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ATTEMPTS TO INTERFERE WITH THE SENSITIZATION AND SKIN-TEST RESPONSE OF DELAYED HYPERSENSITIVITY TO SIMPLE CHEMICALS IN THE GUINEA PIG

by

Baldwin Heng Tom

A Dissertation Submitted to the Faculty of the DEPARTMENT OF MICROBIOLOGY AND MEDICAL TECHNOLOGY In Partial Fulfillment of the Requirements For the Degree of DOCTOR OF PHILOSOPHY WITH A MAJOR IN MICROBIOLOGY In the Graduate College THE UNIVERSITY OF ARIZONA

1970
I hereby recommend that this dissertation prepared under my direction by Baldwin Heng Tom entitled ATTEMPTS TO INTERFERE WITH THE SENSITIZATION AND SKIN-TEST RESPONSE OF DELAYED HYPERSENSITIVITY TO SIMPLE CHEMICALS IN THE GUINEA PIG be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY.

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ABSTRACT

Antisera were produced in rabbits against normal guinea pig serum, guinea pig serum and autologous rabbit serum conjugated with 2,4-dinitrofluorobenzene (DNFB), serum from DNFB-sensitized guinea pigs, and against lymphocytes from normal guinea pigs and from guinea pigs sensitized with DNFB.

Guinea pigs were passively immunized with the antisera by the intraperitoneal (IP), subcutaneous (SQ), or the intradermal (ID) routes in an effort to alter or inhibit the sensitization process or the subsequent skin test response to DNFB skin painting.

Antilymphocytic sera (produced against cells from both normal and sensitized animals), given IP, were effective in altering the sensitization process but not the skin test response. The skin test response was partially inhibited by antiserum to dinitrophenyl-conjugated guinea pig serum when antiserum was given SQ or ID. All other antisera were ineffective in altering either the sensitization or skin test response process by any of the above routes of administration.

It was also determined that the minimum dose of DNFB necessary to sensitize a guinea pig to a skin test dose of 0.5% DNFB was 3 μg.
CHAPTER 1

INTRODUCTION

Sensitization of animals with halogenated dinitrophenyl compounds [dinitrofluorobenzene (DNFB) and dinitrochlorobenzene (DNCB)] is readily accomplished by painting alcoholic solutions of the chemical onto a shaved area on the back of the animal (1).

Eisen, Orris, and Belman (2) demonstrated that 2,4-dinitrophenyl compounds are able to sensitize only if the chemicals conjugate to proteins. Although the chemicals bind to the epidermis, especially to the epsilon-amino groups of lysine (2, 3), most of the hapten appears to arrive at the lymph nodes unconjugated (4).

Other studies indicate that sensitization may occur only if dinitrophenyl (DNP) groups attach to cells in the Malpighian layer at the epidermo-dermal junction (5). There is also evidence that DNP conjugation to proteins is not a sole requisite for sensitization since injection of DNP-conjugated homologous and heterologous serum proteins in complete Freund's adjuvant was ineffective in producing contact sensitivity to the chemical hapten (4). These results may reflect the requirement that the chemical must be able to penetrate lymph nodes in order to initiate sensitization (4). Frey and Wenk (6) determined that intact lymphatic drainage to the regional nodes is required in the sequence of events leading to sensitization. However, Macher and Chase
(7) have recently suggested that intact blood vessels and not the lymphatics are the prime path of chemical distribution.

The "activated antigen" resulting from the sensitization has not yet been identified. Possible candidates include the conjugated serum proteins, skin proteins, and "sensitized" leucocytes. These leucocytes are sensitized either by direct conjugation with free chemical onto protein moieties found at the surface of cells, or by some processing mechanism involving one or more cell types (8).

Much information can be obtained from the studies on passive transfer of delayed-type hypersensitivities for use in inhibiting such sensitivities. Bloom and Chase (9) and Turk (10) have reviewed the work done in this area.

Landsteiner and Chase (11) were the first to demonstrate passive transfer of picryl chloride sensitivity in guinea pigs with viable peritoneal exudative white cells. Chase (12) later showed passive transfer of tuberculin sensitivity again with white cells. Confirmation of these studies and studies using different cell populations have been performed in various laboratories (9, 10). From these experiments on the passive transfer of sensitivity to chemicals with cells from various sources, it has been determined that lungs, peritoneal exudates, spleens, lymph nodes, peripheral blood, thymuses, and lymph from thoracic ducts are sites where sensitized cells may be found. A report by Guthrie et al. (13) suggested that in the sensitization process reactivity to the chemical allergen might first develop at the regional lymph nodes and then spread out to the other cellular sites.
The ability of cell-free extracts to transfer passively contact and tuberculin sensitivities has been reported. Jeter, Tremaine, and Seebohm (14) used sonically disrupted peritoneal cells to transfer contact sensitivity in guinea pigs. Cummings, Patnode, and Hudgins (15) were able to transfer tuberculin sensitivity in animals using sonicated peritoneal exudative or spleen cells. Bloom and Chase (9), however, were unable to repeat the above experiments. Turk (10) concluded that the ability to transfer contact sensitivity and tuberculin sensitivity in experimental animals with cell-free extracts is yet unsubstantiated. However, a paper by Guthrie, Ellis, and Brock (16) presented evidence suggesting that reports of unsuccessful passive transfer with extracts may be due to the timing of cell collection and the handling of the cells.

In man, Lawrence (17, 18, 19) demonstrated that viable and disrupted blood leucocytes transferred sensitivity to tuberculin and streptococcal M substance. He showed that material in cell extracts responsible for transfer was not DNA, RNA, or a typical protein. The material was found to be dialyzable and about 10,000 MW (20) and was termed "transfer factor."

Early indications that a transfer factor in contact sensitivity in experimental animals existed were found in the report of Crepea and Cooke (21). They described transfer of sensitivity to poison ivy by use of wash water from spleen cell washings. Kucharski and Favour (22) also described passive transfer with wash fluid from spleen cells of tuberculin-sensitive guinea pigs. Guthrie, Ellis, and Brock (16)
reported passive transfer to DNFB with incubation fluid from sensitized peritoneal exudative cells.

One of the first characterizations of transfer material from animal hypersensitivities was made by Jeter, Laurence, and Seebohm (23). They demonstrated an electrophoretic fraction in cell extracts of tuberculin and chemical sensitive guinea pigs which was not found in normal animals. This material resembled alpha-1 serum globulin. More recently Burger (24) reported the partial characterization of a transfer factor obtained from cells of animals sensitized to DNFB. He described the transfer factor as protein in nature, dialyzable, and about 10,000 MW.

At this time, it is not certain whether there is one or more transfer factors involved in cell-mediated immunities. In fact, there are some indications that transfer factors obtained from human delayed hypersensitivities are not the same as those obtained from animal sensitivities (25).

The events which occur in the elicitation or skin test response phase in sensitized animals probably include the processing of the skin test chemical into an active molecule capable of reacting with sensitized cells or molecules present in the animal. This interaction may trigger a sequence of events leading to the release of various specific mediators of the hypersensitivity (25, 26), perhaps including transfer factor and nonspecific chemical substances. These activities combine to give the characteristic inflammatory reaction of delayed hypersensitivity (27).
Contact sensitivity can be inhibited by various means (10). The feeding of the sensitizing chemical to an animal prior to sensitization can produce a permanent unresponsiveness to sensitization (28, 29, 30). Intravenous injection of DNCB or dinitrobenzene-sulphonic acid after sensitization with DNCB will inhibit the skin test response up to 48 hours (31, 32).

Other methods of inhibition are less selective and involve the surgical, physical, or chemical inhibition or destruction of leucocytic cell functioning, e.g., thymectomy, immunosuppressive drug therapy, or x-irradiation.

However, the most direct and possibly least damaging method in attempting to alter any aspect of the sensitization or response phases would be the use of specific antisera produced against various "sensitized" moieties.

It is well documented that the small lymphocyte plays a major role in delayed-type hypersensitivities (10, 33) and that the destruction or removal of small lymphocytes from an animal alters its ability to react to delayed hypersensitivities and other cell-mediated immunities. Therefore, the selective destruction of lymphocytes with antisera would be an ideal method for the specific inhibition of delayed sensitivity. The use of heterologous antilymphocytic sera (ALS) in the study of cell-mediated immunities such as delayed hypersensitivities has been the subject of several recent symposia and reviews (34-38).
Various attempts to inhibit the sensitization and response to chemically induced delayed hypersensitivity with ALS have been moderately successful (39-44).

Inderbitzin (39) used a single intraperitoneal injection of 7-10 ml ALS per 500 gm guinea pig and demonstrated a reduction in the histamine release in the skin test response to DNCB. However, he was unable to show a clear-cut reduction in the intensity of the skin test response. Wilhelm, Fisher, and Cooke (40) reported that the skin test reaction from DNCB sensitivity was reduced from a "distinct" induration to "slight thickening" by the intracardial injection of 1.0 ml, 0.5 ml, and 0.5 ml ALS given at 0 time, 24 hr, and 60 hr, respectively. The 0 time was 7 days after an initial skin test of the animals to obtain a control reaction before ALS treatment. The second skin test was at 60 hr. The results reported above were for a 0.5% DNCB test solution. Similar results were obtained in the studies of Waksman and Arbouys (41) and Waksman, Arbouys, and Arnason (42). They showed that an intraperitoneal injection of 3-4 ml ALS in the afternoon and a second injection the next morning before skin testing were effective in reducing the test response to 0.1% and 1.0% DNCB test solutions. The diameter and the thickness of induration were used as criteria for measuring skin test reactions. The difference between the control and the ALS-treated areas of induration was 4 mm.

Pincus and Flick (43) studied the effect of anti-monoruclear serum (AMS) (monocyte-rich) on guinea pigs multiply immunized with vaccinia virus and sensitized with DNCB. The AMS was administered for 6
consecutive days beginning one day before vaccination and sensitization.

Five-tenths ml of AMS was injected intramuscularly into a leg and 0.5 ml was injected subcutaneously into the same leg under the area of vaccination or chemical application. One ml of 0.5% DNCB was painted on the sensitizing site for 4 consecutive days. On day 5, 1 drop of 0.1% DNCB was used as a skin test dose applied 3 cm away from the sensitizing site. Their results showed that the skin test sensitivity was inhibited locally (in the treated leg), but was only slightly inhibited at a distant site. They did not describe their criteria for a positive skin test reaction to DNCB.

Long (45) demonstrated that passive transfer of DNCB with cell extracts could be inhibited by incubating antisera with the extract prior to transfer. Antisera to cellular extracts and to serum from both normal and sensitive animals were effective in the inhibition. These same antisera and antisera to peritoneal exudative cells from normal or sensitive animals did not inhibit transfer of sensitivity by viable cells.

Turk (44) recently reported that antiwhole guinea pig serum or antipolymorphonuclear neutrophil sera were not effective in inhibiting DNCB skin test. However, antithymocyte and antilymphocytic sera were effective.

The use of ALS has drawbacks. Antilymphocytic sera have been shown to have deleterious effects on antibody production and to affect nonspecifically other cell-mediated immunities (46, 47). Host defense
processes requiring cell-mediated components are also affected (48). Consequently, the use of a more specific, selective ALS against only those cells carrying the chemical sensitivity would be indicated. So far, the use of this approach has not been reported.

Finally, alteration or inhibition of the response might be possible by producing antisera against transfer factor (TF) (46). Since it is known that transfer factor from sensitized cells has specificity for the sensitizing agent, anti-TF would be a much more refined antiserum. The problem involved in producing anti-TF is that TF (MW 10,000) is not readily antigenic by itself (49, 50) and as yet no laboratory has reported a biologically active anti-TF. Burger (24) demonstrated an antibody produced in rabbits against a relatively pure transfer factor isolated from DNFB-sensitized cells. This anti-TF was shown to produce low level precipitin, latex particle agglutination, and tanned-cell, passive hemagglutination titers. The biological activity of this antiserum was not tested. Lawrence (51) is now attempting to increase the antigenicity of TF by conjugating it to methylated albumin.

From the foregoing discussion it is evident that the sensitization and the skin test response processes of chemical delayed hypersensitivities are complex. The exact sequence of events which occurs in chemical sensitivities is uncertain. Consequently, it is not known which steps in the process are more susceptible to manipulation than others.

In all of the above studies, the antileucocytic sera were produced against normal leucocytes. Consequently, these antisera,
employed to immunize passively guinea pigs against sensitization with DNFB, would be directed against normal cells in the sensitized animals.

The purpose of this project was to determine whether anti-lymphocytic serum produced against cells from DNFB-sensitive animals might not be a better antiserum than normal ALS in inhibiting DNFB sensitivity in guinea pigs. We also wanted to study further the use of antisera against normal guinea pig serum, guinea pig serum and autologous rabbit serum conjugated with DNFB, and serum from DNFB-sensitized guinea pigs in passively inhibiting the initial sensitization or the subsequent skin test response process of DNFB reactivity.
CHAPTER 2

MATERIAL AND METHODS

Experimental Animals

Guinea Pigs

Outbred Rockefeller and Amana strain albino guinea pigs of both sexes, weighing 400-600 g, were obtained from the colonies of the Department of Microbiology and Medical Technology, The University of Arizona. The animals were maintained on Purina guinea pig chow. The diet was supplemented with biweekly rations of fresh cabbage and ascorbic acid (0.3%) was added to the drinking water daily.

Rabbits

New Zealand white male rabbits weighing between 2.5 and 3.5 kg were purchased from a local supplier. As a precautionary measure against subclinical infections, the incoming rabbits were placed on a 10-day holding period in which terramycin (25 mg/liter) was added to their drinking water each day. The rabbits were given Purina rabbit chow supplemented biweekly with fresh cabbage.

Chemicals

One-fluoro-2,4-dinitrobenzene (DNFB, Eastman Organic Chemicals) was recrystallized three times from 95% ethyl alcohol at -20 C and stored in a dark bottle at room temperature.
Protein Determination

Serum and conjugated serum samples diluted in saline were analyzed for protein content by using a modification of Lowry's Folin-Ciocalteau method (52). The absorbence of the colored reaction product was read at 660 nm in a Coleman Jr. spectrophotometer using bovine serum albumin as a standard for comparison.

Conjugate Preparation

A modification of Sanger's procedure (53) was used to conjugate DNFB to guinea pig or rabbit serum. Two-tenths ml of DNFB was added to 500 mg of serum protein contained in 100 ml of 0.15 M sodium chloride. The pH was adjusted to 8.0 with sodium bicarbonate and the mixture was incubated at room temperature for two hr with gentle stirring by a magnetic stirrer. After storage overnight at 4 C, the excess chemical was removed from the mixture by desalting on a column of Sephadex G-25 coarse, using phosphate buffered saline, pH 7 (54). The effluent containing the conjugated protein was concentrated by using dry Sephadex G-25 coarse (55, 56). The collected conjugate was mixed with dry G-25 and stirred. The slurry produced was transferred into 50-ml centrifuge filtration tubes (Seprafuge tube, Occomy Associates, 223 E. Marquette Rd., Chicago 60637). Centrifugation was at 140 x g, 20 min, ambient temperature, in the Model UV International centrifuge. Each conjugate preparation was concentrated from about 100 ml to a final volume of 10-15 ml. Protein determinations were performed on the final product. The number of dinitrophenyl groups conjugated to the serum protein was determined by ultraviolet absorption of conjugate dilutions at 360 nm.
The optical density reading times the dilution factor was divided by 16 to give the number of \( \mu \) moles DNP groups per ml of conjugate (57, 58). From the protein determinations, \( \mu \) moles DNP/mg serum protein ratios were determined. The DNP-guinea pig sera averaged 0.47 \( \mu \) mole DNP/mg and the DNP-rabbit sera averaged 0.59 \( \mu \) mole DNP/mg protein.

The conjugates were sterilized by Millipore filtration (0.45 \( \mu \)) and stored at -20 °C.

**Sensitization of Guinea Pigs**

Donor animals were sensitized by topical application of 5 drops (0.15 ml) of 2% DNFB in 95% ethyl alcohol (1). The chemical was applied for 6 consecutive days to a shaved area on the nape of the neck and gently rubbed in with the round end of a test tube. The diameter of the painted area was about 2.54 cm. Four days after the last painting, the animals were skin tested to determine sensitivity to the chemical (see next section). In the passive immunization studies the animals were sensitized as above or with one drop (0.007-0.020 ml) of a 1% DNFB solution in 95% ethyl alcohol. On day 10 after sensitization, the animals were skin tested.

For determination of minimum sensitizing dose, the following concentrations of DNFB in alcohol were prepared: 0.001%, 0.005%, 0.050%, and 0.100%. Exactly 0.1 ml of each concentration was painted onto a shaved area on the nape of the neck of two guinea pigs. On day 10 after sensitization, the guinea pigs were skin tested with 0.5% DNFB in olive oil. After 24 hr the reactions were recorded.
Skin Test of Guinea Pigs

Four days after the last sensitizing dose or on day 9, the animals were skin tested to determine reactivity to the chemical. Twenty-four hours prior to skin testing, the guinea pigs were carefully shaved on their right flank. Animals which were actively sensitized were skin tested with 0.1% DNFB in olive oil. These animals used in the passive immunization studies were skin tested with 0.1, 0.5, or 1.0% DNFB in olive oil. One drop (0.03 ml) of the test material was placed onto the shaved flank and gently rubbed in as before. The test area was about 2 cm in diameter. The skin test reactions were read in 24 hr and graded:

0  no detectable reaction
+  questionable erythema
1+ patchy erythema
2+ homogenous erythema
3+ homogenous erythema, edema
4+ erythema, edema, induration; center area sometimes blanched and edges marked by red rim.

Collection of Lymphocytes

Guinea pigs were anesthesized with ether and exanguinated by cardiac puncture. In chemically sensitized animals, only the regional scapular and axillary nodes were aseptically excised and placed into saline or minimum essential medium, Eagles with Earles salts (MEM). Only animals showing a 2+ to 3+ skin test with 0.1% DNFB test chemical were used as sensitized lymphocyte donors. When normal lymphocytes inguinal region were also included.
The fatty tissue was trimmed away and the nodes were placed on a sterile stainless steel wire screen of 40 mesh. The nodes were cut, gently minced, and the cells rinsed through the screen with MEM. These lymphoid cells were collected by centrifugation at 210 x g, 4 C, 10 min, in the PR-2 International centrifuge and washed twice. The cells were then resuspended in 5 ml of MEM and stored at 4 C until used. Total counts and viability of the lymphocytes were made using a hemocytometer and the trypan blue dye exclusion technique. Differential counts were made using Wright-Giemsa and Janus green B-neutral red supravital staining (59).

Qualitative Precipitin Titration

Undilute antisera were pipetted into the bottom of 2-mm (i.d.) glass tubing. Twofold dilutions of antigen were layered onto the surface of the antisera. For controls, saline was layered onto the antisera and antigen was layered onto pretreatment normal autologous rabbit sera. The results were read at 15, 30, and 60 min. Precipitation at the interface was recorded as a positive reaction. The reciprocal of the highest antigen dilution which gave a positive reaction was the titer.

Production of Antisera

Antisera to Serum and Serum Conjugates

Twenty-five mg of serum protein (whole serum or conjugated serum) was emulsified with an equal volume of incomplete Freund's adjuvant. This emulsion was injected subcutaneously into the back of a
rabbit using a 20 ga needle. No more than 0.2 ml of emulsion was injected into any one site. Twenty-one days later, the rabbit was bled and precipitin titers were determined. A booster dose of 25 mg protein in incomplete Freund's adjuvant was given as before if the titer was less than 2560. Seven to ten days after the booster the animal was exanguinated by cardiac puncture. Twenty-four hours prior to sacrifice of the animal, feed was removed in order to reduce the amount of lipid in the serum. The collected serum was sterilized by Millipore filtration (0.45 μ) and stored at -20 C.

Antisera to Lymphocytes (ALS)

Depending on the number of guinea pigs utilized, from 20 to 800 million cells were used for the immunizing dose. The cells were injected subcutaneously into the back of a rabbit with no more than 0.2 ml at any one site. Ten days later the animals were boosted subcutaneously with 10 to 1,500 million cells. Seven days after the booster, the animals were test bled. Lymphocytotoxic titers were determined. If the titer was less than 256, they were boosted intravenously with 10 million cells. After seven days, the animals were sacrificed. The sera were collected, titrated, sterilized by Millipore filtration (0.45 μ), and stored at -20 C.

Lymphocytotoxicity

Lymphocytes from suprascapular and axillary nodes were collected from homologous guinea pigs and suspended in MEM to contain one million cells in 0.8 ml. Lymphocytotoxic titers were determined utilizing both
sensitized and normal lymphocytes. One-tenth ml of heat-inactivated 
(56 C, 30 min) antilymphocytic sera dilutions and 0.1 ml undilute 
guinea pig complement were added to each 0.8 ml of cells. The 1 ml re-
action mixtures were shaken and incubated at 37 C, 60 min. The reac-
tions were stopped by plunging the tubes into an ice-water bath.
Utilizing the trypan blue dye exclusion technique, lymphocytotoxic 
titers were determined from the viable cells counted in a hemacytometer.
Using the total number of viable cells in a normal serum control as 
100% viability, the reciprocal of the ALS dilution producing 20% cell 
death was recorded as the titer [modified from Woodruff, Anderson, and 
Abaza (60)].

**Hemagglutination**

Normal guinea pig blood was collected in an equal volume of Al-
sever's solution and stored for three days at 4 C before use. The red 
blood cells were collected by centrifugation (125 x g, 4 C, 10 min), 
washed three times with buffered saline (pH 7.2), and resuspended to 
0.5% in the buffered saline. The supernatant fluids and buffy coats 
were discarded from the washes. Five-tenths ml of the 0.5% red cell 
suspension was incubated at 37 C for 60 min with 0.5 ml of heat-
inactivated twofold dilutions of antiserum. The procedure was modified 
from Campbell et al. (61) and the hemagglutination reactions were read 
as follows:

+ compact granular agglutination of diffuse film of 
  agglutinated cells covering the bottom of the tube; 
  edges of film either folded or somewhat ragged.
+ Narrow ring of cells surrounding a diffuse film of agglutinated cells.
- Heavy ring of cells or discrete smooth button of cells in center of tube.

The reciprocal of the highest dilution of antiserum which produced a positive reaction was expressed as the titer.

**Complement Fixation**

The titration of guinea pig complement was performed as described by McKee and Jeter (62). The protocol for the complement fixation test was follows: Two-tenth ml of twofold dilutions of heat-inactivated ALS, 0.2 ml of antigen (250,000 normal or sensitized lymphocytes), and 0.2 ml of two full units guinea pig complement were mixed together and incubated at 37 C for 30 min or overnight at 4 C. At the end of this period, 0.4 ml of sensitized sheep red blood cells (in Mg-saline) was added to the 0.6 ml mixture and incubated at 37 C for 30 min. The reciprocal of the highest dilution of antiserum which inhibited lysis of the red cells was expressed as the titer.

**Passive Immunization**

**Intraabdominal Route**

To test whether the sensitization process could be altered by antiserum, 1 ml of undilute or a 1:2 dilution of heat-inactivated antiserum was injected intraabdominally into two animals on days -1, 0, 1, 2, 3, 4, 5, 7, and 9 for a total of 9 injections. Initially sensitization by skin painting began on day 0 and proceeded until day 5. In
later experiments, only 1 drop of a 1% solution was used for sensitiza-
tion. Skin testing was on day 9. The attempt to inhibit the skin test
response was carried out by the intraabdominal injection of 1 ml of a
1:2 dilution of heat-inactivated antiserum into pairs of animals on
days 8 and 9 only. The antisera used in the above experiments were
rabbit anti-guinea pig serum (pooled), anti-DNP guinea pig serum, anti-
lymphocytic serum from sensitized cells (ALS), and antilymphocytic serum
from normal cells (NALS). The guinea pigs employed in the experiments
using anti-guinea pig sera included the original donors of sera against
which the antisera was made.

Subcutaneous and Intradermal Routes

In one experiment 0.5 ml undilute ALS was injected subcutaneously
(SQ) into the flank of shaved, sensitized guinea pigs in an attempt to
inhibit the skin test response. Five-tenths ml of normal rabbit serum
was injected SQ into another site. The animals were immediately skin
tested on top of the injection sites with 1 drop of a 0.5% DNFB test so-
lution. The reactions were read in 24 hr.

The intradermal (ID) injections of 0.1 ml ALS and 0.1 ml NRS
were made into the skin of additional sensitized guinea pigs. The bleb
from the ID injection was allowed to subside for 48 hr before skin test-
ing on the injection site. In several animals, skin test painting was
done immediately onto the bleb.
Test for Antiserum Specificity

Homotransplantation of Skin Grafts

The skin grafting technique described by Billingham and Medawar (63) and modified by Siebeling (64) and Lowke (65) was utilized. Skin donor and recipient guinea pigs were anesthetized with an intraperitoneal injection of sodium nembutal (30 mg/kg) and supplemented with ether. The abdominal area of the skin donor and the right and left dorso-lateral chest walls of the skin recipients were clipped and shaved free of hair. These areas were cleansed with 2% Amphyl followed by 70% alcohol.

The donors included a Rockefeller strain, Hartley strain, and a heterologous Amana guinea pig. A strip of abdominal skin approximately 3 x 7 cm was removed from each donor with the resulting wound closed with 11 mm Michelle wound clips. The superficial fascia was trimmed from the strips and these were cut into individual grafts approximately one cm square. The grafts from each donor were kept in separate sterile Petri dishes containing MEM.

Each of the twelve recipients received four grafts—three donor grafts and an autograft. Graft beds were prepared by removing a one cm square, full thickness portion of skin. Care was taken so that the blood vessels and nerves lying above the panniculus carnosus layer were not destroyed. The grafts were placed orthotopically onto the dorsal surface of the recipients with the grafts just posterior to the scapula and 2-3 cm away from the spinal column. Two grafts were placed on each side. To prevent slippage and dehydration the graft was held in place with 3M-Blenderm surgical tape, which was applied directly over the
graft. Gauze pads were then taped over this area to secure and protect the graft site.

After the fourth post-surgical day, the grafts were inspected daily for any changes in coloration and in the integrity of the graft epidermis. The protective gauze and Blenderm tape was not needed after the eighth post-surgical day.

**Adsorption of Antilymphocytic Sera**

Lymphoid cells from axillary and suprascapular nodes were collected from unsensitized and DNFB-sensitized Amana guinea pigs using the procedure previously described.

Ten ml of heat-inactivated ALS, produced with cells from chemically sensitized Amana guinea pigs, were adsorbed with either normal lymphocytes or sensitized lymphocytes.

The 10 ml ALS was incubated with 100-150 million lymphoid cells for 10 min at 37 C. The cells were removed from the serum by centrifugation at 210 x g at 4 C for 10 min. This adsorption was repeated twice more with fresh cells.

Lymphocytotoxic titers were determined for the whole ALS, normal cell adsorbed ALS, and sensitized cell adsorbed ALS.

Control antisera for the homograft system was kindly supplied by Mr. Aftab A. Ahmed. These sera were prepared in rabbits against lymphoid cells from Amana recipients which had been exquisitely sensitized with Rockefeller donor grafts. The antiserum, ALS-22, was tested by Mr. Ahmed (66) and was found to prolong Rockefeller donor grafts in Amana
recipients. A portion of this ALS-22 was adsorbed as above with cells from chemically sensitized animals (ALS-22S).

**Passive Immunization With Antilymphocytic Sera**

To test the effect of the various antisera on the first set rejection of skin grafts, the following protocol was used:

All of the antisera were diluted 1:2. One ml was injected intraperitoneally into each recipient on days 0, 1, 2, 3, 5, 7, 9, 11, and 13. The grafting was performed on day 0. Two animals were employed for each serum sample. In addition, one animal received normal rabbit serum and one received no serum at all.

**Immunoelectrophoresis**

The electrophoretic separation of antigens was carried out on glass plates $3\frac{1}{2}'' \times 8''$ or $7\frac{1}{2}'' \times 8''$. A Heathkit Model IP-17 regulated high voltage supply was utilized for the power source and custom chambers designed to handle the large glass plates were used. The chemicals and materials employed in the electrophoretic procedure are described in the manual (1-6800A-E02) accompanying the LKB 6800A immunoelectrophoretic apparatus. Due to the large size of the plates, several processing changes were made. Immunodiffusion was carried out for 24-48 hr. Also, the thorough rinsing of excess protein from the agar required 3 to 4 days. For the $3\frac{1}{2}'' \times 8''$ plates, about 30 ml of agar were required to coat the plate. With this size plate, it was possible to test 14 antigens and 13 antisera altogether in one immunoelectrophoretic run. The $7\frac{1}{2}'' \times 8''$ plates doubled the capacity to 28
antigens and 26 antisera. About 60 ml of agar were required for this plate.

**Cellulose Acetate Electrophoresis**

The Gelman electrophoresis chamber No. 51101 and Sepaphore III cellulose acetate strips were used. Electrophoresis of serum was carried out according to the Gelman manual No. 70176-B. After staining the completed strips with Ponceau S, and rinsing, the strips were allowed to dry between two blotters. The relative amount of protein found in each band was determined by elution with 0.1 N sodium hydroxide. An empty portion of the strip was eluted for a control blank. Adsorption of the eluted material was determined by reading the optical density at 525 nm on the Coleman Jr. spectrophotometer.
CHAPTER 3

RESULTS

Attempts to alter the sensitization process and the skin test response with antisera to normal guinea pig sera or to sera conjugated with the dinitrophenyl group (DNP) were unsuccessful when injected by the intraabdominal route. Antisera to serum from chemically sensitized guinea pigs or DNP-conjugated autologous rabbit serum were also ineffective (Table 1). The precipitin titers of the antisera ranged from 2000 to 5600.

Since the antisera to serum and serum conjugates had no effect on sensitization or skin tests, we next attempted to determine whether antilymphocytic sera would alter these processes. It is uncertain what in vitro criteria, if any, are applicable for assessing the biological activity of ALS. Thus, the usefulness of titrating antilymphocytic sera at present is questionable. Nevertheless, lymphocytotoxic, hemagglutination, complement fixation, and precipitin titers were routinely determined for the various sera to give some evaluation of immunizing efficacy (Table 2).

Lymphocytotoxic titers of whole ALS ranged from 40 to 640. Sera adsorbed with lymphoid cells had reduced titers of 8 and 16. Hemagglutination titers were done in order to determine the amount of anti-guinea pig erythrocyte antibodies produced along with the antibodies to lymphocytes. No hemagglutination titers were greater than 16.
Table 1. Effect of antisera to serum and serum conjugates on sensitization and response of guinea pigs to 2,4-dinitrofluorobenzene.

<table>
<thead>
<tr>
<th>Antiserum to:</th>
<th>Effect on Sensitization</th>
<th>Effect on Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal guinea pig serum</td>
<td>3+ (6)</td>
<td>3+ (6)</td>
</tr>
<tr>
<td>Sensitized guinea pig serum</td>
<td>3+ (6)</td>
<td>3+ (6)</td>
</tr>
<tr>
<td>DNP-normal guinea pig serum</td>
<td>2.5+ (6)</td>
<td>3+ (6)</td>
</tr>
<tr>
<td>DNP-auto-rabbit serum</td>
<td>4+ (2)</td>
<td>4+ (2)</td>
</tr>
<tr>
<td>Normal rabbit serum</td>
<td>3+ (3)</td>
<td>3.3+ (3)</td>
</tr>
<tr>
<td>Untreated</td>
<td>3.5+ (4)</td>
<td>3.5+ (3)</td>
</tr>
</tbody>
</table>

*Sensitization with 5 drops, 2% DNFB in alcohol for 6 days. Animals treated with antisera for 9 days, intraabdominal injections.

*Animals treated twice with antisera prior to skin testing. Skin test of all animals on day 10 after start of sensitization with 1 drop, 0.5% DNFB in olive oil.

*Average skin test reactions for number of animals in parentheses.
Table 2. In vitro titrations of antisera made against lymphocytes from normal (NALS) and from 2,4-dinitrofluorobenzene-sensitized guinea pigs (ALS).

<table>
<thead>
<tr>
<th>Antiserum^a</th>
<th>Lymphocytotoxicity Titer^b</th>
<th>Hemagglutination Titer</th>
<th>Complement Fixation Titer</th>
<th>Precipitin Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS-1</td>
<td>512</td>
<td>undilute</td>
<td>256 (1)^c</td>
<td>2560^d</td>
</tr>
<tr>
<td>ALS-2</td>
<td>40</td>
<td>8</td>
<td>128 (1,2)</td>
<td>2560</td>
</tr>
<tr>
<td>ALS-3</td>
<td>40</td>
<td>16</td>
<td>128 (1,2)</td>
<td>40</td>
</tr>
<tr>
<td>ALS-3N</td>
<td>8</td>
<td>16</td>
<td>not done</td>
<td>20</td>
</tr>
<tr>
<td>ALS-3S</td>
<td>16</td>
<td>not done</td>
<td>not done</td>
<td>not done</td>
</tr>
<tr>
<td>ALS-4</td>
<td>640</td>
<td>16</td>
<td>128 (1)</td>
<td>160</td>
</tr>
<tr>
<td>NALS-1</td>
<td>40</td>
<td>8</td>
<td>128 (1,2)</td>
<td>0^e</td>
</tr>
<tr>
<td>NALS-2</td>
<td>80</td>
<td>16</td>
<td>256 (1)</td>
<td>0</td>
</tr>
<tr>
<td>ALS-R1</td>
<td>80</td>
<td>4</td>
<td>128 (1)</td>
<td>2560^d</td>
</tr>
<tr>
<td>ALS-R2</td>
<td>80</td>
<td>16</td>
<td>128 (1,2)</td>
<td>640</td>
</tr>
<tr>
<td>NALS-R1</td>
<td>80</td>
<td>0</td>
<td>64 (1,2)</td>
<td>0</td>
</tr>
<tr>
<td>NALS-R2</td>
<td>80</td>
<td>2</td>
<td>128 (1,2)</td>
<td>640</td>
</tr>
</tbody>
</table>

^a An N after a serum number (ALS-3N) means that serum was adsorbed with normal homologous lymphoid cells; S means adsorption with sensitized lymphoid cells. R preceding a number (ALS-R1) refers to Rockefeller strain cells used for ALS. All others from Amana strain.

^b Titors determined with normal homologous lymphoid cells.

^c Neisser-Wechsberg phenomenon. Lysis occurred in tubes 1 (1:2 dilution of antiserum) and/or 2 (1:4 dilution).

^d Precipitin titers determined with homologous serum from sensitized guinea pigs. Sensitized sera were used for all Rockefeller precipitin titers.

^e NALS-1 and NALS-2 titers determined with normal homologous sera.
Neisser-Wechsberg phenomena (67) were demonstrated at the lower dilutions in the complement fixation titrations. High concentrations (1:2 and 1:4 dilutions) of antilymphocytic sera apparently inhibited the binding of the antisera to lymphocytes. The excess complement was therefore available for lysing the sensitized red cells.

Precipitin titrations ranged from 0 to 2560. Since it was shown by immunoelectrophoretic studies that ALS contained antibodies against guinea pig sera, it was not unexpected that precipitin titers were also present. The immunoelectrophoretic analysis of several sera against normal or sensitized guinea pig sera demonstrated some precipitin arcs in the anodal (albumin) region. This was not consistently observed. In one antiserum, faint arcs also appeared in the gamma region. Since lymphocytes can synthesize serum proteins, the presence of titers in precipitin titrations between ALS and guinea pig sera is not surprising. Antisera produced against lymphocytes containing large amounts of serum proteins would subsequently yield ALS which would have high precipitin titers against the proteins. Low precipitin titers would indicate that the cells used in producing the ALS contained minimal amounts of serum proteins. Cellulose acetate electrophoresis of eight antisera showed a 2.3% increase in the gamma region of antisera over pre-immunization sera. The protein concentrations of 17 antisera were also determined and averaged 70 mg protein per ml of serum.

There are difficulties in recording skin test reactions to chemical sensitization. The graded skin test reactions are arbitrary and vary with different concentrations of skin test material. Consequently,
the interpretation as to whether ALS alters a response depends on both the sensitization protocol and the concentration of the skin test reagent. In Table 3, the results of passive immunization of guinea pigs with ALS are recorded. We have interpreted reduction of reactivity as a difference of 1.5 between the test reaction and the untreated skin test reaction. On this basis, there was some inhibition of the sensitization process in group 1 with both 0.1% and 1.0% skin test doses. There was also some inhibition of the sensitization in group 4. Anti-lymphocytic sera did not demonstrate any inhibition of the skin test response process except at the 0.1% skin test dose level (groups 1 and 3). It is interesting to note that normal rabbit serum also was able to partially reduce skin test reactivity.

One experiment was performed using both ALS-3 and anti-guinea pig serum together in the passive immunization protocol. One ml of a 1:2 dilution of each was injected intraabdominally into 2 guinea pigs following the prescribed injection schedule. No inhibition of either the sensitization or the skin test response process was detected.

In several experiments, undilute ALS or antiserum to DNP-conjugates was injected intradermally (ID, 0.1 ml) or subcutaneously (SQ, 0.5 ml) into the skin test site of sensitized guinea pigs (Table 4).

No inhibition of the skin test reactions were noted with ALS injected SQ or ID when compared to the normal rabbit serum reaction sites on the untreated animals. There appeared to be some inhibition of the skin reaction with DNP-guinea pig antisera. However, this result
Table 3. Effect of intraabdominal injection of antisera to lymphocytes from normal and 2,4-dinitrofluorobenzene-sensitized guinea pigs on sensitization and skin test response of guinea pigs to the chemical.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Effect on Sensitization</th>
<th>Effect on Skin Test Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1%</td>
<td>0.5%</td>
</tr>
<tr>
<td><strong>Group 1 (1:2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS-1 (2)</td>
<td>0.5+</td>
<td>-</td>
</tr>
<tr>
<td>NRS (2)</td>
<td>2.0+</td>
<td>-</td>
</tr>
<tr>
<td>Untreated (2)</td>
<td>4.0+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Group 2 (1:2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS-2 (4)</td>
<td>2.0+</td>
<td>2.5+</td>
</tr>
<tr>
<td>NALS-1 (4)</td>
<td>2.0+</td>
<td>2.0+</td>
</tr>
<tr>
<td>NRS (2)</td>
<td>2.0+</td>
<td>2.0+</td>
</tr>
<tr>
<td>Untreated (2)</td>
<td>2.0+</td>
<td>2.0+</td>
</tr>
<tr>
<td><strong>Group 3 (1:1)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS-R2 (2)</td>
<td>1.0+</td>
<td>-</td>
</tr>
<tr>
<td>NALS-R1 (2)</td>
<td>1.5+</td>
<td>-</td>
</tr>
<tr>
<td>NRS (2)</td>
<td>1.0+</td>
<td>-</td>
</tr>
<tr>
<td>Untreated (2)</td>
<td>2.0+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Group 4 (1:2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS-4 (3)</td>
<td>-</td>
<td>1.8+</td>
</tr>
<tr>
<td>NALS-2 (3)</td>
<td>-</td>
<td>1.3+</td>
</tr>
<tr>
<td>ALS-R1 (3)</td>
<td>-</td>
<td>1.0+</td>
</tr>
<tr>
<td>NALS-R2 (3)</td>
<td>-</td>
<td>2.2+</td>
</tr>
<tr>
<td>NRS (2)</td>
<td>-</td>
<td>2.0+</td>
</tr>
<tr>
<td>Untreated (2)</td>
<td>-</td>
<td>2.7+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Animals were skin tested with 0.1, 0.5, or 1.0% DNFB skin test dose or simultaneously with two concentrations.

<sup>b</sup> Ratio in parentheses is the antiserum dilution. Groups 1 and 2 sensitized with 5 drops, 2% DNFB for 6 days. Groups 3 and 4 sensitized with 1 drop of 1% DNFB.

<sup>c</sup> Number of animals represented in results.

<sup>d</sup> Results carried over from sensitization column.
Table 4. The effect of subcutaneous and intradermal injection of anti-lymphocytic serum into the skin test site of 2,4-dinitrofluorobenzene-sensitized guinea pigs.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Subcutaneous Injection</th>
<th>Intradermal Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin Test&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Skin Test&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALS-R2 (4)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2+ (2+)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2+ (2+)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALS-R1 (4)</td>
<td>2+ (2+)</td>
<td>2+ (2+)</td>
</tr>
<tr>
<td>DNP-guinea pig serum (4)</td>
<td>1+ (1+)</td>
<td>1+ (1+)</td>
</tr>
<tr>
<td>Untreated (4)</td>
<td>3+ (2+)</td>
<td>3+ (2+)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Skin tested with 0.5% DNFB in olive oil immediately after injection of antiserum; reaction read at 24 hr.

<sup>b</sup>Skin tested with 0.5% DNFB in olive oil after bleb subsided (48 hr). These animals are not the same ones used for the immediate skin test.

<sup>c</sup>Number of animals used in each of the three groups.

<sup>d</sup>Reaction of same guinea pigs to normal rabbit serum injected at another site.
might be questioned because of the reduced skin test reactivity also shown by the normal rabbit serum sites. This might be due to leakage of antisera into the normal rabbit serum sites. An additional observation in the intradermal studies was that 48-hr skin test reactions were one grade lower than 24-hr skin tests, except for the animals which received DNP-guinea pig serum. In these animals, the 48-hr skin test reaction increased one grade. This might indicate that the antiserum has delayed the onset of skin reactivity.

In order to test whether ALS produced against lymphocytes from chemically sensitized animals was specific to the chemical system, an experiment was set up using the ALS in the homograft system (Table 5). We chose the homograft system because it has also been shown to be cell-mediated and that ALS has been effective in inhibiting homograft rejection. These aspects are discussed in some detail in the proceedings of the Conference on Antilymphocytic Serum (35). Each Amana guinea pig received a heterologous Amana graft, a Hartley graft, and a Rockefeller graft. Autografts were used as controls. In every case, the autografts were accepted and healed into place. The ALS produced against chemically sensitized cells did not demonstrate any apparent biological activity in the homograft system. Only one animal showed a prolongation of an Amana graft more than two days longer than the controls. This might be due to the guinea pig's genetic compatibility to the Amana donor. The control ALS-22 prolonged all of the grafts. The adsorbed ALS-22S did not act differently from the unadsorbed ALS-22.
Table 5. Effect of intraabdominal injection of antilymphocytic sera produced against cells from 2,4-dinitrofluorobenzene-sensitive animals on homograft rejection.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Rejection Day</th>
<th>Amana Graft</th>
<th>Hartley Graft</th>
<th>Rockefeller Graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS-22</td>
<td>35</td>
<td>28</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>ALS-22</td>
<td>38+</td>
<td>23</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>ALS-22S</td>
<td>20</td>
<td>23</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>ALS-22S</td>
<td>18</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>ALS-3</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>ALS-3</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ALS-3N</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ALS-3N</td>
<td>18</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>ALS-3S</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ALS-3S</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>NRS</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Amana guinea pigs received heterologous Amana grafts, Hartley grafts, and Rockefeller grafts. The antisera were given intraabdominally for a series of thirteen injections.

\textsuperscript{b}The letter \textit{S} after the serum number means that the serum was adsorbed with chemically sensitized cells: \textit{N}, with normal homologous cells. ALS-22 is from homograft system.

\textsuperscript{c}Grafting day is day 0.
In the course of the experiments the minimum skin sensitizing dose for DNFB was determined. The results are given in Table 6. They show that 0.1 ml of a 0.05% DNFB sensitizing dose was enough to produce sensitivity in guinea pigs to a skin test dose of 0.5% DNFB. The optical densities of several concentrations of DNFB were determined by U.V. absorption at 360 nm. The amount of 0.05% chemical contained in 0.1 ml was calculated from the OD$_{360}$ reading of 0.26 for 0.005% DNFB. Therefore, the OD$_{360}$ for a 0.05% solution would be 2.6. The OD$_{360}$ value was divided by 16 to get the number of μmole DNP/ml in a 0.05% solution. This was calculated to be 0.1625 μmole DNP/ml in the solution or 0.01625 μmole DNP in 0.1 ml. Utilizing the molecular weight of the dinitrophenyl group (MW = 167), we then determined that the number of micrograms represented by this amount was 2.7 μg DNP. Therefore, about 3 μg DNFB (0.016 μmoles) was sufficient to render a guinea pig sensitive to a subsequent skin test dose of 0.5% DNFB.
Table 6. The determination of the minimum sensitizing dose required to sensitize guinea pigs to 2,4-dinitrofluorobenzene.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Skin Test Reaction</th>
<th>OD (360 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001% (v/v)</td>
<td>0/0^c</td>
<td>0.07</td>
</tr>
<tr>
<td>0.005</td>
<td>0/1+</td>
<td>0.26</td>
</tr>
<tr>
<td>0.010</td>
<td>0/0</td>
<td>0.53</td>
</tr>
<tr>
<td>0.050</td>
<td>2+/3+</td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>2+/2+</td>
<td></td>
</tr>
</tbody>
</table>

^aOne-tenth ml of the sensitizing dose was painted on day 1 (DNFB diluted v/v in 95% ethyl alcohol).

^bSkin test with one drop 0.5% and 1.0% DNFB/olive oil on day 10.

^cDiagonal separates the reactions of two guinea pigs. The reaction intensities for 0.5% and 1.0% were identical.
There are few reports on the use of antisera in the suppression of the sensitization or response processes in delayed hypersensitivities to chemicals. The results we obtained demonstrating ALS inhibition of the skin test response process agree with those of Inderbitzen (39), Wilhelm, Fisher, and Cooke (40), Waksman, Arbouys, and Arnason (42), and Turk (44). In addition, we demonstrated that ALS was effective in partially inhibiting the sensitization process. Antisera produced against cells from DNFB-sensitized animals were no more effective than the ALS against cells from normal animals in causing inhibition of the sensitization process.

As with some of the results of other workers (39, 40, 42, 43), there is uncertainty regarding the effectiveness of the antisera in inhibiting or reducing the reactivity of DNFB sensitization in our experiments.

The prime reason for this uncertainty is the subjective nature of recording skin test reactions. In addition, we do not have any in vitro means to determine the immunosuppressive ability of antilymphocytic sera. Consequently, we do not know whether the effect of the antisera was due to poorly immunosuppressive antisera or to antisera with antigenic specificities directed to determinants not directly involved in the sensitization process. Other factors involved in
affecting the efficacy of the ALS are the amount of ALS administered, the route and timing of ALS administration, and any combination of these factors.

De Weck and Frey (68) have made a speculative list of required amounts of DNCB to elicit sensitization and response reactions. Theoretically, one should be able to inhibit each step by making the proper amount of antibody against the required amount of chemical needed for sensitization. However, the problem is complicated by the fact that DNP chemicals probably conjugate to most available proteins--epidermal, serum, and cellular. As a result, antigenic specificities may vary greatly. From our studies, we showed that 3 μg DNFB was sufficient to sensitize a guinea pig. Therefore, when more than minimal sensitizing doses are used, a tremendous excess of chemical is being applied. Consequently, antisera used to inhibit passively the sensitization process might be neutralized by the excess chemical before it arrives at the sensitization sites.

If a prerequisite for sensitization is the penetration of the chemical into lymph nodes (4), then ALS may not be effective in inactivating subsequent "sensitized" cells within the nodes, because it has been suggested by Levey and Medawar (69) that ALS does not affect lymphocytes in central lymphoid organs, but only those in the circulation.

In the past few years, the studies on chemical delayed sensitivities have centered around the skin and skin proteins (70, 71). And the most direct attempts at studying the role of skin proteins in
contact sensitivity have been made by Parker, Aoki, and Turk (70). They extracted proteins from guinea pig skin which had been previously sensitized with DNFB. Some of these extracts were found able to sensitize other guinea pigs. They also showed that DNP-serum protein conjugates were capable of sensitizing guinea pigs. In light of these results, it is interesting that Pincus and Flick (43) were able to show an inhibition to the sensitization process by a series of intradermal and subcutaneous injections of anti-monomonuclear serum under the skin test site. When these guinea pigs were skin tested directly on the injected site, no skin test reaction developed. We were able to demonstrate that intradermal and subcutaneous administrations of antisera to DNP-guinea pig serum were effective in producing partial skin test inhibition in DNFB-sensitized animals. Unlike Pincus and Flick (43), however, we were unable to show any inhibition with our antilymphocytic sera injected SQ or ID. Turk (44) demonstrated that anti-guinea pig serum injected intraabdominally was ineffective in inhibiting sensitization. We showed that antiserum against both normal and sensitized guinea pig serum was ineffective.

Another reason for only partial effectiveness of inhibiting the chemical sensitivities with specific antisera is the fact that there are a number of heterogenous epidermal proteins which form DNP-conjugates in the animal. In addition, sensitized cells, DNP-serum proteins, and cell products are undoubtedly involved. Consequently, one would need equally diverse antisera to inhibit completely the chemical sensitivity. From the recent study of Macher and Chase (7),
it appears that DNP-epidermal proteins may well play the prime role in initiating sensitization. In another report, Fichtelius, Groth, and Liden (71) described the skin as a "first level" lymphoid organ. They defined a "pure first level" lymphoid organ as one which was involved with cell-mediated functions (as with lymphocytes), but not with humoral (antibodies) functions. They proposed a theory relating the role of the skin in allergic contact sensitivity. They added that the epidermis and its lymphocytes may indeed function as the site for sensitizing noncommitted lymphocytes.

In conclusion, any future studies on altering either the sensitization or the skin test response process using antisera will also require the consideration of the roles of the epidermis, epidermal proteins, and epidermal lymphocytes.
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