THE EFFECT OF 1-CHLORO-2, 4 DINITROBENZENE ON TISSUE TRANSPLANTATION IN THE GUINEA PIG

by

Preston Harold McKee

A Thesis Submitted to the Faculty of the DEPARTMENT OF MICROBIOLOGY AND MEDICAL TECHNOLOGY

In Partial Fulfillment of the Requirements For the Degree of MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1968
STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Preston Harold McFee

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

WAYBURN S. JETER
Professor of Microbiology

Date
ACKNOWLEDGMENTS

The author wishes to thank Dr. Wayburn S. Jeter for his guidance, constructive criticism, and encouragement during the course of this investigation.

Appreciation is also extended to former graduate student, Dr. Ronald J. Siebeling, for his advice on grafting techniques.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>3</td>
</tr>
<tr>
<td>Experimental animals</td>
<td>3</td>
</tr>
<tr>
<td>Sensitizing agent</td>
<td>3</td>
</tr>
<tr>
<td>Anaesthesia</td>
<td>4</td>
</tr>
<tr>
<td>Skin grafting procedure</td>
<td>4</td>
</tr>
<tr>
<td>Observational procedures</td>
<td>5</td>
</tr>
<tr>
<td>EXPERIMENTAL RESULTS</td>
<td>6</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>19</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>23</td>
</tr>
<tr>
<td>LIST OF REFERENCES</td>
<td>24</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table  | Page

1. Survival of Skin Homografts Transplanted 24 hours after Application of 2, 4 DNC1B on Skin to be Grafted | 8
2. Survival of Skin Homografts Transplanted Immediately after Application of 2, 4 DNC1B on Skin to be Grafted | 13
3. Survival of Skin Autografts from Untreated Animals Transplanted Immediately after Application of 2, 4 DNC1B on Skin to be Grafted | 15
4. Responses of Guinea Pigs to Sensitizing Dose of 1% 2, 4 DNC1B in Alcohol after Receiving Painted Homografts | 16
5. Responses of Guinea Pigs to Sensitizing Dose of 1% 2, 4 DNC1B in Alcohol after Receiving Painted Autografts | 17
# LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Experimental design for first set skin grafts made 24 hours after 2, 4 DNC1B was painted on the skin to be grafted.</td>
<td>7</td>
</tr>
<tr>
<td>2.</td>
<td>Experimental design for first set skin grafts made immediately after 2, 4 DNC1B was painted on the skin to be grafted.</td>
<td>11</td>
</tr>
</tbody>
</table>
ABSTRACT

The effects of combining chemical sensitization with transplantation in guinea pigs were studied. These included the effects of 2, 4 DNC1B sensitization of guinea pigs and painting of grafts on graft survival and histological appearance as well as the sensitization of previously untreated guinea pigs by chemically painted grafts.

Groups of out-bred guinea pigs received autografts or orthotopic skin homografts from a donor of another strain. Both chemically painted and nonpainted grafts were used. Grafts were transplanted to sensitized and nonsensitized recipients either immediately or after 24 hours.

Chemically painted grafts transferred to sensitive guinea pigs remained white grafts. Painted grafts transferred to other recipients usually demonstrated initial homograft acceptance as did normal grafts. Painted autografts were permanently accepted.

Sensitivity to 2, 4 DNC1B was transferred to untreated recipients by chemically painted homografts if they were transplanted immediately after painting. Painted autografts resulted in slightly greater sensitivity.
INTRODUCTION

The events which occur in homotransplantation of tissues are thought to be related to immunological phenomena with the graft recipient becoming sensitized to the tissue antigens of the donor (1). A first set graft is initially accepted and then later rejected following sensitization. A second set graft is rejected in an accelerated fashion. Unlike homografts, autografts are permanently accepted.

Another response which, like the transplantation response, is immunological in nature, is delayed-type hypersensitivity to simple chemicals. An animal sensitized to such compounds will elicit an intense, red inflammatory reaction upon topical application of the sensitizing agent (2).

While there has been much investigation on both transplantation reactivity and chemical sensitivity, there has been little work on how the two phenomena might influence each other in combination. Silverberg (3) treated mouse homografts with 20-methylcholanthrene and observed the histological appearance of the grafts. Bauer and Stone (4) transferred chemically treated homografts between guinea pigs but tested only the donors for sensitivity.

The purpose of this investigation was to determine the interrelationship between delayed-type hypersensitivity to simple chemical
compounds and tissue transplantation. The guinea pig was chosen as an appropriate experimental animal for study. The chemical 1-chloro-2, 4-dinitrobenzene was used as the sensitizing agent. Skin was employed as the tissue for transplantation. By using this experimental system it was our intent to determine:

1. the effect of prior sensitization to chemical on skin graft survival;
2. the effect of skin testing in sensitive and normal donor animals on graft survival;
3. and the effect of transplanting chemically sensitive and tested skin as a means of recipient sensitization.
MATERIALS AND METHODS

Experimental animals

Outbred Rockefeller and Amana strain albino guinea pigs of both sexes were used. The animals were obtained from the colonies of the Department of Microbiology, University of Arizona. The age of the animals varied from about two to five months. Their weight ranged from 500 to 1,000 grams. The animals were individually housed in stainless steel cages. They were maintained on Purina guinea pig chow and water containing ascorbic acid. In addition, the diet included fresh cabbage given every other day.

Sensitizing agent

A solution of 1-chloro-2, 4 dinitrobenzene (2, 4 DNC1B) in 95% ethyl alcohol was used as the sensitizing agent in all experiments. The chemical was applied by dropper to an area 3 cm in diameter on the nape of the neck which had first been clipped and shaved free of hair. Donor guinea pigs to be sensitized received topical application of five drops of 2% 2, 4 DNC1B applied daily for six days. When the agent was applied to a graft one drop of a 1% solution was painted on the graft area either 24 hours or immediately before grafting. In the case of sensitized animals the chemical was applied one week after the
sensitizing procedure was completed. Control animals for sensitization experiments were treated topically with one drop of the 1% solution on the shaved nape of the neck.

**Anaesthesia**

Sodium pentobarbital (nembutal) was used as the anaesthetic agent. It was given intra-abdominally as a solution containing 15 mg/ml. One tenth ml was injected for each 50 grams of animal.

**Skin grafting procedure**

All equipment used in grafting was sterile. The surgical instruments were kept in a 2% amphy solution. Saline and gauze pads were autoclaved.

The grafting technique utilized was similar to the one of Billingham and Medawar (5). The abdominal area of the skin donor and the left or right lateral chest walls of the skin recipients were clipped and shaved free of hair. The shaved skin surfaces were then cleaned with 70% alcohol. Full thickness skin grafts approximately one cm$^2$ were cut by scalpel and scissors from the donor's abdomen. The wound was then closed with metal wound clips. The fat was trimmed from the grafts and they were stored raw surface down on saline soaked filter paper. A graft bed was then prepared at shoulder level on the recipient's rib cage. The graft bed was made to approximate the size of the graft. Any bleeding in the graft bed was stopped.
before the graft was applied. The graft was then transplanted to the bed and secured in place with a strip of 3-M Blenderm Surgical Tape applied directly over the graft and surrounding area. Gauze pads were then placed over the graft and the animal was wrapped with surgical tape.

**Observational procedures**

The grafts were observed starting from one to five days after grafting. Observations included healing of the edges, color, inflammation of the surrounding graft bed, and bleeding of the graft when scraped with a scalpel. Cessation of bleeding was used to determine graft rejection. Animals receiving 2, 4 DNClB on a graft were also tested for sensitivity as were controls receiving the chemical on the nape of the neck. One week after their first contact with the chemical one drop of a 1% solution of 2, 4 DNClB in alcohol was applied to the shaved flank. One day after the animals were tested for sensitivity they were observed for delayed hypersensitivity as characterized by erythema and edema. An irregular patch of erythema was designated a +1 reaction, solid erythema a +2 reaction, and solid erythema with raised edges a +3 reaction.
EXPERIMENTAL RESULTS

The first experiments were conducted to determine the effect of 2, 4 DNC1B on guinea pig homografts transplanted 24 hours after application of the chemical to donor graft sites. The design of these first experiments is shown in Fig. 1. One drop of a 1% solution in alcohol of 2, 4 DNC1B was applied to appropriate graft sites. Homografts were transplanted 24 hours later from sensitive and untreated recipients. In addition, grafts from the same donors were transplanted to sensitive and untreated recipients from sites to which the chemical had not been applied. This was done to determine whether the application of chemical had any effect on tissue to which it had not been directly applied. The results of these experiments are demonstrated in Table 1. The grafts painted with 2, 4 DNC1B were not as well accepted by the host as those that were not painted. All seven of the painted grafts transplanted from sensitive donors to sensitive recipients were white grafts. The grafts remained loose on their beds, yellowish-white in color, and exhibited no bleeding at any time. In addition the graft edges did not heal into the edges of the graft bed.

When 2, 4 DNC1B painted grafts were transplanted from the sensitive donors to untreated recipients, on the other hand, four of 11 grafts were initially accepted by the host. They were more pale in
Fig. 1.--Experimental design for first set skin grafts made 24 hours after 2, 4 DNC1B was painted on the skin to be grafted.

Sensitive animals were topically treated with 2, 4 DNC1B for six days, eight days prior to grafting.

\(^a\) Number of animals.
TABLE 1. --Survival of Skin Homografts Transplanted 24 Hours after Application of 2, 4 DNC1B on Skin to be Grafted.

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of Graft</th>
<th>Donor State</th>
<th>Recipient State</th>
<th>Number of Grafts Rejected at Various Times after Transplantation</th>
<th>Average Rejection Time in Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Painted</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>7[^c]</td>
<td>White Graft</td>
</tr>
<tr>
<td>11</td>
<td>Painted</td>
<td>Sensitive</td>
<td>Untreated[^d]</td>
<td>1 2 1</td>
<td>8.5</td>
</tr>
<tr>
<td>3</td>
<td>Painted</td>
<td>Untreated</td>
<td>Untreated</td>
<td>1 1 1</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>Painted</td>
<td>Untreated</td>
<td>Sensitive</td>
<td>3 1 1</td>
<td>8.3</td>
</tr>
<tr>
<td>4</td>
<td>Nonpainted</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>1 1 2</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Nonpainted</td>
<td>Sensitive</td>
<td>Untreated</td>
<td>1 2 3</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Nonpainted</td>
<td>Untreated</td>
<td>Sensitive</td>
<td>1 1 1</td>
<td>9.7</td>
</tr>
</tbody>
</table>

[^a]: One drop of 1% 2, 4 DNC1B in alcohol.

[^b]: Animals sensitized by six consecutive paintings with 2% DNC1B eight days prior to grafting.

[^c]: White grafts when first observed at five days after operation.

[^d]: No prior treatment with chemical.
color than normal homografts, however, and bled less when scraped with a scalpel. The other seven grafts were white grafts very similar to those transplanted to sensitive recipients.

Grafts from untreated donors were accepted better than those from sensitive donors. When three grafts were transplanted from an untreated donor to an untreated recipient one was rejected at 11 days, one at eight days, and a third was a white graft. Of four grafts transplanted from untreated donors to sensitive recipients three were rejected at eight days and one at nine days. The grafts bled on scraping and became pink in color.

In contrast to grafts exposed to the chemical those from non-painted areas of the donor demonstrated the typical homograft response of initial acceptance. These included four grafts from the sensitive donors to sensitive recipients, six grafts from sensitive donors to untreated recipients, and four grafts transplanted from untreated donors to sensitive recipients. Thus, the results with first set grafts to which the chemical had not been applied were comparable to regular homografts. They bled when scraped with a scalpel, became pink in color, and healed into the graft bed.

Second set grafts were transplanted from the original donor 14 days after the first set on two occasions. A single drop of 1% 2, 4 DNBClB was applied to some of the donor graft sites as before. Then
chemically painted grafts were placed on untreated recipients receiving either chemically painted or nonpainted first set grafts. They were also transplanted to sensitive recipients receiving nonpainted first set grafts from a sensitive donor. Non-chemically painted second set grafts were transplanted to the same three types of recipients. The results were the same regardless of the recipient treatment or whether the graft was chemically painted. All of the second set grafts remained white grafts, demonstrating no bleeding, coloration, or healing in at the edges.

A second group of experiments was done to determine what experimental results would be obtained if the grafts were transplanted immediately after chemical application instead of waiting 24 hours. The design of the second group of experiments is shown in Fig. 2. As in the first experiments one drop of 1% 2, 4 DNC1B in alcohol was applied on skin to be grafted. Chemically painted homografts were again transplanted from sensitive donors to sensitive and untreated recipients. Chemically painted homografts were also transplanted from untreated donors to sensitive and untreated recipients. This was done to determine whether 2, 4 DNC1B painted sensitized tissue reacted differently than painted nonsensitized tissue in the homograft reaction. The effect of 2, 4 DNC1B application to autografts was also determined. Autografts painted with chemical were transferred from the abdomen
<table>
<thead>
<tr>
<th>Donors</th>
<th>Sensitive</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homografts</td>
<td>Painted</td>
<td>Painted</td>
</tr>
</tbody>
</table>

Recipients Sensitive (9) Untreated (22) Sensitive (3) Untreated (4) Their Beds (10) To Shoulder (16)

Fig. 2.--Experimental design for first set skin grafts made immediately after 2, 4 DNC1B was painted on the skin to be grafted.

Sensitive animals were topically treated with 2, 4 DNC1B for six days, seven days prior to grafting.
to the shoulder of untreated animals. Other painted autografts were merely cut from the shoulder and placed back on their bed.

Painted homografts transferred immediately from sensitive donors to sensitive recipients were not accepted as demonstrated in Table 2. The gross anatomy of the grafts was the same as that of those transplanted after 24 hours except for a halo of inflammation around the grafts. This halo was always present and measured two mm in width on the average. It appeared on the first day after grafting and lasted two to four days. The inflammation did not consistently appear except with 2, 4 DNC1B painted grafts immediately transferred from sensitive donors to sensitive recipients. It never appeared with grafts transferred 24 hours after application of chemical and never appeared with the same intensity around other grafts transplanted immediately after 2, 4 DNC1B application.

When 2, 4 DNC1B painted homografts were immediately transferred from sensitive donors to untreated recipients 16 of the 22 grafts demonstrated typical first set homograft responses. The 16 grafts bled, and became pink in color. However, a layer of dead skin was usually present on the graft surface. The other six grafts remained white grafts.

Transplantation of 2, 4 DNC1B painted grafts from untreated donors to sensitive recipients immediately after chemical application resulted in three white grafts and no first set responses. When
TABLE 2.—Survival of Skin Homografts Transplanted Immediately after Application of 2, 4 DNC1B on Skin to be Grafted.

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of Graft</th>
<th>Donor State</th>
<th>Recipient State</th>
<th>Number of Grafts Rejected at Various Times after Transplantation</th>
<th>Average Rejection Time in Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Painted a</td>
<td>Sensitive b</td>
<td>Sensitive</td>
<td>9 c</td>
<td>White Grafts</td>
</tr>
<tr>
<td>22</td>
<td>Painted</td>
<td>Sensitive</td>
<td>Untreated d</td>
<td>6 2 4 2 2 2 4 2</td>
<td>9.75</td>
</tr>
<tr>
<td>3</td>
<td>Painted</td>
<td>Untreated</td>
<td>Sensitive</td>
<td>3</td>
<td>White Grafts</td>
</tr>
<tr>
<td>4</td>
<td>Painted</td>
<td>Untreated</td>
<td>Untreated</td>
<td>2 2</td>
<td>9.5</td>
</tr>
</tbody>
</table>

a One drop of 1% 2, 4 DNC1B in alcohol.
b Animals sensitized by six consecutive paintings with 2% 2, 4 DNC1B seven days prior to grafting.
c White grafts when first observed at five days after operation.
d No prior treatment with chemical.
untreated recipients were used, however, all four grafts transplanted showed first set homograft responses. The gross morphology was the same as that of viable grafts from sensitive donors to untreated recipients.

Application of 2, 4 DNC1B did not affect acceptance of autografts as demonstrated in Table 3. All autografts attempted were permanently accepted whether they were transferred from the abdomen to the shoulder or lifted from the shoulder and placed back on their bed. Their edges healed in, they developed a pink color, and bled on scraping with a scalpel.

In addition to possibly altering graft morphology and acceptance by the host, we thought that painting grafts with 2, 4 DNC1B might confer delayed sensitivity to 2, 4 DNC1B in untreated graft recipients. The animals were tested and graded as described in the Materials and Methods. The results are tabulated in Tables 4 and 5. Here it can be seen that 2, 4 DNC1B sensitivity was not transferred to recipients when the grafts were transplanted 24 hours after the chemical was applied. This was true whether sensitive or untreated donors were used. Likewise, grafts transplanted from sites to which 2, 4 DNC1B had not been applied did not transfer sensitivity to the chemical.

Only when the grafts were transplanted to the recipients immediately after 2, 4 DNC1B application did the animals become sensitive. Even then there was not a sharp distinction between most of the
TABLE 3. -- Survival of Skin Autografts from Untreated Animals Transplanted Immediately After Application of 2, 4 DNClB on Skin to be Grafted.

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of Graft</th>
<th>Rejection Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Painted(^a) grafts lifted from shoulder and placed back on bed</td>
<td>Accepted Permanently</td>
</tr>
<tr>
<td>16</td>
<td>Painted grafts transplanted from abdomen to shoulder</td>
<td>Accepted Permanently</td>
</tr>
</tbody>
</table>

\(^a\)One drop of 1% 2, 4 DNClB in alcohol.
### TABLE 4. --Responses of Guinea Pigs to Sensitizing Dose of 1% 2, 4 DNC1B in Alcohol after Receiving Painted Homografts.

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of Graft</th>
<th>Time From Painting Until Grafting</th>
<th>Donor State</th>
<th>Recipient State</th>
<th>Number of Animals Showing Sensitivity Response 0+1+2+3</th>
<th>Average Sensitivity Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Painted(^{a,b})</td>
<td>Immed.</td>
<td>Sensitive(^{c})</td>
<td>Untreated</td>
<td>1 4</td>
<td>+1.8</td>
</tr>
<tr>
<td>5</td>
<td>Painted</td>
<td>Immed.</td>
<td>Untreated(^{d})</td>
<td>Untreated</td>
<td>4 1</td>
<td>+2.2</td>
</tr>
<tr>
<td>4</td>
<td>Painted</td>
<td>24 Hours</td>
<td>Sensitive(^{e})</td>
<td>Untreated</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Painted</td>
<td>24 Hours</td>
<td>Untreated</td>
<td>Untreated</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{a}\) One drop of 1% 2, 4 DNC1B in alcohol.

\(^{b}\) Control animals received an application of one drop of 1% 2, 4 DNC1B in alcohol followed by an equal application one week later on the shaved flank. The 20 animals so tested had an average sensitivity response of +1.8.

\(^{c}\) Animals sensitized by six consecutive paintings with 2% 2, 4 DNC1B seven days prior to grafting.

\(^{d}\) No prior treatment with chemical.

\(^{e}\) Animals sensitized by six consecutive paintings with 2% 2, 4 DNC1B eight days prior to grafting.
TABLE 5. --Responses of Guinea Pigs to Sensitizing Dose of 1% 2, 4 DNC1B in Alcohol after Receiving Painted Autografts.

<table>
<thead>
<tr>
<th>No.</th>
<th>Time From Painting Until Grafting</th>
<th>Animal State</th>
<th>Number of Animals Showing Sensitivity Response 0+1+2+3</th>
<th>Average Sensitivity Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Painted (^a) grafts lifted from shoulder and placed back on bed</td>
<td>Immed.</td>
<td>Untreated (^b)</td>
<td>5 5</td>
</tr>
<tr>
<td>16</td>
<td>Painted grafts transplanted from abdomen to shoulder</td>
<td>Immed.</td>
<td>Untreated</td>
<td>1 6 1 0 3</td>
</tr>
</tbody>
</table>

\(^a\) One drop of 1% 2, 4 DNC1B in alcohol.

\(^b\) No prior treatment with chemical.
grafts and controls which received a sensitizing dose of one drop of a 1% solution of the chemical followed by another application one week later. Animals receiving painted grafts from sensitive donors demonstrated the same degree of sensitivity as did the controls. Animals receiving painted grafts from untreated donors had a slightly higher degree of sensitivity than the controls. Autografts resulted in the best sensitivity with autografts transplanted from the abdomen to the shoulder resulting in slightly greater sensitivity than autografts cut from the shoulder and placed back on their beds.
DISCUSSION

These results indicate a considerable difference in graft acceptance, depending on the type of graft transplanted and the type of recipient to which it was transferred. Grafts from sensitive donors painted with 2,4-DNC1B and then transferred to sensitive recipients were white grafts. Painted grafts transferred immediately from untreated donors to sensitive recipients were also white grafts. Transfer of 2,4-DNC1B painted grafts from sensitive donors to untreated recipients resulted in some first set responses and some white graft reactions. Typical first set homograft responses occurred with the other homografts. Autografts painted with 2,4-DNC1B were accepted by the host the same as nonpainted autografts.

These observed differences in graft acceptance seem to be the result of several factors. One factor appears to be the chemical's toxicity for homograft tissue. Grafts painted with 2,4-DNC1B were not as well accepted as those which were not painted. Even when typical first set homograft or autograft reactions did take place, a layer of dead skin was usually to be found on the surface of the graft. The fact that white grafts sometimes resulted when 2,4-DNC1B painted skin was transplanted immediately from sensitive donors to untreated recipients supplies the best evidence for chemical toxicity. Since the grafts were
transplanted immediately, there would be little opportunity for donor reaction against the graft. There would be little reason to suspect host reaction against the graft either, since the host was untreated.

Another factor, however, seemed to be a specific reaction by sensitive animals against chemically painted skin. None of the sensitive recipients accepted chemically painted skin unless it was transplanted from untreated donors 24 hours after painting. By that time any chemical influence on the graft may have been diminished. Most of the untreated recipients accepted painted skin. Better acceptance was also observed when painted grafts from sensitive donors were transplanted to untreated recipients immediately. In this case a time interval before transplanting might allow sensitive donors to react against painted grafts.

Finally, 2,4 DNC1B had to be painted directly onto the grafted skin to exert its effect. Almost all nonpainted grafts demonstrated the typical homograft response of initial acceptance, regardless of whether the animal was sensitized or not.

Immediate transplantation of 2,4 DNC1B painted grafts was shown to affect not only the homograft response, but also to confer sensitivity to 2,4 DNC1B in previously untreated animals. The sensitivity conferred was of a low degree, being about equal for homograft recipients and the controls receiving one drop of 1% 2,4 DNC1B.
The autograft recipients demonstrated a slightly higher degree of sensitivity, being half to three-fourths of a grade scale higher.

Grafts transplanted 24 hours after 2, 4 DNC1B was applied to them did not result in sensitized recipients. This fact suggests that uncombined 2, 4 DNC1B complexing with host tissue is responsible for the sensitivity resulting from immediate transplantation of 2, 4 DNC1B painted grafts. Grafts transplanted immediately would possess a large amount of free, uncombined 2, 4 DNC1B that had not been adsorbed to the tissues or absorbed into the blood stream. If transplantation took place after 24 hours the chemical would have had considerable time to be adsorbed to the tissues or absorbed into the blood stream. The fact that the controls became sensitive with only one drop of 2, 4 DNC1B supports this theory as it proves that the guinea pigs can be sensitized by a very small amount of free chemical. Thus, diffusion of 2, 4 DNC1B from the graft into the surrounding host tissue and combination with the host tissue appears to be the mechanism whereby the host becomes sensitive.

This theory could explain the halo of inflammation that consistently surrounded only 2, 4 DNC1B painted grafts transplanted immediately to sensitive recipients. The inflammation would be caused by uncombined 2, 4 DNC1B diffusing into the surrounding host tissue. It could cause inflammation just as if one drop of 2, 4 DNC1B had been applied to the sensitive animal.
Grafts transplanted 24 hours after painting would not contain any free chemical to diffuse into surrounding host tissue and would not result in inflammation.

When grafts were transplanted immediately to untreated recipients, no inflammation occurred just as none would occur when one drop of 2, 4 DNOP is applied to an untreated animal.

The fact that autografts resulted in the greatest sensitivity could be explained by better host acceptance of autografts than homografts. If the blood supply to the autograft developed sooner and more extensively than to a homograft it would result in better contact between the 2, 4 DNOP and tissue surrounding the graft. This in turn would result in greater sensitivity in the animal.
SUMMARY

Chemically painted and normal skin grafts were transplanted to sensitive and untreated recipients. Painted grafts transplanted from sensitive donors to sensitive recipients remained white grafts. Normal grafts and the majority of painted grafts were initially accepted in untreated recipients. Painted autografts were permanently accepted.

Chemical sensitivity was transferred to previously untreated animals by both homografts and autografts transplanted immediately after painting. Grafts transplanted 24 hours after chemical application did not transfer sensitivity.
LIST OF REFERENCES


