STUDIES OF MOUSE HYPERSENSITIVITIES

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STUDIES OF MOUSE HYPERSENSITIVITIES

THESIS

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By

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CHAPTER I

INTRODUCTION

An examination of current literature concerning the immunological responses of mice to various stimuli found to be antigenic in numerous other laboratory animals reveals a lack of agreement. This is not necessarily due to a lack of volume since considerable laboratory work has been carried out using the mouse as an experimental animal. Until recently proper stimulation and elicitation of immunological responses in the general category of hypersensitivities was not presented in the published data (4, 8). Early workers had not doubted the ability of mice to respond to antigens, but they firmly believed those responses were too weak for the accumulation of conclusive data (9).

Possibly the negative attitude toward the ability of the mouse to respond immunologically was due to accumulative misinterpretation rather than to a single causative factor. It has been noted that a relatively large number of genetically specific strains of laboratory mice has been available to
those interested in their use (6). It has also been found that their response patterns differ considerably, depending upon the genotype (5, 12). The available techniques and equipment were relatively unsuited to mice, so larger animals such as guinea pigs or rabbits were used (19). Unfortunately the protocol of immunologists lacked the inclusion of mice as suitable experimental animals. This was probably generated by the quest for "positive" results, which could be more easily obtained with other animals. Recently, however, numerous workers have realized the possibilities of using such small, inexpensive and convenient animals as mice. Following this observation, considerable activity in this area of immunology has begun.

The results of the accumulated data indicated that a revision of some ideas incorporated into the science of immunology might be needed. One criterion for questioning involves the theories concerned with the sequence of events in hypersensitized individuals. One concept is that delayed hypersensitivity may be an initial event in hypersensitization followed in time with proper stimulation by the immediate type (11, 20). The second theory involves an early type delayed hypersensitivity preceding immediate hypersensitivity, yet considerably different from the classical tuberculin-type
delayed hypersensitivity (13, 18). This early type delayed hypersensitivity has been referred to as the "Jones-Mote" type (17). In the mouse quite the reverse of both these has been found to occur (7). Immediate hypersensitivity precedes rather than follows the appearance of delayed type hypersensitivity (8). No doubt the sensitization procedure in the mouse can affect the strength and longevity of such reaction, but not their appearance (7).

Numerous other immunological phenomena have been studies by various techniques. The published data are by no means congruent concerning reaction patterns, however, since the same workers at times seem to contradict themselves. Crowle, for example, stated in 1964, that immediate hypersensitivity to dinitrochlorobenzene is rarely encountered (8, p. 133). In data published in 1961, an exact description of positive immediate hypersensitivity was given by the same worker (6, p. 306).

Many new concepts in the field have been brought about as a result of experimentation using the mouse. Fink in 1954, concluded from his work with variations in sensitivity to anaphylaxis and to histamine in inbred strains of mice that circulating antibody might not be the causative factor of mouse anaphylaxis (12). Favour, also in 1954, examined the possibilities of reversing the tuberculin anergy in mice by
adrenalectomy (10). This seemed quite likely, according to Favour, since small animals like the mouse have proportionally larger adrenals than those of heavier animals such as guinea pigs and rabbits. He found this hypothesis to be inaccurate upon experimentation.

Passive transfer of immunocompetent cells, either leucocytes, spleen cells or lymph nodes, has given positive delayed hypersensitivity tests in the recipient animals (19, p. 334). In conjugation with this work, theories concerned with antibody formation have been projected. By injecting mice with either high or low doses of antigen from the fifth week of life to adulthood, unresponsiveness to the antigen could be found in the ones injected with high concentrations. This, of course, is a type of immune paralysis. Transfer of cells from unresponsive spleens and lymph nodes to normal animals, with or without antigenic stimulation of the recipient, did not result in antibody formation. Recovery from unresponsiveness took place when levels of antigen in the blood became undetectable. No further challenge was required for inducement of antibody synthesis. This caused the workers to suggest that "paralyzed animals do not produce antibody, but rather their cells are specifically inhibited " (21). Mice have not only been used in the work already cited, but their
importance in research concerning homograft immunity has also been noted (4, p. 154). One might draw the conclusion that the work done using the mouse as an experimental tool is highly justified.

**Dimethyl Sulfoxide As A Solvent**

The data already collected by workers in the field of mouse immunology need not be considered completed. By reworking the experimental design to include new variables, numerous new significant data might be obtained. Most of the experimental work done to examine the mechanisms of delayed and immediate hypersensitivity to contact allergens such as dinitrochlorobenzene or dinitrofluorobenzene have used acetone as a solvent or carrier of low concentrations of stimulant. By using a solvent of somewhat different character, one might find the immunological response altered. A solvent which seems to lend itself to immunological studies in dimethyl sulfoxide.

Dimethyl sulfoxide is quite similar in chemical structure to acetone, in that the carbonyl carbon of acetone is replaced by a sulfur atom. Since the early 1940's, this chemical, which was first synthesized by Alexander Saitzeff in 1867 (2), has been recognized as a valuable product. Specifically it is defined as a highly polar chemical which is water miscible and highly hygroscopic.
Innumerable uses for dimethyl sulfoxide have been devised since the rediscovery of its useful characteristics in the 1940's. Industrial applications have long been recognized for dimethyl sulfoxide (DMSO), as it is an important component in many economic processes. It is used in the polymerization and spinning of polyacrylonitrile in the synthetic fabric industry (24). It is an intermediate or important ingredient in the production of numerous synthetic chemicals. It has also been found to be an efficient carrier for dyes and pigments, a paint stripper, a resin solvent and has been applied indiscriminantly to any number of other processes.

Only until very recently were its pharmaceutical and medicinal attributes recognized. The original medicinal application of DMSO was as a preservative of various frozen cell types, in which case it was found to increase the longevity of these cells under low temperature conditions (16). Newer applications and uses include its potentials as a penetrant carrier, local analgesic, anti-inflammatory adjunct, bacteriostat, diuretic, and tranquilizer (24, p. 345). Most of the work of pharmaceutical and medicinal properties has been at the clinical level rather than the experimental level, so published scientific data is not as common as one might expect.
The plant sciences have used DMSO as a solvent for numerous pesticides and fungicidal agents. It has been found to be extremely effective in both enhancing and expediting the activity of the agents. Many of the solutes of pesticide nature were previously considered almost insoluble, but the ease with which they may be suspended in dimethyl sulfoxide has done away with this observation. Exemplification of every other use of dimethyl sulfoxide is almost an impossibility, but a consideration of its applicability to an immunological study might be of more importance.

A close examination of the literature indicates that dimethyl sulfoxide is not only important as a solvent, but also as a skin penetrant (15). Horita and Weber (14) found this substance to be an excellent penetrant of biological membranes. In their study, increased absorption of heparin, sulfadizine, salicylic acid, trypan blue, and insulin was found when a DMSO-buffer solution was used in conjugation with the bladder perfusion technique (14, p. 1389).

The main problem in the use of dimethyl sulfoxide as a solvent in contact with living organisms is its toxicity (23; 2, p. 345). Numerous studies have been made to show what effect DMSO has upon living systems; however, the results might thus far be considered without an accepted conclusion.
Rabbits with abraded and unabraded skin were exposed to one-half milliliter of DMSO on one-inch gauze-covered squares. Examination at twenty-four and seventy-two hours showed no irritation. Other rabbits tested were treated with fifteen treatments of ten milliliters each over a hundred square centimeter segment of shaved back skin; all completed in a time period of twenty days. The only results were dried, cracked, and scaly skin (25).

Sommer dipped the tails of mice into absolute DMSO for five and one-half hours, with the production of no discernable signs of local damage (22). Brown and associates applied daily doses of undiluted DMSO to the backs of clipped guinea pigs for a period of twenty-eight days, with the demonstration of "no macro or microscopic signs of injury" (3). Male AH mice were also treated on unshaven dorsal scapula skin with twice weekly paintings of DMSO for thirty weeks, again with no sign of toxicity (3).

Willson, Brown and Timmons determined the oral and intravenous toxicity of DMSO in mice and rats with the finding that some toxicity did exist (23, p. 111). Twenty-four intraperitoneal injections in rats resulted in anemia and peritoneal inflammation. Low level doses over the same period showed no
fatalities or pronounced toxicity. These workers concluded a low systemic toxicity for mice and rats (23, p. 111).

Ashwood-Smith has done a comprehensive study on toxicity produced in various tissues frozen with various concentrations of DMSO (1). Generally, he concludes, low concentrations of DMSO added to freeze mediums enhance the viability of the frozen cells, with little or no toxic effects.

The toxicity for human use has been of more concern recently than the above data might lead one to believe. The carefree clinical use of DMSO under uncontrolled conditions has resulted in some unexplainable results. The Food and Drug Administration has recently curtailed the use of DMSO and investigated those who have been using it in the last few years (26). Until more scientific data can be accumulated, its clinical use will probably be prevented.

Not only has there been considerable work done on the toxicity of DMSO, but the allergenic effects of its use have also been investigated. Several studies have been published concerning the possibility of adverse allergenic responses to DMSO as a solvent injected intracutaneous. At the Laboratory of Vitamin Technology, one-tenth per cent solutions of DMSO in water were administered to white male guinea pigs. After ten sensitizing injections of the solution, no evidence
of reaction during the sensitization was noted. After two weeks, a challenge dosage gave no indication of allergy (25, p. 2). Other work by Brown (3, p. 2) likewise indicated no allergenic response in guinea pigs of both sexes.

If one concludes from the data previously collected that the toxicity of DMSO is low enough and that it has no allergenic effects, then its use as a solvent in immunological systems seems quite possibly rewarding. If it does in fact increase membrane permeability and transport its solute more rapidly to the immunocompetent cells, then it could be an excellent carrier for antigens. The mechanism of this action has been studied, showing some chemical characteristics which might well explain its transporting activities.

Most of the mechanistic studies have been in conjunction with topical therapeutic application, from which pertinent facts have evolved. DMSO displays exceptional ionizing powers, which actually should decrease penetration of biological membranes since less ionized molecules pass more easily (2, p. 462). Many organic compounds form complexes with DMSO, which again would show increased polarity. When DMSO is added to a solution, complex formation with metallic salts is noted. Many of the salt complexes are found to be highly hygroscopic;
DMSO itself is highly hygroscopic; both factors may be involved in membrane penetration (15, p. 462).

These pertinent facts have stimulated some theories:

a. Its solvency towards both lipoidal and non-lipoidal materials, including polysaccharides, free fatty acids, and sterols, allowing for rapid penetration and permitting disruption of the matrix such that other compounds are able to penetrate more easily;

b. Its ability to form chelates with metallic elements known to be present in the cellular membranes; thus altering membrane porosity;

c. Its semi-polar nature and its small molecular size;

d. The fact that DMSO is hygroscopic and that as it penetrates the skin, it may be picking up moisture from the surrounding tissue; the increase in moisture content of the skin in the 'microenvironment' of DMSO molecules could allow for more rapid absorption through the skin. Of course, one might again consider the 'shell of hydration' surrounding the molecule as influencing transport;

e. The heat of dissolution generated when water is added to DMSO causing an increase in solvent power, an increase in diffusion; in short an increased permeability (2, p. 464).

Having considered the data collected thus far concerning immediate and delayed hypersensitivity reactions in the mouse, the experimentation following is devised essentially to follow the pattern of Crowle (6, pp. 303-304; 8, p. 133) in the production and testing for immunoresponse. Instead of using the normal solvent, that is, acetone, this work is also intended to partially evaluated the use of dimethyl sulfoxide as a solvent for the contact allergin, dinitrofluorobenzene.
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CHAPTER II

MATERIALS AND METHODS

Mice of the C57Bl/6J strain were obtained from Jax Memorial Laboratories, Bar Harbor, Maine, and were maintained on an unsupplemented diet of Purina Dog Chow and water. Each experimental series involved forty mice which were kept in lots of thirteen or fourteen animals in small plastic pans. These pans were cleaned at least once weekly. Other than group sensitization, the mice were maintained under as nearly the same conditions as possible.

1-fluro-2,4-dinitrobenzene (DNFB) obtained from Eastman Kodak Company was twice crystallized in absolute ethanol and used as a sensitizing agent. This was suspended in absolute dimethyl sulfoxide (DMSO) obtained from Matheson Scientific Products Company, at a final concentration of two-tenths percent. This concentration was used as a result of the experimentation of Crowle, in which stronger concentrations of DNFB were found to be somewhat toxic to the test mice (3).

Three experimental series utilizing forty male mice each, were composed of three groups, referred to hereafter as
Group I, Group II, and Group III. In experimentation series II, the proposed technique of Crowle (2) was used for sensitization of the mice. The mice were clipped with animal clippers forty-eight hours prior to sensitization. However, this procedure was found to be unsatisfactory due to severe reactions consisting of bloody lesions in the Group I mice, probably a result of either solute or solvent toxicity of the clipped skin which exhibited minor abrasions. Shaving seventy-two hours prior to challenge was also attempted, but growth of hair was so profuse by the second day of the five day painting sequence that clipping actually served little or no purpose. The following technique was utilized in both experimental series I and III, and found to be more satisfactory.

The thirteen mice of Group I were sensitized with two-tenths per cent DNFB in DMSO administered with a number four camel hair brush. The brush was dipped in the sensitizing solution, drained of excess liquid by pressing against the neck of the solution container, and then stroked from the base of the skull to mid-back on the unclipped mouse. Sensitization continued for five consecutive days, after which a period of seven days passed without painting. Five more paintings were made after this interval, followed by another lapse of seven days. This continued until three series, or a total of fifteen paintings, were administered.
The thirteen mice of Group II were painted unclipped with absolute DMSO. A number four camel hair brush was again used, and the same sequence as in Group I was followed. Group II thus served as a solvent control group. Group III, composed of fourteen mice, was the normal control group, and no sensitization of any kind was administered.

**TABLE I**

**SENSITIZATION AND TEST SCHEDULE**

<table>
<thead>
<tr>
<th>Sensitization and mice numbers</th>
<th>Delay period and mice numbers</th>
<th>Testing and mice numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 1-5; 40 mice</td>
<td>Days 6-12; 40 mice</td>
<td>Day 13; 13 mice</td>
</tr>
<tr>
<td>Days 13-17*; 27 mice</td>
<td>Days 18-24; 27 mice</td>
<td>Day 25; 13 mice</td>
</tr>
<tr>
<td>Days 25-29; 14 mice</td>
<td>Days 30-37; 14 mice</td>
<td>Day 38; 14 mice</td>
</tr>
</tbody>
</table>

*Series II delayed due to nonspecific reactions*

Table I shows the sequence of sensitizations noted as day numbers, the day numbers on which tests were made, and the numbers of mice present in each portion of the experiment. Testing was carried out to determine if either delayed or immediate hypersensitivity resulted from the sensitization of the mice.
Although numerous techniques for testing of immediate hypersensitivity in the mouse have been proposed in the literature, none had been shown to be more satisfactory than the others (1). Of the several testing procedures discussed, production or lack of production of systematic anaphylactic shock served as a criterion for determining the presence of immediate hypersensitivity. Skin testing using DNFB in acetone has been reported to show the presence or absence of immediate hypersensitivity (2, p. 306; 3, p. 133). Even though this is similar to the technique used to test for delayed hypersensitivity, it was considered less satisfactory for an immediate hypersensitivity test due to the lack of consistency in the reports of its use (2, p. 306; 3, p. 133).

The mice from all three tests groups were challenged on the days indicated in Table I by injecting three-hundredths milliliters of two-tenths per cent DNFB in DMSO into the tail vein of the mice by means of a twenty-seven gauge inoculating needle. The individual mice to be tested were inserted head first into a test tube about the same circumference of the mouse. The bottom of the tube was packed with cotton to support the mouse in the upper half of the tube. The tail was placed through a notch carved in the side of a number six rubber stopper which was used to hold the mouse firmly in the test tube. With
the mouse in place, the tail was rubbed with xylene, which resulted in the enlargement of the tail vein. The challenging procedure was accomplished in less than two minutes, and none of the mice showed any ill effects from the mechanics of the innoculation process.

Challenged mice were observed closely for a period of forty-eight hours. Hourly examinations were made for the first four hours. Following this, observations were made every six to eight hours. Systemic anaphylaxis in the mouse assumes somewhat a different course from the type experienced in guinea pigs (5). In most cases death does not occur within the first few minutes after challenge, but the mouse may exhibit symptoms of unresponsiveness to irritation, watery eyes, ruffled fur, a wheezing breath, or other minor reactions (4). A mouse exhibiting any of these characters was considered as an immediate hypersensitized animal.
### TABLE II

**TESTING PROCEDURE**

<table>
<thead>
<tr>
<th>Day</th>
<th>0.03 cc 0.2% DNFB in DMSO inoculated into tail vein</th>
<th>Skin painted with 0.25% and 0.10% DNFB in olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>2 mice; Group I 2 mice; Group II 1 mouse; Group III</td>
<td>3 mice; Group I 3 mice; Group II 2 mice; Group III</td>
</tr>
<tr>
<td>25</td>
<td>2 mice; Group I 2 mice; Group II 1 mouse; Group III</td>
<td>3 mice; Group I 3 mice; Group II 2 mice; Group III</td>
</tr>
<tr>
<td>38</td>
<td>2 mice; Group I 2 mice; Group II 1 mouse; Group III</td>
<td>3 mice; Group I 3 mice; Group II 3 mice; Group III</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Challenge for the demonstration of delayed hypersensitivity was carried out by the following procedure on test days indicated in Table I. The mice were clipped with Oster A-2 Small Animal Clippers twenty-four hours prior to testing, except in experimental series III, in which they were shaved forty-eight hours prior to testing. On the test dates, the clipped skin on the unpainted rump and right flank of mice from Groups I, II and III was challenged with twice crystallized DNFB in olive oil. Each mouse was tested with a twenty-five hundredths percent DNFB-olive oil solution on the flank, and a one-tenth percent DNFB-olive oil solution on the rump. Prior to testing,
the clipped skin was cleaned of body oils with absolute acetone on a cotton swab. After the skin dried, one drop of each test solution was placed in position on the skin with a standard eyedropper. This was followed by vigorous massage of the area with small round-tipped glass rods. Separate rods were used for each concentration. Readings of the skin tests were made at three, twenty-four, and forty-eight hours after challenge. The readings after three hours were made in order that any immediate hypersensitivity reactions might be detected. Readings after twenty-four and forty-eight hours consisted of examination for the onset of slight edematous swelling, followed by inflammation, and possible central necrosis as described by Crowle (2, pp. 305-306). The animals exhibiting any of these reactions were interpreted as delayed hypersensitized animals.
CHAPTER BIBLIOGRAPHY


CHAPTER III

EXPERIMENTAL RESULTS

Cautious examination of various techniques, one of which hopefully would produce repeatable experimental results, has caused an unavoidable variety of observations and results in the three experimental series carried out. A refinement of the techniques offered in the literature was necessary so that inaccuracies would not be incurred as a result of inappropriate technique. Essentially the techniques employed in all three experimental series were the same, with the variations noted in experimental procedures. Slight manipulations in series I and II, however, warrant individual consideration and explanation.

The pilot study, series I was intended to determine the feasibility of the experimental design previously conceived. Results in Table III as well as the tables representing results for series II and series III are noted as the number of positive test animals over the total number of test animals. Various workers report the strength of immediate and delayed reactions as plus one, two, three or four, but these results are only
designated as either positive or negative since no clear variety of degree was observed.

TABLE III

RESULTS SERIES NO. I

<table>
<thead>
<tr>
<th>Day</th>
<th>Immediate Test</th>
<th>Delayed Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>0/2 Group I</td>
<td>0/3 Group I</td>
</tr>
<tr>
<td></td>
<td>0/2 Group II</td>
<td>0/3 Group II</td>
</tr>
<tr>
<td></td>
<td>0/1 Group III</td>
<td>0/2 Group III</td>
</tr>
<tr>
<td>25</td>
<td>0/2 Group I*</td>
<td>0/3 Group I*</td>
</tr>
<tr>
<td></td>
<td>0/2 Group II</td>
<td>0/3 Group II*</td>
</tr>
<tr>
<td></td>
<td>0/1 Group III</td>
<td>0/2 Group III</td>
</tr>
<tr>
<td>38</td>
<td>1/2 Group I</td>
<td>0/3 Group I*</td>
</tr>
<tr>
<td></td>
<td>0/2 Group II</td>
<td>0/3 Group II*</td>
</tr>
<tr>
<td></td>
<td>0/1 Group III</td>
<td>0/3 Group III</td>
</tr>
</tbody>
</table>

*Reactions described in text

Challenge of the test animals with three-hundreths milliliters of two-tenths per cent DNFB in DMSO resulted in the death of one animal of Group I on day thirty-eight. The animal was given the same treatment in testing as the other animals, yet it was the only one which within seconds after the inoculation exhibited extreme muscle spasms, restricted breathing and death within ten seconds. It is believed that mechanical injury could not have been the cause of this animal's death. The possibility of systemic anaphylactic shock very similar to the type found in guinea pigs may therefore occur in mice (1, p. 471). Over
an extended period of forty-eight hours, none of the other animals showed symptoms indicative of systemic anaphylaxis.

After the forty-eight hour period of observation, another attempt was made to incite anaphylactic response. This time, inoculations of the same amounts were made directly into the heart. Although death or apparent shock did result in some cases, equally large numbers of positive reactions were found among solvent control and normal control animals. It was concluded that fatalities and apparent shocks were probably a result of mechanical injury rather than immediate hypersensitivity to the challenge dose.

Testing of twenty-five animals for delayed hypersensitivity resulted in no positive tests. No inflammation or soft swellings were encountered in any animals after forty-eight hours. After the forty-eight hour observation period, this group of animals was tested for anaphylaxis. After twenty-four hours, two of the three animals of Group I and one of the three animals of Group II in the twenty-five day test group, and one of the three animals of Group I and two of the three animals of Group II in the thirty-eight day test group developed irregular bordered inflammations. These appeared as a darkened area on the clipped skin which had been tested for delayed hypersensitivity, and lasted from one to two days. An
explanation of the cause of these reactions was not available at that time. Even though no reactions of this type were found in the normal controls, it should be noted that these normal control animals were sacrificed shortly after completion of the delayed test. The animals of Group II, however, which had only been sensitized with DMSO, showed some positive reactions to either the DNFB, DMSO or both.

It was decided at this time to maintain watch over the animals tested for delayed reactions in series II for a period of seventy-two hours, even though reactions are seldom if ever reported to require this much time. If no reactions appeared in seventy-two hours, the animals were to be given inoculations.

The modification suggested by Crowle (2), which called for clipping of the mice before sensitization, was attempted in experimental series II. Paintings one through five were made as before, but forty-eight hours before the sixth painting, the backs of the mice were clipped. Paintings six and seven were made, and on day fifteen lesions had begun to form on the backs of the animals in Group I. Paintings of Group I and Group II were delayed because of these severe reactions. The reactions progressed in intensity over the next three days, until bloody lesions completely through the skin revealed the muscles of the back. Other than clean cages, nothing was done to prevent
infection, yet infection did not occur. The mice could not be painted again until these lesions healed. No further shaving before sensitization was attempted. On day thirty, painting of Group I and Group II was continued. Three paintings on days thirty, thirty-one and thirty-two finally brought the total to ten. On day thirty-eight the second tests were made, and the third painting sequence was begun. Supplementary tests showed the Crowle modification unsuitable for C57Bl / 6J mice when using the described techniques.

### TABLE IV
RESULTS SERIES NO. II

<table>
<thead>
<tr>
<th>Day</th>
<th>Immediate Test</th>
<th>Delayed Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>0/2 Group I</td>
<td>0/3 Group I</td>
</tr>
<tr>
<td></td>
<td>0/2 Group II</td>
<td>0/3 Group II</td>
</tr>
<tr>
<td></td>
<td>0/1 Group III</td>
<td>0/2 Group III</td>
</tr>
<tr>
<td>38</td>
<td>1/2 Group I</td>
<td>1/3 Group I</td>
</tr>
<tr>
<td></td>
<td>2/2 Group II</td>
<td>2/3 Group II</td>
</tr>
<tr>
<td></td>
<td>0/1 Group III</td>
<td>0/2 Group III</td>
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<tr>
<td>51</td>
<td>0/2 Group I</td>
<td>2/3 Group I</td>
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<tr>
<td></td>
<td>0/2 Group II</td>
<td>1/3 Group II</td>
</tr>
<tr>
<td></td>
<td>0/1 Group III</td>
<td>0/3 Group III</td>
</tr>
</tbody>
</table>

The animals exhibiting positive immediate hypersensitivity were all in the thirty-eight day test group. All three animals showed slightly ruffled fur, possibly indicating anaphylaxis,
for approximately forty-eight hours after onset of symptoms. These were interpreted as positive tests, even though they were not as pronounced as in series I.

The same type delayed hypersensitivity reactions as had been seen in series I were found to occur three days after initiation of testing. These reactions became obvious without further testing which had seemed influential in series I. These were observed in both Groups I and II in the thirty-eight day test groups.

Experimentation series III was begun exactly the same as experimentation series I. Clipping before sensitization, which seemed responsible for the bloody lesions in series II, was omitted, even though Crowle feels this necessary for significant results (3). The clipping procedure in series III preceded the challenge by forty-eight hours, whereas before it preceded the challenge by twenty-four hours. This precaution was initiated as a result of the non-specific reactions to sensitization obtained in series II, and the positive delayed reactions in solvent control animals.
TABLE V

RESULTS SERIES NO. III

<table>
<thead>
<tr>
<th>Day</th>
<th>Immediate Test</th>
<th>Delayed Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>0/2 Group I</td>
<td>0/3 Group I</td>
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<td></td>
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<tr>
<td>25</td>
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<td>0/3 Group I</td>
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<tr>
<td></td>
<td>0/2 Group II</td>
<td>0/3 Group II</td>
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<tr>
<td></td>
<td>0/1 Group III</td>
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</tbody>
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In experimental series III there were no animals exhibiting positive tests to either immediate or delayed challenges. The only known difference between this series and series I is the number of hours separating the clipping procedure and testing.
CHAPTER BIBLIOGRAPHY

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CHAPTER IV

DISCUSSION

The original experimental design was changed somewhat in order to correct certain conditions encountered upon experimentation. Essentially the methods used remained constant. The two major changes concern shaving time before testing, and shaving before sensitization.

Shaving or clipping prior to sensitization was found by Crowle to be valuable in obtaining consistent results (1). A more accurately measured sensitizing dosage could be applied, and interference of hair during testing was avoided by clipping. When this technique was applied to the experimental procedure in sensitization of C57Bl / 6J mice, severe lesions formed. This made further painting inadvisable for fourteen days. In the CF-1 white mice used by Crowle, shaving forty-eight hours prior to sensitization and testing was found to prevent scaling and non-specific inflammation (3, p. 305). Since mention was not made of lesions similar to those encountered in series II of this work, these lesions were considered abnormal. None of the other literature dealing with contact hypersensitivity in
mice made mention of such reactions. In order to avoid these reactions, clipping prior to sensitization was omitted.

The experimental results observed in test mice which had experienced the sensitization reactions might indicate enhanced hypersensitivity. The occurrence of this type reaction during sensitization, however, would change considerably the physiology of the mice involved. Experimentation normally avoids points of variation. Notation of such reactions should probably be made since animals which remain healthy through sensitization might exhibit different reaction patterns. An increase or decrease in response strength was not studied in mice which underwent such reactions during sensitization.

The somewhat unexplainable delayed reactions after three days in Group II mice in both series I and series II were thought to be similar to the non-specific inflammation encountered in sensitization after clipping. These reactions to DNFB in olive oil by mice of the solvent control group sensitized only with DMSO were unexplainable, except as a function of toxicity of solute or solvent. Since clipping had been done twenty-four hours prior to challenge, a forty-eight hour interval was thought to be desirable in preventing non-specific reactions which might be confused as indicative of delayed hypersensitivity.
Even though it can not be stated that clipping twenty-four hours prior to challenge was responsible for the three day reactions, the fact remains that challenge forty-eight hours after clipping in series III resulted in no positive reactions in those animals tested for delayed hypersensitivity.

The observation was made that mice sensitized with two-tenths per cent DNFB in DMSO had yellow stained hair on the painted area. As the number of paintings accumulated, the hair and skin both were stained yellow. This indicates a loss of DNFB to the hair and decreased effective sensitizing dosage.

These experimental results might lead one to believe that immunoresponse in mice to contact allergins might be entirely different from that observed not only in other species, but also in different strains of the same species (4). Crowle and Hu found numerous variations in reactions among six specific strains of mice which they studied (2). The strains of mice which have been highly inbred for years are possible more reliable tools for experimentation due to their isologous genotype than other animals of a more heterogeneous genetic character.

The effects of DMSO upon the response mechanisms in mice to contact allergins is hardly clarified as a result of these experiments. On the basis of the results, one might conclude
that even though DMSO is a good penetrant of skin and biological membranes, it does not enhance immunoresponse in C57Bl/6J mice. An additional observation was made which was not noted in experimental results concerning the mice of Group II. There was no apparent toxicity to the mice which were given fifteen applications of absolute DMSO over a period of thirty days. Microscopic examinations of tissue were not made, and the lack of toxicity was apparent in gross anatomy and lack of behavioral changes in the mice. The skin on the painted backs of the mice increased considerably in thickness. It appeared scalier than the skin on mice in the other groups, and assumed a tough consistency.

The use of DMSO as a solvent in wide-spread studies is premature, since its use in work with mice is not extensive. Until more is known about the effects DMSO alone has on the animals, conclusions concerning immunoresponse will be somewhat difficult to make.

The importance of considering solvent effect in immunological studies has possible not been considered to its fullest extent. Few immunological studies using acetone as a solvent, or other solvents have been reported which have used solvent control animals. This seems unfortunate, since response patterns may be dependent upon effects of the solvent used.
CHAPTER BIBLIOGRAPHY


CHAPTER V

SUMMARY

Even though mice are inexpensive, easily kept, and many strains are isologous, their use in immunological studies has been limited. In this study, C57Bl / 6J mice were painted with the contact allergin dinitroflurobenzene. Dimethyl sulfoxide was used as the solvent. Considerable research has recently been concerned with the activities of DMSO. Its similarity to acetone, along with its properties as a penetrant of skin and biological membranes, qualify it as possibly useful in immunological studies.

Sensitization was attempted in C57Bl / 6J mice by painting the mice fifteen times with a two-tenths per cent solution of DNFB in DMSO. Tests for immediate and delayed hypersensitivities were made in mice which had received five, ten and fifteen doses of the sensitization material. Systemic anaphylaxis served as a criterion for determining the presence of immediate hypersensitivity. Attempts were made to demonstrate delayed hypersensitivity by testing with DNFB in olive oil solutions.
The results indicate the following: (1) Clipping of hair from the backs of C57Bl / 6J mice left the skin with abrasions, making it sensitive to the toxicity of DNFB, DMSO or both. Clipping forty-eight hours prior to testing allowed sufficient time for the skin to heal; (2) Sensitization forty-eight hours after clipping resulted in severe lesions on the mice painted with two-tenths per cent DNFB in DMSO. Even though the schedule of paintings was delayed, more positive immediate and delayed tests were observed in mice having experienced these reactions. These results were considered incongruent with the other results, since the mice in this group had undergone abnormal reactions.

Even though mice may be valuable for immunological studies, consideration must be given to the strain used. Each strain will perhaps exhibit an individual reaction pattern. The use of DMSO in immunology is somewhat premature, but as more is discovered concerning its activity in vivo, its value may increase considerably.
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