A. 1960. Production of tuberculin sensitivity. Nature 187:434-435.

BLOCK, R. L. 1964. Paper chromatography. Academic Press, Inc. New York. pp. 78-91.

BLOOM, B. R., HAMILTON, L. D., CHASE, M. W. 1964. Effects of mitomycin C on the cellular transfer of delayed-type hypersensitivity in the guinea pig. Nature 201:689-691.

BOUGHTON, B., and SCHILD, H. O. 1962. Transfer of delayed hypersensitivity by lymph node cells in testis autosensitization. Immunology 5:522-532.

BOVORNKITTI, S. 1961. The passive transfer of delayed cutaneous hypersensitivity to tuberculin in man. A pilot study using serum from person with active tuberculosis. J.M.A. Thailand. 44:725-734.

BOVORNKITTI, S. 1962. Serum protein changes in tuberculosis in humans. Am. Rev. Resp. Dis. 85:58-65.

BOVORNKITTI, S., KANGSADAL, P., LIMPANICHAKAIN, S., and INDANIYOM, C. 1963a. Serum transfer of delayed cutaneous hypersensitivity to tuberculin in man. Diseases of the Chest 43:393-396.

BOVORNKITTI, S., SUWANIK, R., BHADRAKOM, S., CHULSMAYA, M., CHANTARAKUL, N., and INDANIYOM, C. 1963b. Passive transfer of delayed hypersensitivity with iodine 131 labeled serum. Jap. J. Tuberc. 11:28-35.

CAREY, R. A., McGEHEE, H. A., HOWARD, J. E., and WAGLEY, P. F. 1950. The effect of adrenocorticotrophic hormone (ACTH) and cortisone on drug hypersensitivity reactions. Bull. Johns Hopk. Hosp. 87:354-385.

CARPENTER, R. 1963. In vitro studies of cellular hypersensitivity. I. Specific inhibition of migration of cells from adjuvant immunized animals by purified protein derivative and other protein antigens. Immunology 91:803-818.

CHASE, M. W. 1945. The cellular transfer of cutaneous hypersensitivity to tuberculin. Proc. Soc. Expt. Biol. Med. 59:134-135.

CHASE, M. W. 1948. The allergic state in bacterial and mycotic infections of man. Edited by R. J. Dubos, J. P. Lippincott Co., Philadelphia, Pa. pp. 110-153.

CHASE, M. W. 1953. Immunological reactions mediated through cells. The nature and significance of the antibody response. Edited by A. M. Pappenheimer, Jr., Columbia University Press, N. Y. p. 156.

CHASE, M. W. 1960. Cellular transfer between homozygous and heterozygous guinea pigs with regard to contact-type hypersensitivity and antibody production. Fed. Proc. 19:214.

CHRISTLIEB, A. R., SBARRA, A. J., and BARDAURIL, W. A. 1962. Isolation of highly purified, viable leukocytes from blood. Am. J. Clin. Path. 37:257-262.

CITRON, K. M. 1958. Tissue culture studies of tuberculin hypersensitivity in man. Tubercle. 39:65-75.

CLARK, S. D., and WILSON, R. E. 1963. Localization of RNA responsible for direct transfer activity. Surgical Forum 14:150-155.

COHEN, S. G., and MOKYCHIC, W. E. 1954. The effect of leukopenia (by nitrogen mustard) and reticular endothelial blockage (by thorium dioxide) on the cutaneous tuberculin sensitivity. J. Inf. Dis. 94:39-46.

COLE, L. R., and FAVOUR, C. B. 1955. Correlations between plasma protein fractions, antibody titers, and the passive transfer of delayed and immediate cutaneous reactivity to tuberculin PPD and tuberculopolysaccharide. J. Expt. Med. 101:391-420.

COLE, L. R., PALDING, R. L., HENDERSON, R. W., and WEST, A. F. 1959. Passive transfer of a delayed type skin reactivity to tuberculin PPD. Amer. Rev. Resp. Dis. 80:398-403.

CROWLE, A. J. 1959. Delayed hypersensitivity in mice. Its detection by skin tests and its passive transfer. Science 130:159-160.

CROWLE, A. J. 1961. Immunodiffusion. Acad. Press. New York and London.

CUMMINGS, M. M., HOYT, M., and GOTTSHALL, R. Y. 1947. Passive transfer of tuberculin sensitivity in the guinea pig. Public Health Rept. 62:994-997.

CUMMINGS, M. M., PATNODE, R. A., and HUDGINS, P. C. 1956. Passive transfer of tuberculin hypersensitivity in guinea pigs using cells disrupted by sonic vibration. Am. Rev. Tuberc. Pulm. Dis. 73:246-250.

DAVID, J. R. 1965. Suppression of delayed hypersensitivity in vitro by inhibition of protein sysnthesis. J. Expt. Med. 122:1125-1134.

DAVID, J. R., AL-ASKARI, S., LAWRENCE, H. S., and THOMAS, L. 1964. Delayed hypersensitivity in vitro. I. The specificity of inhibition of cell migration by antigens. J. Imm. 93:264-273.

DAVID, J. R., AL-ASKARI, S., LAWRENCE, H. S., and THOMAS, L. 1964. Delayed hypersensitivity in vitro. II. Effect of sensitive cells on normal cells in the presence of antigens. J. Imm. 93:274-278.

DAVID, J. R., and PATERSON, P. Y. 1965. In vitro demonstration of cellular sensitivity in allergic encephalomyelitis. Fed. Proc. 24: 182.

DAVID, J. R., LAWRENCE, H. S., THOMAS, L. 1964. The in vitro desensitization of sensitive cells by trypsin. J. Expt. Med. 120:1189-1200.

DEITCH, A. D. 1955. Microspectrophotometric study of the binding of the anionic dye, napthel yellow S., by tissue sections and by purified proteins. Lab. Inv. 4:324-351.

DERBES, V. T., DENT, J. H., WEAVER, N. K., and VAUGHN, D. D. 1950. Response of tuberculin skin test to ACTH and cortisone in tuberculous guinea pigs. Proc. Soc. Expt. Biol. 75:423-426.

DIENES, L. 1928. Further observations concerning the sensitization of tuberculous guinea pigs. J. Imm. 15:153-174.

DIENES, L., and MALLORY, T. B. 1932. Histological studies of hypersensitive reactions. Part I. The contrast between the histological responses in the tuberculin (allergic) type and the anaphylactic type of skin reaction. Am. J. Path. 8:689-700.

EHRENKRANZ, N. J., and WAKSMAN. B. H. 1956. Failure to transfer tuberculin sensitivity passively with plasma fractions containing alpha globulin. J. Expt. Med. 104:935-945.

EISEN, H. N. 1959. Cellular and humoral aspects of the hypersensitive state. Paul B. Hoeber Inc., New York. p. 89.

EKAMI, Y. and SAKURAHAYASHI, T. 1956. Passive transfer of tuberculin allergy by concentrated serum. Jap. J. Allergy 5:144-154.

EPSTEIN, W. L., and KLIGMAN, A. M. 1957. Transfer of allergic contact-type delayed sensitivity in man. J. Inv. Derm. 28:291-304.

FABRIZIO, A. M. 1952. Effect of purified fractions of tuberculin on leukocytes from normal and tuberculous animals in tissue cultures. Am. Rev. Tuberc. 65:250-271.

FAVOUR, C. B. 1947. Lytic effect of bacterial products on lymphocytes of tuberculous animals. Proc. Soc. Expt. Biol. Med. 65:269-272.

FAVOUR, C. B. 1951. In vitro effect of tuberculin on cells. Bibliotheca. Tuber. Suppl. Schweiz. Z. Tuberk. U. Pneumonol. 5:219-235.

FAVOUR, C. B., HARRISON, B. A., and OSGOOD, C. K. 1950. Increased in vitro tuberculin leucocyte cytolysis following a tuberculin skin test. Proc. Soc. Expt. Biol. Med. 73:122-124.

FEINBERG, J. G. 1957. Identification, discrimination, and quantification in Ouchterlony gel plates. Int. Arch. Allergy II 1:129-152.

FELDMAN, J. D., and NAJARIAN, J. S. 1963. Dynamics and quantitative analysis of passively transferred tuberculin hypersensitivity. Imm. 91:306-312.

FISHMAN, M., and SILVERMAN, M. S. 1957. Bactericidal activity of rat leucocytic extracts. J. Expt. Med. 105:521-528.

FOLLIS, R. H., JR. 1940. Studies on the cellular response in the early stages of the tuberculin reaction. Bull. Johns Hopk. Hosp. 66: 245-252.

FONG, J., CHIN, D., and ELBERG, S. S. 1962. Studies on tubercle bacillus histiocyte relationship and passive transfer of cellular resistance. J. Expt. Med. 115:475-489.

FONG, J., CHIN, D. K., and ELBERG, S. S. 1963a. Studies of tubercle bacillus histiocyte relationships. VI. Induction of cellular resistance by ribosomes and ribosomal RNA. J. Expt. Med. 118:371-386.

FONG, J., CHIN, D. K., and VICKREY, H. M. 1963b. Studies on tubercle bacillus histiocyte relationships. VII. Homologous and heterologous transfer of cellular resistance. J. Expt. Med. 118:727-742.

FREEDMAN, S. O., FISHER, J. P., and COOKE, R. A. 1957. A study of leukocytic antibodies in allergic patients. J. Allergy 28:501-513.

FREIGANG, B. F., BOYD, R. P., and ELLIOTT, G. B. 1963. Serum protein electrophoresis in tuberculosis. Canad. Med. Assoc. J. 88:240-242.

GANGAROSA, E. J., INGLEFIELD, J. T., THOMAS, C. G. A., and MORGAN, H. R. 1955. Studies on hypersensitivity of human tissues in vivo. I. Tuberculin hypersensitivity. J. Expt. Med. 102:425-433.

GELL, P. G. H., and BENACERRAF, B. 1959. Studies on hypersensitivity. Delayed hypersensitivity to denatured proteins in guinea pigs. Immunology 2:69-70.

GELL, P. G. H., and BENACERRAF, B. 1961. Delayed hypersensitivity to simple protein antigens. W. H. Taliaferro and J. H, Humphrey eds. Academic Press, New York. Adv. in Imm. 1:319-344.

GELL, P. G. H., and HINDE, I. T. 1951. The histology of the tuberculin reaction and its modification by cortisone. Br. J. Exp. Path. 32:516-529.

GELL, P. G. H., and SILVERSTEIN, A. M. 1962. Delayed hypersensitivity to hapten protein conjugates. I. The effect of carrier protein and site of attachment to hapten. J. Expt. Med. 115:1037-1051.

GEORGE, M., and VAUGHN, J. H. 1962. In vitro cell migration as a model for delayed hypersensitivity. Proc. Soc. Expt. Biol. Med. 111: 514-521.

GLASGOW, L. A., and MORGAN, H. R. 1957. Studies of delayed hypersensitivity in vitro. II. Delayed hypersensitivity in experimental mumps virus infections. J. Expt. Med. 106:45-52.

GOOD, R. A., KELLY, W. D., ROTSTEIN, J., and VARCO, R. L. 1962. Immunological deficiency diseases. Agammaglobulinemia, hypgammaglobulinemia, Hodgkins disease and sarcoidosis. Prog. Allergy 6:187-319. P. Kallo's and B. Waksman eds. Karger, Basel, N. Y.

GOOD, R. A., and ZAK, S. J. 1956. Disturbances in gamma globulin synthesis as "experiments of nature". Pediatrics 18:193-194.

GRABAR, P. 1959. Immunoelectrophoretic analysis in methods of biochemical analysis. Vol. 7, Ed. D. Glick, Interscience Publishers, Inc. New York.

GRABAR, P., and WILLIAMS, C. A. 1953. Methode permettant l'etude conjugee des proprietes electrophoretiques et immunochimiques d'un melange de proteins. Application au serum sanguin. Biochim. Biophys. Acta 10:193-194.

GRIDLEY, M. F. 1957. Manual of histologic and special staining techniques. Armed Forces Institute of Pathology. Washington, D.C. pp. 30-33.

GRUNER, O. 1909. Uber die Herabsetzung der tuberkulinempfindlichkeit bei tuberkulosen wahrend der masern. Munch Med. Wschr. 56: 1681.

GUTHRIE, R. K., LOWKE, G. E., FERGUSON, J. K., and ELLIS, W. L. 1966. Contact hypersensitivity to simple chemicals. Time after donor sensitization as a factor in passive transfer. J. Inv. Derm. 46:224-229.

HAGERMAN, G. 1954. How is epidermal hypersensitivity transmitted through lymphocytes. Acta Dermato-Venereol. 34:51-56.

HALL, H. E., and SCHERAGO, M. 1957. The sensitivity of human leukocytes to old tuberculin. Am. Rev. Tuberc. 75:807-822.

HAMILTON, L. D., and CHASE, M. W. 1962. Labeled cells in the cellular transfer of delayed hypersensitivity. Fed. Proc. 21:40.

HARRIS, S., and HARRIS, T. N. 1950. Effect of cortisone on some reactions of hypersensitivity in laboratory animals. Proc. Soc. Expt. Biol. Med. 74:186-189.

HARRIS, S., HARRIS, T. N., and FARBER, M. B. 1954. Studies of the transfer of lymph node cells. I. Appearance of antibody in recipients of cells from donor rabbits injected with antigen. J. Imm. 72:148-160.

HARRIS, T. N., HARRIS, S., OGBURN, C. A., and FARBER, M. G. 1960. Neonatal rabbits as donors of transferred lymph node cells. J. Imm. 88:206-216.

HAXTHAUSEN, H. 1947. Studies of the role of the lymphocytes as "transmitters" of the hypersensitiveness in allergic eczema. Acta Derm. Vener. 27:275-286.

HAXTHAUSEN, H. 1951. Passive transmission of dinitrochlorobenzene allergy with white blood cells from sensitized guinea pigs. Acta Dermat-Verereol. 31:655-659.

HEILMAN, D. 1962. Cellular sensitivity to tuberculin in adjuvant sensitized animals. Bact. Proc. 97.

HEILMAN, D. H., and FELDMAN, W. H. 1946. Specific cytotoxic action of tuberculin. Studies on tissues of tuberculous rabbits in which negative cutaneous reactions to tuberculin have developed. Am. Rev. Tuberc. Pulm. Dis. 54:312-318.

HEILMAN, D. H., FELDMAN, W. H., and MANN, F. C. 1944. Specific cytotoxic action of tuberculin. Am. Rev. Tuberc. 50:344-356.

HEILMAN, D. H., HOWARD, D. H., and CARPENTER, C. M. 1958. Tissue culture studies on bacterial allergy in experimental brucellosis. I. The effect of Brucella suis whole antigen on cultures of spleen from normal and Brucella infected guinea pigs. J. Expt. Med. 108:319-332.

HELMHOLZ, H. F. 1909. Uber passive Uebertragung der Tuberkulinempfindlichkeit bei Meerschweinchen. Z. Immunitatsforsch. 3:371-375.

HOLST, P. M. 1922. Studies on the effect of tuberculin. (4) Experiments with phagocytosis. Tubercle. 3:337-353.

HOYER, J. S., HOYER, L. W., GOOD, R. A., and CONDIE, R. M. 1962. The effect of 6-mercaptopurine on delayed hypersensitivity in guinea pigs. J. Expt. Med. 116:679-685.

HOYLE, C. 1954. Skin sensitivity in sarcoidosis. Lancet 2:164-168.

HUDGINS, P. C., CUMMINGS, M. M., and PATNODE, R. A. 1956. Electrophoretic distribution of serum proteins in rabbits, guinea pig and rat following BCG administration. Proc. Soc. Expt. Biol. Med. 92:75-77.

HUDGINS, P. C., and PATNODE, R. A. 1957. Electrophoretic distribution of serum protein and glycoprotein in tuberculous rat, rabbits, and guinea pig. Proc. Soc. Expt. Biol. Med. 95:181-183.

HUSSEY, R. G. 1961. General leucocytic response of the guinea pigs during the reaction of artifical immunity in experimental tuberculous infection. J. Expt. Med. 33:337-348.

JANKOVIC, B. D., and DVORAK, H. F. 1962. Enzymatic inactivation of immunologically competent lymph node cells in the transfer reaction. J. Imm. 89:571-581.

JANOWSKY, D. S., ROSENAU, W., and MOON, H. D. 1964. Isolation of immunologically competent lymphocytes from sensitized mouse spleens. Proc. Soc. Expt. Biol. Med. 115:77-79.

JENCKS, W. P., JETTON, M. R., and DURRUM, E. L. 1955. Paper electrophoresis as a quantitative method. Serum proteins. Biochem. J. 60:205-215.

JENSEN, K., PATNODE, R. A., CUMMINGS, M. M., and TOWNSLEY, H. C. 1962. Simultaneous, multiple, passive transfer of the delayed type of hypersensitivity to human, battery and avian tuberculin has been accomplished in man. Am. Rev. Resp. Dis. 85:373-377.

JETER, W. S., LAURENCE, K. A., and SEEBOHM, P. M. 1957. Analysis of leukocytic extracts from guinea pigs hypersensitive to tuberculin and 2,4 dinitrochlorobenzene. J. Bacteriol. 74:680-683.

JETER, W. S., TREMAINE, M. M., and SEEBOHM, P. M. 1954. Passive transfer of delayed hypersensitivity to 2,4 dinitrochlorobenzene in guinea pigs with leucocytic extracts. Proc. Soc. Expt. Biol. Med. 86:251-253.

JOHNSON, R. W., and SCHERAGO, M. 1960. The effect of histoplasmin in vitro on the migration of leukocytes from guinea pigs experimentally infected with Histoplasma capsulatum. Am. Rev. Resp. Dis. 81:96-99.

JOHNSON, R. W., and SCHERAGO, M. 1963. The effect of PPD on the in vitro migration of leukocytes from guinea pigs passively sensitized to old tuberculin. Am. Rev. Resp. Dis. 87:739-747.

JOHNSON, T. M., and GARVIN, J. E. 1959. Separation of lymphocytes in human blood by means of glass wool column. Proc. Soc. Expt. Biol. Med. 102:333-335.

JONES, V. E., and LESKOWITZ, S. 1964. Production of immunologic unresponsiveness in delayed hypersensitivity. Fed. Proc. 23:453.

JULIANELLE, L. A. 1930. Reactions of rabbits to intracutaneous injections of pneumococci and their products. IV. The development of skin reactivity to derivatives of pneumococcus. J. Expt. Med. 51:625-657.

KALLOS, P., and KENTZLER, J. 1932. Tuberkulose und innere sekretion. Beite Klin. Tuberk. 79:584.

KANTOR, F. S., and SMITH, P. 1965. Desensitization of leukocytes from delayed sensitive guinea pigs by exposure to antigen in vitro. Fed. Proc. 24:182.

KIND, P. D., BOCOBO, F. C., CURTIS, A. C., and BULALA, P. 1965. Cellular passive transfer of contact hypersensitivity to parahenylenediamine and to 2,4 dinitrochlorobenzene in guinea pigs. J. Investigative Dermatology 44:1-11.

KIRCHHEIMER, W. F., HESS, A. R., and SPEAR, R. G. 1951. Attempts at passive transfer of tuberculin type of hypersensitivity with living granulocytes. Am. Rev. Tuberc. Pulm. Dis. 64:516-519.

KIRCHHEIMER, W. F., and WEISER, R. S. 1947. The tuberculin reaction. I. Passive transfer of tuberculin sensitivity with cells of tuberculous guinea pig. Proc. Soc. Expt. Biol. Med. 66:166-170.

KIRCHHEIMER, W. G., and WEISER, R. S. 1948. The tuberculin reaction. II. Cytotoxicity of tuberculin for spleenic explants of desensitized tuberculous guinea pigs. Proc. Soc. Expt. Biol. Med. 68:407-410.

KIRCHHEIMER, W. F., WEISER, R. S., and VANLIEW, R. 1949. Transfer of systemic tuberculin sensitivity with cells of tuberculous guinea pigs. Proc. Soc. Expt. Biol. Med. 70:99-102.

KOCH, R. 1891. Weitere Mitteilung uker das Tuberkulin. Dtsch. Med. Wschr. 17:1189.

KOIW, E., and GRONWALL, A. 1952. Straining of protein bound carbohydrate after electrophoresis of serum on filter paper. Scand. J. Clin. Lab. Inv. 4:244-246.

KUCHARSKI, W., and FAVOUR, C. B. 1964. Transfer of cutaneous hypersensitivity to tuberculin with a cell free extract of spleenic tissue. Proc. Soc. Expt. Biol. Med. 115:374-379.

LANDSTEINER, K., and CHASE, M. W. 1942. Experiments on transfer of cutaneous sensitivity to simple compounds. Proc. Soc. Expt. Biol. Med. 49:688-690.

LAWRENCE, H. S. 1949. The cellular transfer of cutaneous hypersensitivity to tuberculin in man. Proc. Soc. Expt. Biol. Med. 71: 516-522.

LAWRENCE, H. S. 1952. The cellular transfer in humans of delayed cutaneous reactivity to hemolytic streptococci. J. Imm. 68:159-178.

LAWRENCE, H. S. 1954. The transfer of a generalized cutaneous hypersensitivity of the delayed tuberculin type in man by means of the constituents of disrupted leucocytes. J. Clin. Invest. 33: 951-952.

LAWRENCE, H. S. 1955. The transfer in humans of delayed skin sensitivity to streptococcal M. substance and to tuberculin with disrupted leukocytes. J. Clin. Inv. 34:219-230.

LAWRENCE, H. S., AL-ASKARI, S., DAVID, J., and FRANKLIN, E. C. 1963. Transfer of immunological information in human with dialysates of leukocyte extracts. Trans. Assoc. Amer. Phy. 76:84.

LAWRENCE, H. S., PAPAPORT, F. T., CONVERSE, J. M., and TILLETT, W. S. 1960. Transfer of delayed hypersensitivity to skin homografts with leukocyte extracts in man. J. Clin. Inv. 39:185-198.

LAWRENCE, H. S., and PAPPENHEIMER, A. M., JR. 1956. Transfer of delayed hypersensitivity to diphtheria toxin in man. J. Expt. Med. 104: 321-336.

LAWRENCE, H. S., and PAPPENHEIMER, A. M., JR. 1957. Effect of specific antigen on release from human leucocytes of the factor concerned in transfer of delayed hypersensitivity. J. Clin. Inv. 36:908-909.

LESKOWITZ, S. 1963a. Delayed hypersensitivity to a conjugate of poly-tyrosine and its inhibition by haptens. Nature 199:291-292.

LESKOWITZ, S. 1963b. Immunochemical study of antigenic specificity in delayed hypersensitivity. II. Delayed hypersensitivity to poly-tyrosine-azobenzeneaisonate and its suppression by haptens. J. Expt. Med. 117:909-923.

LESKOWITZ, S. 1963c. Use of oxidized proteins in the examination of immunochemical specifically in delayed hypersensitivity to hapten-protein conjugates. Nature 199:85-86.

LOMANITZ, R., and HALE, J. M. 1963. Production of delayed hypersensitivity to Cryptococcus neoformans in experimental animals. J. Bacteriol. 86:505-509.

LONG, J. B., and FAVOUR, C. B. 1950. The ability of ACTH and cortisone to alter delayed type bacterial hypersensitivity. Bull. Johns Hopk. Hosp. 87:186.

LONG, K. R., and JETER, W. S. 1961. Antiserum inhibition of passive transfer of delayed hypersensitivity to 2,4 dinitrochlorobenzene. Bact. Proc. 107.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the folin phenol reagents. J. Biol. Chem. 193:265-275.

LUBAROFF, D. M., and RITTS, R. E. 1964. In vitro cytotoxicity in delayed type hypersensitivity and immunologic tolerance. Proc. Soc. Expt. Biol. Med. 116:823-827.

LURIE, M. R., HARRIS, T. N., ABRAMSON, S., and ALLISON, J. M. 1949. Constitutional factors in resistance to infection. II. The effect of estrogen on tuberculin skin sensitivity and on the allergy of the internal tissues. Am. Rev. Tuberc. 59:186-197.

MANNICK, J. A., and EGDAHL, R. H. 1962. Transformation of nonimmune lymph node cells to state of transplantation immunity by RNA. Am. Surg. 156:356-366.

MARKS, J. 1958. The demonstration of tuberculin hypersensitivity in vitro. J. Path. Bact. 75:39-53.

MARKS, J., and JAMES, D. M. 1953. The effect of tuberculin on sensitized and normal leucocytes. J. Hyg. 51:340-346.

MAXWELL, K. W., DIETZ, T., and MARCU, S. 1964. An in vitro method for harvesting guinea pig alveolar macrophages. Am. Rev. Res. Dis. 89:579-580.

MC CLUSKEY, R. T., BENACERRAF, B., and MC CLUSKEY, J. W. 1963. Studies on the specificity of the cellular infiltrate in delayed hypersensitivity reactions. J. Imm. 90:466-477.

MERCHANT, D. J., and CHAMBERLAIN, R. E. 1952. A phagocytosis inhibition test in infection hypersensitivity. Proc. Soc. Expt. Biol. Med. 80:69-70.

METAXAS-BUHLER, M. 1951. Passive transference of bacterial allergy from guinea pigs infected with Brucella abortus. Internat. Arch. Allergy 1:325-332.

METAXAS, M. N., and METAXAS-BUHLER, M. 1948. Passive transfer of local cutaneous hypersensitivity to tuberculin. Proc. Soc. Expt. Biol. Med. 69:163-165.

METAXAS, M. N., and METAXAS-BUHLER, M. 1955. Studies on the cellular transfer of tuberculin sensitivity in the guinea pig. J. Imm. 75:333-347.

MEYER, K., and LOEWENTHAL, H. 1927. Untersuchungen uber anaphylaxie and gewebekulturen. A. Immunitaloforsch. 54:420-438.

MICHELAZZI, L., NANNI, G., BALDINI, I., and NOVELLI, A. 1964. Presence of RNA immuno-carrier in immune rabbit serum. Experientia 20: 447-449.

MILLER, J. M., and FAVOUR, C. B. 1951. The lymphocytic origin of a plasma factor responsible for hypersensitivity in vitro of tuberculin type. J. Expt. Med. 93:1-12.

MILLER, J. M., FAVOUR, C. B., WILLSON, B. A., and UMBARGER, M. A. 1949a. Nature of the plasma factor responsible for in vitro lysis of leucocytes by tuberculoprotein. Proc. Soc. Expt. Biol. Med. 70:738-740.

MILLER, J. M., FAVOUR, C. B., WILLSON, B. A., and UMBARGER, M. A. 1949b. Nature of the plasma factor responsible for in vitro lysis in leucocyte M tuberculoprotein. Proc. Soc. Expt. Biol. Med. 71: 287-289.

MITCHELL, A. G., WHERRY, W. B., EDDY, B., and STEVENSON, F. E. 1928. Studies in immunity. I. Non-specific factors influencing the reaction of the skin to tuberculin. Am. J. Dis. Child. 36:720-724.

MOEN, J. K. 1936. Tissue culture studies on bacterial hypersensitivity. II. Reactions of tissue from guinea pigs infected with group C hemolytic streptococci. J. Expt. Med. 64:355-368.

MOEN, J. K., and SWIFT, H. F. 1936. Tissue culture studies on bacterial hypersensitivity. I. Tuberculin sensitive tissues. J. Expt. Med. 64:339-353.

MOORE, B. D., and SCHERAGO, M. 1964. The effect of histoplasmin on the in vitro migration of leukocytes from dogs injected with Histoplasma capsulatum. Am. Rev. Resp. Dis. 90:806-808.

MOSCHOS, T. D., and SERRI, F. 1963. Passive transfer of allergic eczematous contact type sensitivity. Acta Allergologica 18:156-167.

MYRVIK, Q. N., LEAK, E. S., and FARISS, B. 1961. Studies on pulmonary alveolar macrophages from the normal rabbit, a technique to procure them in a high state of purity. J. Imm. 86:128-132.

NAJARIAN, J. S., and FELDMAN, J. D. 1961. Passive transfer of tuberculin sensitivity by tritiated thymidine-labeled lymphoid cells. J. Expt. Med. 114:779-790.

NAJARIAN, J. S., and FELDMAN, J. D. 1963a. Passive transfer of contact sensitivity by triatiated thymidine labelled lymphoid cells. J. Expt. Med. 117:775-780.

NAJARIAN, J. S., and FELDMAN, J. D. 1963b. Specificity of passively transferred delayed hypersensitivity. J. Expt. Med. 118:341-352.

NOLBANT, J. P. 1937. The effect of contagious diseases on pulmonary tuberculosis and on the tuberculin reaction in children. Am. Rev. Tuberc. 36:773-777.

O'NEILL, E. F., and FAVOUR, C. B. 1955. Tissue culture analysis of tuberculin hypersensitivity in man. Am. Rev. Tuberc. Pulm. Dis. 72:577-600.

O'NEILL, E. F., and FAVOUR, C. B. 1957. Comparison between the degree of cutaneous tuberculin reactivity with the in vitro toxicity of tuberculin for peripheral blood leucocytes. Tuberculology 16:158-165.

OPRESCU, C. C. 1962. Induction of "delayed" hypersensitivity. Nature 193:492.

OUCHTERLONY, O. 1949. Antigen-antibody reactions in gels. Acta Path. Microbiol. Scand. 26:507-515.

OUDIN, J. 1952. Specific precipitations in gels and its application to immunochemical analysis. Methods in Med. Research 5:335-378.

PEPYS, J. 1955. The relationship of nonspecific and specific factors in the tuberculin reaction. Am. Rev. Tuberc. Pulm. Dis. 71:49-73.

PATERSON, P. Y. 1960. Transfer of allergic encephalomyelitis in rats by means of lymph cells. J. Expt. Med. 111:119-136.

PATNODE, R. A., and HUDGINS, P. C. 1959. Sonic fragility of leukocytes from guinea pigs vaccinated with BCG. Am. Rev. Tuberc. Pulm. Dis. 79:323-328.

PINCUS, W. B., and FLICK, J. A. 1963. Inhibition of the primary vaccinial lesion and of delayed hypersensitivity by an antimononuclear cell serum. J. Infectious Diseases 113:15-21.

PORTER, H. 1955. Congenital agammaglobulinemia: A sex-linked genetic trait and demonstration of delayed skin sensitivity. Am. J. Dis. Children 90:617-618.

PORTIER, P., and RICHET, C. 1902. De l'action anaphylactique de certains venins. Compt. Rend. Soc. Biol. 54:170-172.

PRAUSNITZ, C., and KUSTNER, H. 1921. Studien uber die ueberemfindlichkeit. Zentr. Bahteriol. Parasiten. 88:160-169.

RABINOWITZ, Y. 1964. Separation of lymphocytes, polymorphonuclear leukocytes and monocytes on glass columns, including tissue culture observations. Blood 23:811-828.

RAFFEL, S. 1948. The components of the tubercle bacillus responsible for the delayed type of "infectious" allergy. J. Infectious Dis. 82:267-293.

RAFFEL, S. 1953. Immunity, hypersensitivity, serology. Appleton-Century-Crofts Inc. New York.

RAFFEL, S. 1954. Delayed hypersensitivity. Prog. in Allergy 4:173-198.

RAFFEL, S., and FORNEY, J. E. 1948. The role of the "wax" of the tubercle bacillus in establishing delayed hypersensitivity. I. Hypersensitivity to a simple chemical substance, picryl chloride. J. Expt. Med. 88:485-502.

RAPAPORT, F. T., LAWRENCE, H. S., MILLAR, J. W., PAPPAGIANIS, D., and SMITH, C. E. 1960. Transfer of delayed hypersensitivity to coccidioidin in man. J. Imm. 84:358-367.

RAPAPORT, F. T., and MILLAR, J. W. 1959. Failure to transfer delayed tuberculin sensitivity from human to animal species. Proc. Soc. Expt. Biol. Med. 102:714-717.

RAUCH, H. D., and FAVOUR, C. B. 1960. Passive transfer of allergic reactions to tuberculin with plasma protein fractions from hypersensitive guinea pigs. Ann. N. Y. Acad. Sci. 87:231-248.

RICH, A. R. 1944. The pathogenesis of tuberculosis. Charles C. Thomas, Baltimore.

RICH, A. R., and LEWIS, M. R. 1928. Mechanism of allergy in tuberculosis. Proc. Soc. Expt. Biol. Med. 25:596-598.

RICH, A. R., and LEWIS, M. R. 1932. The nature of allergy in tuberculosis as revealed by tissue culture. Bull. Johns Hopk. Hosp. 50: 115-131.

RILEY, J. F. 1963. Functional significance of histamine and heparin in tissue of most cells. Ann. N. Y. Acad. Sci. 103:164.

RITTS, R. E., and FAVOUR, C. B. 1955. In vivo uptake of isotope-tagged tuberculin by leukocytes. J. Imm. 75:209-213.

ROBERTS, J. C., and DIXON, F. J. 1955. The transfer of lymph node cells in the immune response to foreign proteins. J. Expt. Med. 102: 379-392.

SALVIN, S. B. 1958. Occurrence of delayed hypersensitivity during the development of Arthus type hypersensitivity. J. Expt. Med. 107: 109-124.

SALVIN, S. B. 1962. Specificity of allergic reactions. V. Observations on the systemic delayed reactions in guinea pigs sensitive to purified protein conjugates. J. Imm. 89:910-919.

SALVIN, S. B., and SMITH, R. F. 1959. Delayed hypersensitivity in the development of circulating antibody. The effect of x-irradiation. J. Expt. Med. 109:325-338.

SALVIN, S. B., and SMITH, R. F. 1960. The specificity of allergic reactions. J. Expt. Med. 111:465-483.

SALVIN, S. B., and SMITH, R. F. 1961. The specificity of allergic reactions. III. Contact hypersensitivity. J. Expt. Med. 114:185-194.

SANDAGE, C., and BIRKELAND, J. M. 1950. Effect of intravenous tuberculin injections on subsequent tuberculin skin reactions in hypersensitive rabbits. Am. Rev. Tuberc. Pulm. Dis. 61:556-559.

SCHWERGER, O., LOW, B., and TOMOSANYI, A. 1962. Studies on the early changes in leukocytes in tuberculous infection. Am. Rev. Resp. Dis. 85:66-71.

SEGRE, D., and SHARP, J. B. 1965. Quantitation of delayed hypersensitivity in guinea pigs by a local passive transfer reaction. Int. Arch. All. 27:82-101.

SEVER, J. L. 1960. Passive transfer of resistance to tuberculosis through use of monocytes. Proc. Soc. Expt. Biol. Med. 103:326-329.

SHEA, J. D., and MORGAN, H. R. 1957. Delayed hypersensitivity in vitro. I. Effects of tuberculoproteins on tissue from sensitive guinea pigs. Proc. Soc. Expt. Biol. Med. 94:436-439.

SHELLEY, W. B. 1963. Rapid technic for obtaining leukocytes from small samples of blood. Tech. Bulletin Reg. Med. Tech. 33:51-53.

SHETLAR, M. R., CAHILL, C., STIDWORTHY, G., and SHETLAR, C. L. 1956. Paper electrophoresis techniques for glycoproteins. Proc. Soc. Expt. Biol. Med. 93:44-52.

SHETLAR, M. R., FOSTER, J. V., and EVERETT, M. R. 1948. Determination of serum polysaccharides by the tryptophane reaction. Proc. Soc. Expt. Biol. Med. 67:125-130.

SKOG, H. 1955. Experimental studies on hypersensitivity to 2,4 dinitrochlorobenzene and tuberculin in animals. I. Passive transfer of hypersensitivity to 2,4 dinitrochlorobenzene. Acta Dermato-Venereol. 35:93-106.

SKOG, H. 1956. Experimental studies on hypersensitivity to 2,4 dinitrochlorobenzene and tuberculin in animals. V. Passive transfer experiments with lysed cells. Acta Dermato-Venereol. 36:1-10.

STAVITSKY, A. B. 1948. Passive cellular transfer of the tuberculin type of hypersensitivity. Proc. Soc. Expt. Biol. Med. 67:225-227.

TREVELYAN, W. E. PROCTER, D. P., and HARRISON, J. S. 1950. Detection of sugar on paper chromatograms. Nature 166:444-445.

TSUJI, S., OSHIMA, S., OSHIRO, M., and IZUMI, T. 1964. Studies on the "transfer factor" of tuberculin hypersensitivity in animals. J. Imm. 93:838-849.

TURK, J. L. 1960. Some quantitative aspects of the uptake of antigens in vitro by the lymphocytes of hypersensitive guine pigs. Int. Arch. Allergy 17:338-351.

TURK, J. L. 1961. Passive transfer of contact sensitivity to picryl chloride in guinea pigs with subcellular material. Nature 191: 915-916.

TURK, J. L. 1962. The passive transfer of delayed hypersensitivity in guinea pigs by the transfusion of isotopically labeled lymphoid cells. Immunology 5:478-488.

TURK, J. L., and ASHERSON, G. L. 1962. Attempts to transfer contact sensitivity passively with subcellular fractions in the guinea pigs. Int. Arch. Allergy 21:321-325.

TURK, J. L., and CORT, J. 1963. A histological study of the early stages of the development of the tuberculin reaction after passive transfer of cells labeled with H_3 thymidine. Immunology 6:140-147.

TURK, J. L., and LEIBOWITZ, S. 1964. Failure of cellular transfer of tuberculin sensitivity between guine pig of different strains. Nature 202:713-714.

UHR, J. W. 1960. Development of delayed type hypersensitivity in guinea pig embryos. Nature 187:957.

UHR, J. W., PAPPENHEIMER, A. M., and YONEDA, M. 1957a. Delayed hypersensitivity. I. Induction of hypersensitivity to diphtheria toxin in quinea pigs by infection with Corynebacterium diphtheriae. J. Expt. Med. 105:1-10.

UHR, J. W., SALVIN, S. B., and PAPPENHEIMER, A. M. 1957b. Delayed hypersensitivity. II. Induction of hypersensitivity in guinea pigs by means of antigen-antibody complex. J. Expt. Med. 105:11-24.

UHR, J. W., and SCHARFF, M. 1960. Delayed hypersensitivity. V. The effect of x-irradiation on the development of delayed type and antibody formation. J. Expt. Med. 112:65-76.

URBACH, F., SONES, M., and ISRAEL, H. L. 1952. Passive transfer of tuberculin sensitivity to patients with sarcoidosis. New Eng. J. Med. 247:794-797.

VON PIRQUET, C. 1906. Allergic. Munch. Med. Wochschi. 53:1457-1458.

VON PIRQUET, C. 1909. Quantitative experiments with the cutaneous tuberculin reactions. J. Pharmacal. Exp. Ther. 1:151.

VON PIRQUET, C., and SCHICK, B. 1905. Die Serumkrankheit. Deuticke, Leipzig.

WAALKEN, T. P., and COBURN, H. 1960. The role of histamine and serotonin during anaphylaxis in the mouse. J. Allergy. 31:151-161.

WAKSMAN, B. H. 1953a. Studies of cellular lysis in tuberculin sensitivity. Am. Rev. Tuberc. Pulm. Dis. 68:746-759.

WAKSMAN, B. H. 1953b. Specific white cell lysis produced by combination of rabbit antiserum to purified protein (ovalbumin, bovine gamma globulin) with homologous antigen. The role of non-precipitating antibody. J. Imm. 70:331-344.

WAKSMAN, B. H. 1959. The toxic effects of the antigen-antibody reaction on the cells of hypersensitive reactors. In Cellular and Humoral Aspects of the Hypersensitive States. Edited by H. S. Lawrence. Hoeber-Harper, New York. pp. 123-250.

WAKSMAN, B. H.., ARBOUYS, S., and ARNASON, B. G. 1961. The use of specific "lymphocyte" antisera to inhibit hypersensitive reaction of the delayed type. J. Expt. Med. 114:997-1022.

WAKSMAN, B. H., and MATOLTSY, M. 1958a. The effect of tuberculin on peritoneal exudate cells of sensitive guinea pigs in surviving cell culture. J. Imm. 81:220-234.

WAKSMAN, B. H., and MATOLTSY, M. 1958b. Quantitative study of local passive transfer of tuberculin sensitivity with peritoneal exudate cells in the guinea pig. J. Imm. 81:235-241.

WALLACE, J. H. 1958. Leukocytic transfer of delayed sensitivity to normal guinea pigs from rats infected with Mycobacterium lepraemurium. J. Imm. 80:362-366.

WARNATZ, H., and SCHEIFFARTH, F. 1964. Cellular fixed antibody in transplantation immunity. Nature 201:408-409.

WARWICK, W. J., ARCHER, O., and GOOD, R. A. 1960. Passive transfer of delayed sensitivity to the newborn rabbits. Proc. Soc. Expt. Biol. Med. 105:459-461.

WARWICK, W. J., PAGE, A., and GOOD, R. A. 1956. Passive transfer of circulating leukocytes of delayed hypersensitivity to cat scratch antigen. Proc. Soc. Expt. Biol. Med. 93:253-255.

WARWICK, W. J., SMITH, R. T., and GOOD, R. A. 1960. Failure of passive transfer of delayed hypersensitivity in newborn human infants. J. Lab. Clin. Med. 56:139-147.

WASSERMAN. J. 1962. Specific and nonspecific inhibition of the in vitro migration of leukocytes from mumps-virus-sensitized and tuberculin-sensitized guinea pigs. Am. Rev. Res. Dis. 80:19-25.

WASSERMAN, J., and PACKALEN, TH. 1959. The in vitro effect of specific and non-specific bacterial products on the migration of leukocytes from tuberculin-sensitized guinea pigs. Am. Rev. Res. Dis. 80:19-25.

WESSLEN, T. 1952a. Passive transfer of tuberculin hypersensitivity by viable lymphocytes from the thoracic duct. Acta Tuber. Scand. 26:38-45.

WESSLEN, T. 1952b. A histological study of the tuberculin reaction in animal with passively transferred hypersensitivity. Acta Tuber. Scand. 26:175-182.

WIELHEML, R. E., FISHER, J. P., and COOKE, R. A. 1958. Experimental depletion of mononuclear cells for the purpose of investigating reactions of the allergic contact type. J. Allergy 29:493-500.

YOUMANS, G. P., and EARLSON, A. C. 1947. Steptomycin sensitivity of tubercle bacilli. Am. Rev. Tuberc. Pulm. Dis. 55:529-535.

ZINSSER, H., and GRINNELL, F. B. 1925. Further studies on bacterial allergy. Allergic reactions to the hemolytic streptococcus. J. Imm. 10:725-730.