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DELAYED HYPERSENSITIVITY TO 7,12 DIMETHYLBENZ(a)
ANTHRACENE IN THE GUINEA PIG

by
John Milton Doll

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

1974
THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by John Milton Doll entitled DELAYED HYPERSENSITIVITY TO 7,12 DIMETHYLBENZ(a) ANTHRACENE IN THE GUINEA PIG be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY.

Robert J. Jansen
Dissertation Director

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:

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ACKNOWLEDGMENTS

The author wishes to thank both Dr. Wayburn S. Jeter and Dr. Robert J. Janssen for their patient guidance through the course of this investigation.

Sincere thanks is given to Andrew Paquet and other fellow graduate students for their assistance.

Finally the author would like to express the deepest gratitude to his wife Shirley, and daughter Karol, whose constant understanding and encouragement have made these years of graduate study possible.
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ABSTRACT

Successful transfer of contact sensitivity to 7,12-dimethylbenz(a)anthracene (DMBA) was accomplished in three strains of outbred guinea pigs using peritoneal exudative cells and lymph node cells. When whole, viable peritoneal exudative and lymph node cells were incubated in Hanks' balanced salt solution for 4 hours at 37 C, the fluid was able to transmit the reactivity. Dialysates of these cell free incubation fluids were capable of transferring the contact reactivity to DMBA in two out of four attempts. The transfer material was dialysible and it was associated with the alpha globulin fraction.

Sensitivity was also transferred passively with 20 ml amounts of plasma from sensitized donors only after they had been injected with 15 ml of undilute antilymphocytic serum (ALS) 4 hours previously.

Topical application of DMBA, or another chemical carcinogen, 3-methylcholanthrene (3MC) as a 0.5% solution in acetone, did not cause tumors after consecutive bi-weekly application for up to 240 days. ALS did not increase the tumor induction by either of these materials when injected every third day for a period of up to 147 days with DMBA, or 240 days in those animals treated with 3MC. There was,
however, significant mortality in animals topically treated with DMBA and simultaneously given ALS.

Tumors were induced by DMBA when injected subcutaneously with 0.5 ml benzene containing 20 mg DMBA. A tumor was produced 100 days after a neonate was injected and 210 days after injection of an adult. Tumors were not produced with the same dosage of carcinogen when olive oil, dimethylsulfoxide, or tri-n-capryllin were the carriers, but mortality occurred in several cases.

Treatment with ALS had no effect on the number of tumors produced by injections made in neonatal or adult guinea pigs.
CHAPTER 1

INTRODUCTION

The study of induction of delayed hypersensitivity to simple chemical compounds and their specificity dates back to Landsteiner and Jacobs (1), in 1935. Most modern research concerning contact sensitivity is based on their observations.

The evaluative criteria of contact reactions were reviewed by Bloom and Chase (2). They state that there is a delay between contact and expression of the reaction in the sensitive individual; the reaction becoming maximal in 16 to 48 hours. Histologically, the response is characterized by early mononuclear infiltration which may cause induration, and reactions occur only in those tissues where antigen is retained in high concentration during the course of the reaction. Transfer of sensitivity from an allergic animal to a normal animal is accomplished by living lymphoid cells but not by serum.

Passive Transfer

Landsteiner and Chase (3), in 1942, first reported successful transfer of delayed hypersensitivity in guinea pigs to simple chemicals using peritoneal exudative leucocytes. Subsequently, Chase (4) was able to transfer
tuberculin reactivity in the same species. Haxthausen (5), in 1947, confirmed these results with transfer of dinitrochlorobenzene sensitivity in guinea pigs, using peritoneal exudate cells, and Cummings and Gottshall (6) as well as Kirchheimer, Weiser, and Van Liew (7) repeated transfer of tuberculin hypersensitivity using exudative cells.

The in vivo transfer of reactivity between sensitized and normal homologous test animals, using viable lymphoid cells free from humoral antibody, has become a principal criterion for distinguishing delayed type hypersensitivity from antibody-mediated phenomena (2).

Sub-cellular transfer has also been accomplished using extracts of whole viable cells. Crepea and Cooke (8), 1948, reported the transfer of poison ivy sensitivity using extracts of guinea pig spleen cells. Jeter, Tremaine, and Seebohm (9), in 1954, described passive transfer of dinitrochlorobenzene reactivity in guinea pigs with cellular extracts from sonically disrupted peritoneal exudative cells. Lawrence (10) used a preparation from human leucocytes, disrupted by distilled water or freezing and thawing, that would transfer tuberculin and streptococcal M substance reactivity in human beings. This material was later termed "transfer factor" (11). Lawrence (12) has described tuberculin transfer factor from human beings as a dialysable substance with a molecular weight of approximately 10,000 daltons.
Jeter, Laurence, and Seebohm (13) described an electrophoretic component in extracts of leucocytes from tuberculin and dinitrochlorobenzene sensitized animals that resembled serum alpha-1-globulin. It was related to the ability to sensitize passively.

Guthrie, Ellis, and Brock (14), using peritoneal exudative cells from guinea pigs sensitive to dinitrofluorobenzene, found incubation of the cells would cause release of substances capable of passively transferring contact sensitivity. Burger and Jeter (15) investigated incubation fluids obtained from peritoneal exudative cells, lymph node cells, and alveolar cells taken from animals sensitive to dinitrofluorobenzene and dinitrochlorobenzene, and found the best passive transfer activity in fluids incubated at 37°C for 4 hours.

Burger et al. (16), in 1971, demonstrated contact sensitivity to 7,12-dimethylbenz(a)anthracene (DMBA) in guinea pigs and succeeded in passively transferring the sensitivity using whole cells. However, they did not demonstrate the transfer using extracts of cellular material.

Ahmed and Jeter (17), in 1971, studied control of the release of transfer material from guinea pig leucocytes and Jeter et al. (18) investigated the use of antilymphocytic serum to release transfer factor in vivo for successful transfer of tuberculin hypersensitivity in guinea pigs.
DMBA Carcinogenesis

In addition to its ability to induce contact hypersensitivity, DMBA is a potent chemical carcinogen capable of inducing tumors in several species (19). The susceptibility of the guinea pig to tumor production by this chemical is of lower degree compared to that observed in rats and mice (19). At the same time the manifestation of contact dermatitis in guinea pigs is dramatic compared to the mouse, which readily forms tumors when treated with DMBA (20). Some authors have attributed the lack of contact reactivity in the mouse to depressed activity in skin rather than immunological deficiency (21).

Several reasons have been given for the lessened susceptibility of guinea pigs to tumor induction by chemical carcinogens. These range from the theory of Weisburger, Weisburger, and Morriss (22) that the carcinogen metabolism in guinea pig skin was different, to that of Bock (23) that the carcinogen remains in the skin of the guinea pig for a shorter time. More recently, workers have speculated that an allergic condition may exist to produce the refractoriness (24).

Polycyclic hydrocarbons like DMBA are solubilized by proteins as a result of weak and unspecific hydrophobic binding with the protein (25). Since these protein binding forces are nonspecific by nature and chemical carcinogenesis is a highly specific process, Franke (25) believes that
protein binding is of only secondary importance in the process of tumor production with these carcinogens. DMBA also binds with cellular nucleic acids (26). Some investigators feel that alteration of this informational molecule may be the key to the tumorigenic effect of DMBA (27).

Induction of tumors in guinea pigs has been accomplished in most cases by subcutaneous injection rather than by topical application (28), and in neonatal rather than adult guinea pigs (29, 30). It appears unlikely that the increased incidence in neonates is due to skin reactivity alone since histological and histochemical evidence indicate no striking difference between adult and neonatal skin (31). In contrast to induced tumors, the incidence of spontaneous tumors in guinea pigs increases after they are 3 years of age (32), suggesting a breakdown of some component of the immune system in old age (33, 34).

Burnet (35), in referring to the term "immunological surveillance" to explain the host's normal rejection of a spontaneous tumor, quoted a suggestion by Thomas that "The phenomenon of homograft rejection will turn out to represent a primary mechanism for natural defense against neoplasia" (p. 1173). Recognition and rejection of homografts is similar in principle to recognition and rejection of new antigens appearing on neoplastic tissue. Prevention of the recognition and subsequent rejection of these new antigens
appearing on neoplastic tissue (neoantigens) may be a method of increasing tumor production by carcinogens (36, 37, 38).

**Immunosuppression**

In tumor transplantation, immunosuppression by antilymphocytic serum (ALS) allows temporary acceptance of tumor material across strong histocompatibility barriers (39).

The concept of an antiserum directed against lymphocytes was attributed to Metchnikoff (40), in 1899. However, the practical use of this biological immunosuppressant in transplantation and other immunological phenomena was demonstrated by Levy and Medawar (41). The activity of ALS is found in the globulin fraction obtained after ammonium sulfate precipitation (42) and has been clearly demonstrated to reside in the immunoglobulin G fraction of ALS (43).

Thus, an active antibody may be produced against antigens on the surface of lymphocytes, and the antibody has the ability to enhance the phagocytosis of these white blood cells by other leucocytes (44, 45). ALS in the presence of complement will also cause lysis of lymphocytes (46).

The action of ALS appears to affect cell mediated immunity more than humoral immunity (47). Examples of the former include tumor rejection and contact sensitivity. For this reason, ALS plays an important role in the increased
formation of tumors in animals treated with the chemical carcinogens (48, 49).

Purpose

The purpose of these investigations was to effect contact sensitivity to DMBA in guinea pigs, to transfer this reactivity using cells and subcellular materials and to examine the relationship of the contact reactivity to tumor formation.
CHAPTER 2

MATERIALS AND METHODS

Animals
Random bred albino guinea pigs of the Amana, Rockefeller, and Hartley strains were used throughout these experiments. Donors for peritoneal exudative cells (PEC) were 700-1000 grams in weight, and recipients weighed 200-400 grams.

Animals were housed in separate cages and kept at a temperature of 25 C and ambient humidity. They were fed Purina guinea pig chow and given tap water supplemented with 0.05% ascorbic acid. Raw cabbage, approximately 80 gm, was provided daily during sensitization periods, biweekly at other times.

Chemicals
For sensitization and tumor induction, 7,12-dimethylbenz(a)anthracene (DMBA) (Eastman Organic Chemicals, Rochester, N. Y.) and 3-methylcholanthrene (3MC) (Eastman Organic Chemicals, Rochester, N. Y.) were employed without further purification.
Glassware

Glassware used in the collection of cells was acid washed, rinsed five times in tap water, three times in distilled water, and boiled in double distilled water. Drying and sterilization was in the hot air oven at 165°C for three hours.

Sensitization

Guinea pigs were sensitized by topical application of 5 drops of 2% DMBA in acetone. This solution was made fresh daily and applied to the shaved nuchal area of the guinea pigs, then rubbed with a polished glass rod.

Skin Testing

Five days after the last skin painting, donor guinea pigs were skin tested topically with a single drop of 0.5% or 0.1% DMBA in olive oil (or 3MC in some experiments) on contralateral sides, followed by spreading of the oil solution over a 10 cm² area of skin with a polished glass rod.

Recipient animals were tested with 1% solutions of the chemicals in olive oil 48 hours after transfer. All skin tests were read at 24 hours and scored according to the following criteria (15):

- no detectable reaction
- 1+ patchy erythema
- 2+ homogeneous erythema
3+ homogeneous erythema, edema
4+ homogeneous erythema, edema, induration.

Olive oil controls were used to determine specificity of the reaction. Untreated guinea pigs were tested with olive oil and 1% carcinogen in olive oil solutions.

**White Cell Counts**

Total white cell counts were made using a Spencer Bright-line hemocytometer and cell viability was determined by trypan blue exclusion (50).

**Differential Counts**

The neutral red–janus green supravital (51) and Wright-Giemsa stains (52) were employed to determine the differential counts of blood, lymph node, and peritoneal exudate cell preparations by microscopic examination of 100 cells.

**Passive Transfer Technique**

Passive transfer of fresh whole cells was accomplished with mixtures of peritoneal exudate cells (PEC) and lymph node cells (LNC) following the technique of Burger and Jeter (15). Donors showing a 2+ or better skin test reaction to the 0.5% DMBA in olive oil solution were clipped laterally to the margins of the legs. Alcohol was applied to the right ventral posterior side and 20 ml of sterile light mineral oil was injected intraperitoneally. To avoid
leakage from the cavity, the needle puncture site was cauterized.

After 48 hours the donors were anesthetized with ether, exsanguinated by cardiac puncture, and immersed in a 2% amphyl solution. Peritoneal exudates were collected in three 20 ml washings of the abdominal cavity with heparinized (2 units/ml) Hanks' balanced salt solution (HBSS) with a pH of 7.2-7.4 (53).

Normal guinea pig serum was added (10%) to the HBSS fluid for stabilization of the PEC during collection and concentration. Suprascapular, cervical, and axillary node of the animal were used as a supplemental cell source as outlined below.

Cells derived from the above sources were pooled, centrifuged, and redispersed in smaller volumes. These cells were then washed 3 times, the latter two washings in HBSS without the added guinea pig serum. Total, viability, and differential counting techniques were used to characterize these cell populations.

Cells, in quantities of at least $1 \times 10^9$ per ml were inoculated by the intraperitoneal route into 200-400 gm homologous strain recipient guinea pigs. These recipients were then skin tested with 1% carcinogen in olive oil solutions after 48 hours.

Incubation fluid transfer involved similar populations of cells except that the cells were allowed to
incubate in a ratio of $1 \times 10^9$ cells per 7.5 ml of HBSS for 4 hours at 37 C before inoculation of the fluids (15). Incubating cells were gently mixed every half hour. After incubation, contents of the tubes were centrifuged at 500 x G for 30 minutes to sediment the cells, and supernatant fluid expressed through a .45 μ millipore filter using a Swindex adapter.

This cell free fluid was injected in 15 ml amounts into guinea pigs via the intraperitoneal route and recipients were skin tested as above after 48 hours.

**ALS Treated Plasma Transfers**

Guinea pigs showing a 2+ or better reaction to 0.5% DMBA were selected for donors in plasma transfers. They were 500-600 gram pigs of both sexes. Four hours before they were to be exsanguinated, the animals were inoculated with 15 ml of undilute antilymphocytic serum (ALS) by the intraperitoneal route (18).

Before exsanguination, the animals were anesthetized with ether. Blood was collected directly from the heart and placed in tubes containing approximately 10 units of heparin per ml. Plasma was separated immediately and injected intraperitoneally into homologous strain recipients. These animals were skin tested 48 hours later. In some cases, suprascapular, cervical, and axillary nodes were
harvested and processed as described below to serve as a check on released transfer material.

**Harvesting of Lymph Node Cells**

Suprascapular, cervical, and axillary nodes were removed using aseptic techniques, trimmed of fat and unwanted tissue, and minced over a sterile stainless steel screen (54). Cells were rinsed through the screen with HBSS, and the minced nodes pressed lightly over the screen to remove cells left in the node matrix. Cells thus harvested were washed three times in HBSS and resuspended in 1-5 ml volumes of HBSS for injection or used to supplement PEC used in the transfers.

**Dialysis Technique**

Incubation fluids were dialysed against two changes of distilled water at 5 C, each change of distilled water representing 10 volumes of the incubation fluid. Dialysis was allowed for 24 hours to each change. Dialysis tubing (Scientific Products) was seamless regenerated cellulose tubing with an average pore radius of 24 angstroms. The dialysates were pooled and concentrated by lypholization and reconstituted to the original volume of the incubation fluid sample with distilled water.
ALS Preparation

ALS was prepared in essentially the same manner as reported by Ahmed (54). The ALS was prepared in goats or rabbits against lymph node cells from normal and DMBA sensitized animals of each of the three strains of guinea pigs and stored at -20 C until further use. Cytotoxicity and heterophile antibody tests were used to determine the in vitro titers and in vivo lymphocyte depletion in guinea pigs was evaluated on some sera.

Cytotoxicity Tests

In vitro titration of the ALS followed a microtiter modification of the method used by Ahmed (54). Two fold dilutions of 0.025 ml amounts of heat inactivated ALS in saline were added to 0.025 ml containing $10^7$ per ml lymph node cells. Next 0.175 ml of HBSS was added and 0.025 ml of undilute guinea pig serum was added as a complement source. The microtiter plate was then incubated at 37 C with gentle tapping for one hour.

After incubation 0.025 ml of a one-quarter per cent trypan blue solution was added and the microtiter plate further incubated for five minutes. At the end of the second incubation the plates were immersed to the brim in ice water to stop all action. Counts were made by transferring the contents of the wells to the stage of a Brightline hemocytometer and observed under 400 x magnification.
Controls included were: (1) cells only in HBSS (cell control), (2) HBSS and C' with cells (complement control), and (3) normal rabbit (or goat) serum and C' as a normal serum control.

Total and viability counts were made in triplicate to determine the titer and the titer redetermined if variation was more than a 2 fold dilution different between the replicates. The titer was taken as the reciprocal of the highest dilution of serum causing more than a 20% cytotoxicity as compared to the normal serum control.

Rabbit (Rb) Anti Normal Guinea Pig Serum

Rabbits were inoculated with a mixture of 1 ml of normal guinea pig serum emulsified in incomplete Freunds' adjuvant. Injections were made subcutaneously at multiple sites in the nuchal area, with no more than 0.2 ml per site. Rabbits were tested and boosted in 3 weeks with a single injection as above, and a week later were exsanguinated.

Immunoelectrophoresis

Incubation fluids were tested for serum components by using RB anti-normal guinea pig serum in a trough parallel to the migration of a sample of the fluid. Gel medium used was 1% Noble Agar in veronal buffer, 8.6 pH, ionic strength 0.1. The technique used 150 V, 50 ma for approximately 2 hours. Excess protein was then removed from the
gel by soaking in 0.15 M salt over a 48 hour period. The
gel plates were dried and stained with amido schwartz stain
(55).

Injection of Carcinogen

Guinea pigs were injected by the subcutaneous route
with either 15 mg or 20 mg of DMBA in dimethyl sulfoxide
(DMSO) (Mallinckrodt Chemical Co.) (56), benzene (J. T.
Baker, Phillipsburg, N. J.), or tri-n-capryllin (Octanoin,

Each animal was injected once with a 0.5 ml volume
of carrier containing the DMBA, either in the shaved right
flank or the lateral nuchal area. It was necessary when
using tri-n-capryllin to initially triturate the DMBA with
solvent 10-12 times through a 20 gauge needle to break up
lumps of solid carcinogen. This procedure was not necessary
with the other solvents.

After this single injection, guinea pigs were shaved
and observed weekly. Control animals were included which
were handled identically and injected with equal amounts of
the solvent carrier without DMBA.
CHAPTER 3

RESULTS

Initial experiments were done to determine whether three strains of outbred guinea pigs could be sensitized with DMBA by topical application. A 2+ or better reaction when animals were tested with 0.5% chemical was considered as being an adequate level of sensitization for donors used in studies.

In the initial testing, evaluation was made of another chemical carcinogen, 3-methylcholanthrene (3MC). However, only 1 out of 8 animals were positive, so 3MC was discontinued in sensitization studies.

Table 1 shows that, using the above criteria, the ratio of sensitized animals to total number treated ranged from a low of 5/18 (28%) to a high of 12/14 (85%) with slightly better sensitization in the Rockefeller strain. The difference between strains was not considered enough to be critical and the animals were used as they were available.

Having achieved this level of sensitization, the next step was to attempt passive transfer of sensitivity using viable peritoneal exudative (PEC) and lymph (LNC) node cells. The experimental scheme is shown in Figure 1. Passive transfer characteristics were determined in guinea pigs.
### Table 1. Sensitization of Guinea Pigs to 7,12-dimethylbenz(a)anthracene (DMBA) by Topical Application^{a}

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Strain</th>
<th>No. Animals</th>
<th>Positive 24 hr. Skin Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1%</td>
</tr>
<tr>
<td>1.</td>
<td>Amana</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Amana</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>Hartley</td>
<td>16</td>
<td>1</td>
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<tr>
<td>4.</td>
<td>Amana</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>Amana</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>6.</td>
<td>Amana</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>7.</td>
<td>Amana</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>8.</td>
<td>Rockefeller</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>9.</td>
<td>Rockefeller</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Rockefeller</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>11.</td>
<td>Amana</td>
<td>9</td>
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<tr>
<td>14.</td>
<td>Rockefeller</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

^{a}All donors were sensitized with daily application of 5 drops 2% DMBA in acetone followed by skin testing in 5 days with 0.1% and 0.5% DMBA in oil.

^{b}Number of animals showing 2+ skin tests to 0.1% and 0.5% DMBA in 24 hours.

Criteria: -, no reaction; 1+, patchy erythema; 2+, homogeneous erythema; 3+, erythema and edema; and 4+, erythema, edema, and induration.
Figure 1. Experimental Scheme for Study of Contact Sensitivity to 7,12-dimethylbenz(a)anthracene in the Guinea Pig
pigs by a series of whole cell transfers first, then by subcellular extracts.

Passive transfer of DMBA sensitivity by whole cells was accomplished in 7 of 11 attempts (Table 2). Three of the four failures to transfer occurred in replicates of successful transfers. All successful passive transfers were accomplished by a combination of 1.8 to 3.6 x 10^9 peritoneal exudative and lymph node cells injected in 2 to 5 ml volumes.

No positive skin reactions were visible in 4 to 6 hours, but responses to the tests were generally of a high degree of contrast with sharp, well defined borders when read in 18-24 hours.

Pooled PEC and LNC, as determined by differential staining, consisted of 30-40% macrophages, 50-60% lymphocytes, and 10-20% granulocytes. Viability of the cells was between 95 and 98%. Normal siblings were injected with similar volumes of the same batch of HBSS for a control and both test and control animals were skin tested with 1% DMBA in oil on one flank, and with olive oil alone on the contralateral side. Control skin tests were negative.

Passive transfer of cells from unsensitized homologous strain guinea pigs did not result in reactivity, and passive transfer of serum from sensitized donors was unsuccessful. Skin tests were negative when 1% 3MC was used to test guinea pigs passively sensitized to DMBA.
Table 2. Passive Transfer of 7,12-dimethylbenz(a)-anthracene (DMBA) Sensitivity in Guinea Pigs by Peritoneal Exudative and Lymph Node Cells

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Strain</th>
<th>No. of Sensitized Donors</th>
<th>WBC($10^9$)$^a$</th>
<th>Skin Tests$^b$ 1% DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Amana</td>
<td>7</td>
<td>2.3</td>
<td>1+</td>
</tr>
<tr>
<td>1B</td>
<td>Amana</td>
<td>7</td>
<td>2.3</td>
<td>-</td>
</tr>
<tr>
<td>2A</td>
<td>Amana</td>
<td>9</td>
<td>2.6</td>
<td>2+</td>
</tr>
<tr>
<td>2B</td>
<td>Amana</td>
<td>9</td>
<td>2.6</td>
<td>1+</td>
</tr>
<tr>
<td>2C</td>
<td>Amana</td>
<td>9</td>
<td>2.6</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Hartley</td>
<td>8</td>
<td>1.8</td>
<td>2+</td>
</tr>
<tr>
<td>5</td>
<td>Amana</td>
<td>9</td>
<td>5.9</td>
<td>-</td>
</tr>
<tr>
<td>9A</td>
<td>Rockefeller</td>
<td>3</td>
<td>3.6</td>
<td>2+</td>
</tr>
<tr>
<td>9B</td>
<td>Rockefeller</td>
<td>3</td>
<td>3.6</td>
<td>2+</td>
</tr>
<tr>
<td>10A</td>
<td>Rockefeller</td>
<td>4</td>
<td>3.3</td>
<td>2+</td>
</tr>
<tr>
<td>10B</td>
<td>Rockefeller</td>
<td>4</td>
<td>3.3</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$Viable cells consisting of 30-40% macrophages, 50-60% lymphocytes, 10-20% granulocytes.

$^b$Skin tests, 24 hours after topical application of 1% DMBA in olive oil, scored according to the following criteria: -, no reaction; 1+, patchy erythema; 2+, homogeneous erythema; 3+, erythema and edema; and 4+, erythema, edema, and induration.
Having achieved successful whole cell transfers, attention was directed to incubation fluids using cells from the above sources. The factor or factors responsible for transfer could be found in fluids from sensitized cells after they were incubated in HBSS for 4 hours at 37 C. After light centrifugation and removal of the incubation fluids the cells were also tested for transfer activity in some cases.

Passive transfer was accomplished with regularity using incubation fluids (Table 3). In paired experiments, using both whole cell and incubation fluid transfers of the same cell population, successful transfer was accomplished in Amana and Rockefeller strain guinea pigs. Incubated cells, after removal of the incubation fluid transferred successfully in one case.

Since incubation fluids would transfer reactivity it was necessary to determine if dialysates of the fluids would retain the activity after passage through dialysis membrane of 24 angstrom average pore size. Dialysates (Table 4) of the incubation fluids transferred reactivity to DMBA in two out of four instances. The pH of incubation fluids dropped from 7.4 to an average of 6.2 during the 4 hour incubation period.

All animals, including controls which had been inoculated with incubated HBSS, were skin tested with both 1% DMBA in olive oil, and olive oil alone, on opposite sides.
Table 3. Passive Transfer of 7,12-dimethylbenz(a)-anthracene (DMBA) Sensitivity in Guinea Pigs by Cells and Extracts

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Strain</th>
<th>Donors (No.)</th>
<th>WBC($\times 10^9$)</th>
<th>Skin Tests 1% DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Used</td>
<td>Cells$^a$</td>
<td>Fluids$^b$</td>
</tr>
<tr>
<td>5</td>
<td>Amana</td>
<td>9</td>
<td>5.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.8</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Rockefeller</td>
<td>6</td>
<td>5.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Rockefeller</td>
<td>8</td>
<td>3.6</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.6</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Rockefeller</td>
<td>14</td>
<td>3.3</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.3</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.4</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Amana</td>
<td>10</td>
<td>3.1</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.1</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.1</td>
<td>1+</td>
</tr>
</tbody>
</table>

$^a$Whole viable lymph node and peritoneal exudative cells from sensitized donors.

$^b$Incubation fluids using cells from sensitized donors, incubated 4 hours at 37°C, in HBSS.

$^c$Cells after removal of incubation fluids.

$^d$Twenty-four hour readings scored according to the following criteria: -, no reaction; 1+, patchy erythema; 2+, homogeneous erythema; 3+, erythema and edema; and 4+, erythema, edema, and induration.
Table 4. Passive Transfer of 7,12-dimethylbenz(a)-anthracene (DMBA) Sensitivity in Guinea Pigs by Dialysates of Incubation Fluids

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Strain</th>
<th>Skin Tests 1% DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dialysates&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Amana</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>Rockefeller</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Rockefeller</td>
<td>1+</td>
</tr>
<tr>
<td>10</td>
<td>Rockefeller</td>
<td>1+</td>
</tr>
<tr>
<td>12</td>
<td>Amana</td>
<td>d</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dialyzed incubation fluid, reconstituted to 7.5 ml after lypholization.

<sup>b</sup>Dialysis bag contents after 48 hour dialysis against double distilled water, 200 ml, at 4 C.

<sup>c</sup> Twenty-four readings scored according to the following criteria: -, no reaction; 1+, patchy erythema; 2+, homogeneous erythema; 3+, erythema and edema; and 4+, erythema, edema, and induration.

<sup>d</sup>Recipient dead, 4 hours.
Skin tests were unreactive in all control animals, as were the olive oil controls in treated animals.

Incubation fluids were tested against rabbit (RB) anti guinea pig serum by immunoelectrophoresis, and showed a definite precipitin arc in the area corresponding to alpha globulin, but no bands were in the gamma globulin area. The alpha band was not present in the dialysate.

Normal guinea pig serum used as a control in adjacent wells produced strong precipitin lines in the areas corresponding to gamma, beta, and alpha globulin and albumin.

Work in this laboratory has shown that successful transfer can be accomplished using plasma from ALS treated animals sensitive to other allergens. To investigate this type of release of transfer material, antilymphotic serum was injected into sensitized animals and the donor plasma collected 4 hours later. The antilymphotic serum was produced in rabbits or goats, titrated for cytotoxicity and heterophile antibody, and evaluated for its in vivo lymphocyte depletion. Table 5 shows the titers of ALS used in the following experiments.

Guinea pig plasma from sensitized animals was found to transfer DMBA sensitivity (Table 6) only after the animal was treated with ALS. It was necessary to inject large (20 ml) amounts of plasma to achieve transfer. Lesser amounts—experiments 7A and 7B—were unsuccessful. Experiment 11 demonstrates this well, since a 30 ml pool was
Table 5. Anti-Lymphocytic Serum Used in Plasma Transfers and Immunosuppression Experiments

<table>
<thead>
<tr>
<th>ALS Batch&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Produced in</th>
<th>Against Guinea Pig Strain</th>
<th>Cytotoxicity Titer</th>
<th>Lympho Deplet.&lt;sup&gt;b&lt;/sup&gt; (Per Cent)</th>
<th>Utilized in Expt.&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>Goat</td>
<td>Amana</td>
<td>256</td>
<td>--</td>
<td>4</td>
</tr>
<tr>
<td>DSGP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Rabbit</td>
<td>Amana</td>
<td>128</td>
<td>80</td>
<td>7A, 8, 9</td>
</tr>
<tr>
<td>DSGP&lt;sup&gt;e&lt;/sup&gt; (ALG)</td>
<td>Rabbit</td>
<td>Amana</td>
<td>512</td>
<td>--</td>
<td>13</td>
</tr>
<tr>
<td>BC</td>
<td>Rabbit</td>
<td>Amana</td>
<td>256</td>
<td>60</td>
<td>7B</td>
</tr>
<tr>
<td>POOL 3</td>
<td>Rabbit</td>
<td>Rockefeller</td>
<td>128</td>
<td>--</td>
<td>14</td>
</tr>
<tr>
<td>POOL 1</td>
<td>Rabbit</td>
<td>Amana</td>
<td>64</td>
<td>55</td>
<td>Tumor Production</td>
</tr>
<tr>
<td>A.A.&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Rabbit</td>
<td>Amana</td>
<td>256</td>
<td>--</td>
<td>Topical DMBA Applic</td>
</tr>
</tbody>
</table>

<sup>a</sup> Final heterophile ab titer after absorption with SRBC was under 4-8.

<sup>b</sup> Represents per cent reduction of lymphocytes in 4 hours as a result of i.p. injection of 1 ml of undilute ALS.

<sup>c</sup> Indicates experiment(s) in which the ALS was used.

<sup>d</sup> Prepared against DMBA sensitized Amana strain guinea pig.

<sup>e</sup> Globulin fraction after ammonium sulfate precipitation.

<sup>f</sup> Received from Aftab Ahmed December 1970.
Table 6. Passive Transfer of 7,12-dimethylbenz(a)anthracene (DMBA) Sensitivity in Guinea Pigs by Plasma After ALS Treatment of Donors

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Strain</th>
<th>Plasma Vol (ml)</th>
<th>Skin Tests 1% DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Amana</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7A</td>
<td>Amana</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>7B</td>
<td>Amana</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Rockefeller</td>
<td>20</td>
<td>2+</td>
</tr>
<tr>
<td>11A</td>
<td>Amana</td>
<td>20</td>
<td>2+</td>
</tr>
<tr>
<td>11B</td>
<td>Amana</td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Amana</td>
<td>20</td>
<td>Dead</td>
</tr>
<tr>
<td>14</td>
<td>Rockefeller</td>
<td>20</td>
<td>1+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Node cells.

<sup>b</sup> Twenty ml plasma from sensitized, ALS treated animals.

<sup>c</sup> Twenty-four hour readings scored according to the following criteria: -, no reaction; 1+, patchy erythema; 2+, homogeneous erythema; 3+, erythema and edema; and 4+, erythema, edema, and induration.

<sup>d</sup> 10 ml of plasma brought up to 20 ml with saline.
divided and 20 ml injected into one recipient, and 10 ml into another. Only the 20 ml pool transmitted reactivity.

An attempt was made to determine if a more specific ALS would be beneficial in the release of the factor(s). ALS pool DSGP differs from the other ALS used in that it is a rabbit antisera against lymph node cells and peripheral white blood cells from guinea pigs sensitized with DMBA. Controls were of two types: (1) animals injected with a 20 ml pool of pre-sensitized normal plasma, and (2) olive oil controls on both injected and control animals. In all cases, control skin tests were negative.

Some pools of ALS caused a noticeable weakening of the guinea pig within 4 hours of the injection. Their appearance was one of increased lethargy and a roughening of the coat. Blood was very difficult to obtain by cardiac puncture in these animals and it was necessary to use up to 5 animals to obtain the 20 ml of plasma for injection into the recipients.

Carcinogenic Experiments

To see if ALS treatment had any effect on tumor-igenesis induced by DMBA, the following studies were initiated.

Twice weekly painting of normal animals for 240 days with a 0.5% solution of either 3MC or DMBA in acetone produced no tumors (Table 7). In one animal no increase in
Table 7. Effect of Antilymphocytic Serum (ALS) on Topically Applied 7,12-dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (3MC) in the Guinea Pig

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Length Treatment (Days)</th>
<th>ALS Treated</th>
<th>Carcinogen</th>
<th>Disposition of Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>yes</td>
<td>DMBA</td>
<td>wasting, death</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>yes</td>
<td>DMBA</td>
<td>wasting, death</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>yes</td>
<td>DMBA</td>
<td>wasting, death</td>
</tr>
<tr>
<td>4</td>
<td>147</td>
<td>yes</td>
<td>DMBA</td>
<td>wasting, death</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>yes</td>
<td>DMBA</td>
<td>wasting, death</td>
</tr>
<tr>
<td>Ce</td>
<td>240</td>
<td>no</td>
<td>DMBA</td>
<td>sacrificed</td>
</tr>
<tr>
<td>1</td>
<td>240</td>
<td>yes</td>
<td>3MC</td>
<td>sacrificed</td>
</tr>
<tr>
<td>2</td>
<td>240</td>
<td>yes</td>
<td>3MC</td>
<td>sacrificed</td>
</tr>
<tr>
<td>Cf</td>
<td>240</td>
<td>no</td>
<td>3MC</td>
<td>sacrificed</td>
</tr>
</tbody>
</table>

a Amana strain, approximately 10 weeks old.
b 0.5 ml carcinogen 0.5% in acetone 2 X week starting day +5.
c 1.0 ml of ALS i.p. 1:3 dilution in saline day +1 to +7, 1.0 ml of ALS i.p., 1:5 dilution day 10, 13, 16 and every 3rd day.
d Final disposition of animal at end of treatment.
e Control, DMBA only.
f Control, 3MC only.
tumor incidence was noticed after 147 days of continuous heterologous ALS treatment and twice weekly painting with DMBA. In 4 other animals in this series, DMBA and ALS treatments caused a lethal effect in from 12 to 14 days. A period of 2-7 days of weight loss and lethargy was followed by 24-48 hours of highly reduced food and water intake, followed by death. Autopsy revealed no apparent tumor or other abnormality except a patchy erythema on the inside of the peritoneal wall, possible irritation from the many injections. Control animals, treated with DMBA but not immunosuppressed, did not display these symptoms.

With 3MC, no tumors or deaths were seen in 240 days of twice weekly painting of the carcinogen with or without ALS. Necropsy at the time of sacrifice of these animals revealed no tumor or other abnormal condition. To determine whether cutaneous sensitivity of these animals existed, these animals were skin tested on day 45 with 1% solutions of DMBA, 3MC, and 2,4-Dinitrofluorobenzene (DNFB). No sensitization was demonstrated to any of the chemicals.

To evaluate subcutaneous injection as a route for tumor induction by this carcinogen, olive oil, benzene, dimethyl sulfoxide, and tri-n-capryllin oil were utilized as carriers to inject Rockefeller strain guinea pigs as shown in Table 8.

The only carrier and carcinogen combination to prove effective in tumor production was benzene with a dosage of
Table 8. Effect of Subcutaneous Injections of 7,12-dimethylbenz(a)anthracene (DMBA) in Various Carriers on Rockefeller Strain Guinea Pigs

<table>
<thead>
<tr>
<th>No. Animals</th>
<th>Age (Day)</th>
<th>Dose mg of DMBA</th>
<th>Carrier&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Result</th>
<th>Days to Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>20 mg</td>
<td>olive oil</td>
<td>death</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>20 mg</td>
<td>olive oil</td>
<td>death</td>
<td>180</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>20 mg</td>
<td>olive oil</td>
<td>death</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>20 mg</td>
<td>olive oil</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>--&lt;sup&gt;c&lt;/sup&gt;</td>
<td>olive oil</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>20 mg</td>
<td>benzene</td>
<td>death</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>20 mg</td>
<td>benzene</td>
<td>tumor</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>20 mg</td>
<td>benzene</td>
<td>death</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>20 mg</td>
<td>benzene</td>
<td>tumor</td>
<td>210</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>--</td>
<td>benzene</td>
<td>death</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>20 mg</td>
<td>DMSO</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>.05 mg</td>
<td>DMSO</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>.05 mg</td>
<td>DMSO</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>15 mg</td>
<td>Tri-n-capryllin</td>
<td>death</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>15 mg</td>
<td>Tri-n-capryllin</td>
<td>death</td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>15 mg</td>
<td>Tri-n-capryllin</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>--</td>
<td>Tri-n-capryllin</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>a</sup>0.1 ml volume containing DMBA injected sub cu.

<sup>b</sup>Living, no visible tumor at termination of experiment.

<sup>c</sup>Control, no carcinogen.
20 mg of DMBA. The resulting tumors, one in a neonate and the other in an adult, were found at the site of injection. The tumor was first noticed 100 days after injection in the neonate and 210 days after injection in the adult.

The neonate was killed by sodium pentothal overdose and the tumor excised. The main nodule was approximately 1-1.5 cm in diameter, firm, with a whitish color. Surrounding the nodule in a lateral plane of reduced thickness was a 0.5 cm radius of the same consistency. A sample of the tissue was placed in 10% formalin and the remainder of the tumor minced and digested with trypsin for tissue culture. Monolayers of these fibroblast cells could not be maintained in minimum essential medium (MEM)+ fetal calf serum for longer than about 2 weeks.

Other injections of DMBA in benzene and olive oil proved too toxic and resulted in death (Table 8). In each experiment, the carrier alone was injected to determine the side effects. Benzene killed the control in 4 days, but olive oil had no effect. DMBA in DMSO had no effect.

Benzene, with or without DMBA, had a necrotic effect at the site of injection. In those animals surviving until appearance of the tumor, the lesion healed in about 14 days and the site of injection remained unchanged until appearance of the tumor.

Because of the severe reaction encountered by the guinea pigs injected with benzene, and the ensuing
complications that might arise in the interpretation of the tumors, benzene was abandoned in favor of tri-n-capryllin oil.

Adult 9 week old Rockefeller guinea pigs were injected subcutaneously with 0.5 ml of tri-n-capryllin oil containing 15 mg of dissolved DMBA, and half the animals treated with ALS. Skin tests undertaken at 14 days (Table 9) revealed positive reactions in both ALS treated and non-treated animals subcutaneously injected. No tumors were in evidence in this group after 200 days of observation.
Table 9. Effect of Antilymphocytic Serum on Subcutaneous Injection of 7,12-dimethylbenz(a)anthracene (DMBA) in Tri-n-capryllin Oil Carrier, Subcutaneous Injection Adult Rockefeller Strain Guinea Pig

<table>
<thead>
<tr>
<th>No. Animals</th>
<th>Dosage DMBA (mg) in 0.5 ml</th>
<th>ALS&lt;sup&gt;a&lt;/sup&gt; Treated</th>
<th>Positive Skin Test&lt;sup&gt;b&lt;/sup&gt; 0.5% DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>15</td>
<td>yes</td>
<td>(2+), N.D.,&lt;sup&gt;d&lt;/sup&gt; N.D.</td>
</tr>
<tr>
<td>1</td>
<td>--&lt;sup&gt;c&lt;/sup&gt;</td>
<td>yes</td>
<td>N.D.</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>no</td>
<td>(2+), N.D., N.D.</td>
</tr>
<tr>
<td>1</td>
<td>--</td>
<td>no</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

<sup>a</sup>ALS pool 1, 1/2 dilution saline, i.p. day -3 to day +3 every other day thereafter to day 9. DMBA injected day 0.

<sup>b</sup>No. animals skin tested/reactive day 14, DMBA in olive oil, 24 readings according to the following criteria: -, no reaction; 1+, patchy erythema; 2+, homogeneous erythema; 3+, homogeneous erythema and edema; and 4+, homogeneous erythema, edema, and induration.

<sup>c</sup>Tri-n-capryllin only, 1/2 ml on day 1.

<sup>d</sup>Not done.
CHAPTER 4

DISCUSSION

The ability of an animal to resist chemical carcinogenesis has been tied to its ability to evoke delayed hypersensitivity to the chemical (21, 24). Burger et al. (16) reported passive transfer of delayed hypersensitivity to DMBA in the guinea pig using whole, viable cells from sensitized donors. Results shown here demonstrate further that DMBA can produce a highly reactive state of sensitization in the guinea pig when topically applied. Transfer of sensitivity to DMBA was demonstrated in these results not only by use of whole cells, in confirmation of Burger's work, but also by use of subcellular incubation fluids, and by plasma from ALS treated animals. Dialysates from incubation fluids were able to transfer reactivity 50% of the time. Transfer of DMBA sensitivity by incubation fluids, dialysates, and serum from ALS treated animals has not been reported before.

Control animals verified that the skin reactions could not be produced by transfer of materials from normal animals, and olive oil alone was not capable of producing the reaction. Another carcinogen (3MC), of similar chemical structure, was unable to elicit a response in DMBA sensitized animals, thus confirming the specificity of the
reaction mentioned by Burger. Burger and associates detected cross reactivity with Benzpyrene, but not with methylcholanthrene and benzanthracene (16).

Transfer activity was associated with the presence of an alpha globulin in incubation fluids. However, this globulin was not found in dialysates, but was retained in the non-dialysible material. Other authors have reported the appearance of transfer factor associated with an alpha-1-globulin (13).

Tumor rejection, as depicted by the immune surveillance theory of Thomas (cited by Burnet [35]) is a manifestation of cell mediated immunity. The biological immunosuppressant antilymphotic serum is able to suppress effectively this type of immunity (47). In results presented here, however, ALS treatment had no effect on production of tumors by DMBA either through the topical application of DMBA in acetone, or through subcutaneous injection of the material in various carriers. Due to limited amounts of ALS available in any one pool, it was impossible to afford the sera necessary to establish a dosage that would prolong homografts. It is therefore inconclusive whether or not cell-mediated immunity was adequately suppressed.

Tumor production was low in all cases, including studies using 15-20 mg DMBA in tri-n-capryllin oil, a combination found successful by Toth (57). Topical application of DMBA along with ALS treatment did, however,
result in high mortality among adult guinea pigs. Injection of the material in benzene, olive oil, and tri-n-capryllin, even without ALS treatment caused some mortality.

It is possible that the reason for the low rate of tumor induction by the dermal route in the guinea pig is its well developed contact reactivity to carcinogens. Subcutaneous injection of the DMBA (19) and another carcinogen 3-methylcholanthrene (30) resulted in much greater production of tumors than the topical application (19). Subcutaneous injection could circumvent the portion of the skin in which contact dermatitis takes place.

The infiltration of mononuclear cells and tissue destruction that takes place in skin reactions of the delayed type could destroy protein and nucleic acid associations of the carcinogen before neoplastic alteration of the tissues could occur.

Indeed, severe tissue destruction occurs in the skin of animals repeatedly given topical application of 2% DMBA in acetone. That this is due to the acetone is refuted by Stenbäck (31) who noted no histological or histochemical alteration of skin continuously treated with acetone for over 100 weeks.

Subcellular passive transfer of contact sensitivity to DMBA is reported for the first time, and a transfer factor found in the dialysate is demonstrated. Further
characterization is needed to determine its similarity to the transfer factor(s) reported by other authors (11, 13).
REFERENCES CITED


