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The relationship of site to transplantability

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Thesis
THE RELATIONSHIP OF SITE TO TRANSPLANTABILITY
by

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INTRODUCTION

In a number of animals, there appear to be various sites of low resistance to transplants (15,16,24,32). The skin from all appearances, is the antithesis to this. That is to say, in the majority of cases, the fate of tissue transplanted to the skin is ultimate rejection. As stated by F. Wise in 1936 (41):

"As to the skin as a whole, I cannot forebear to quote from two of our contemporaries, '... Medicine will learn to regard the skin as a highly differential parenchymatous organ of special significance in immunologic as well as in physical and chemical protection."

Transplantations to skin and of skin have been for centuries and the work amassed in these endeavors is quite formidable. A relatively few have experimented with a single stratum of the skin, the majority electing to consider the skin as a single unit. In this study, I have attempted to further clarify the role played by a single layer of the skin, the tela subcutanea or "subcutaneous tissue" (25) in the active rejection of homotransplants.

REVIEW OF THE LITERATURE

1. Early History

The first attempts of man to transplant to the skin date far back into the 7th or 8th century B.C. Hindu surgeons at that time were known to use transplantation as

a means of repairing amputated noses. This procedure as described by the Hindu surgeon, Sushruta, (1,7) would be classified today as a form of flap transplantation. Sir Harold Gillies (13) asserts that the earliest endeavor at homografting to the skin of a human being was recorded in 1503 A.D. At this time, a poet named Calenzio had written the praises of a skilled surgeon called Branca who performed homotransplants to the nose. Legend records that at least one of his transplants failed, but undoubtedly, as our present experience shows, they all must have failed. The aforementioned Branca, the elder, and his son, Antonio, left no documents on their surgical methods; however, by word of mouth and by references as to their work by Calenzio and other writers of the day, a surgeon named Tagliacozzi was able to piece the procedure together. This selfsame Tagliacozzi is generally considered the father of plastic surgery as it is known today (14).

2. Problems in Homotransplantation

The field of transplantation in general is fraught with problems of tissue rejection. At this time, I should like to discuss such difficulties as are indigenous to the area of skin homografting. Billingham's work in this area (2) has shown that the skin shares some antigens with every

other tissue of the body with the possible exception of erythrocytes. These antigens appear to be directly responsible for the rejection of skin homografts by most higher animals. The source of this antigenic material is not yet known.

Gaines (12) has clearly outlined the inter-relationships of skin disorders and the malfunctioning of other body systems based upon their mutual phylogenetic and histogenetic derivation from embryonic ectoderm. This, in part, explains the mechanisms involved which lead to the communal sharing of antigens by these systems. Also, Kahn (20) has shown by his work that different body tissues in the same individual have wide variations in their defensive responses to given amounts of foreign antigenic material. The defensive response is described as a "localizing" capacity. For example, the "localizing" capacity of skeletal muscle was found to be one-tenth that of skin. Thus it follows that homograft rejection in skeletal muscle is less than that of skin.

In contradiction to Kahn's work, the results obtained by Teir (36,37,38) seem to show that the skin has a growth stimulating, rather than inhibiting factor.

According to Longmire, (23) homotransplants are only satisfactory when relatively acellular, inert tissues such as bone and cartilage are used. He reasons that this

is due to the inert tissue acting only as a supporting structure for the host's own invading tissue.

Southam showed that live cells of either a normal or a cancerous nature, when implanted under the skin of normal humans, resulted in the eventual rejection of the implant. Conversely, humans having a neoplasm at the time of implantation, do not reject the implant but rather, the latter results in viable tumors (35). Koelsche (21) has tried to explain this phenomenon by asserting that cancer cells will only grow in transplants from one animal to another if the recipient can recognize "self-identity markers" in the transplanted tissue (i.e. The transplant must have properties similar to that of the recipient's normal tissue.)

One of the few cases of a skin homograft success between two normal humans was described by Foster and Hanrahan in 1948 (11). An antihistaminic drug was given to the recipient and a split-thickness graft from a white male was transplanted to a full-thickness defect in a negro female. The case was followed for 60 days post operatively with the graft functioning normally during this time. Unfortunately, this was the only case of this type which the authors had experience with and they drew no conclusions from it.

There are a number of works which deal with the skin and local immune reactions produced by foreign antigens.

Cannon observed that a local subcutaneous injection into previously mobilized histiocytic tissue led to the local formation and retention of immune bodies relatively high in concentration (4). The immune bodies then diffused out and were recovered in lower concentrations in organs elsewhere. This, Cannon claimed, seemed to indicate that local immunization takes place wherever possible. Ørskov (31) determined that the rapid decrease in the amount of virus recovered from the skin of rabbits infected with vaccinia was due to local antibody production. He also showed that a form of virus neutralizing antibody could be recovered from the skin four days after infection. Research by Haxthausen (19) showed that an intracutaneous injection of foreign antigen evoked a stronger hypersensitivity of the cutaneous regions than did a similar injection delivered intravenously. He went on to theorize that substances which are not antigens from a humoral point of view may nonetheless give rise to the formation of antibodies in the skin. Rabinovici (33) X-rayed a series of rats and produced a marked and demonstrable reduction in the circulating lymphocytes of the body. Skin was then homografted to these rats, but the grafts were destroyed in the same manner and rate as in nonirradiated rats. This appears to support the argument that the circulating lymphocytes in the body do not play a part in skin transplant rejection. The experiments by Oakley showed for the first time that there is antibody production in the

fat and voluntary muscle of an animal (30). He also found that there was no consistent evidence for such production in the liver, spleen, kidney, or bone marrow. Williams, using neoplastic grafts in vivo, arrived at the conclusion that subcutaneous tumor transplants are ultimately rejected. Such destruction, he reasoned, is a function of the degree of vascularization of the transplant (40). Lastly, the in vivo work of Thorbecke (39) show that an immunizing injection given subcutaneously produced 22.4% more antibodies than did an injection of identical concentration given intravenously.

3. Theories of Skin Homotransplant Failure

Medawar (29) has limited his speculation on transplant failure to the following three hypotheses: blood incompatibility, genetical-cellular differences, and acquired active immunity. He also expressed the opinion that antibodies which are generated by the introduction of a homograft prevent the completion of cellular mitosis (27). It is for this reason that foreign antigenic material, when transplanted, will not grow successfully. Additional evidence from McMaster (26) and Danforth (5) has suggested that homograft rejection is the result of acquired immunity. This conclusion is based upon both researchers', independent of each other, having found lymphoid cells surrounding the transplant and large numbers of lymphoid cells in the peripheral blood. A theory of tissue incompatibility due to organismal differentials has been advanced by Loeb (22).

Meanwhile, Ehrlich et al. have shown that lymphoid cells may be a precursor for or the carrier of immune bodies (9).

Harris (18) has proposed that transplant rejection takes place in the following manner: the antigens are broken down physiologically into smaller soluble particles which retain their individual groupings characteristic of the original antigenic material. These soluble particles are carried to the regional lymph nodes and lymphatic tissue. In this way, the antigenic particles can stimulate the antibody synthesizing mechanism of the lymphatic tissue. To further complicate matters, the work by Ehrlich (9) and Darey (6) has implicated an additional cell entering into the transplant destruction mechanism with the lymphocyte. This additional cell is very similar to a plasma cell. Rostenberg's theory of transplant rejection is based upon the primitive reticulum cell (34). When this cell comes into contact with an antigen, an enzymatic adaptation takes place. Since this alteration takes place in a primitive reticulum cell, the change can be passed on to descendants of this cell (i.e. lymphocytes and/or plasma cells). These cells, assuming that they are capable of synthesizing globulin, would now synthesize an altered globulin (antibody) because of the inherited enzymatic modifications. Thus, the antibody would have a specificity against the antigen which initially caused the adaptation in the enzyme system of the reticulum cell. The latest work by Medawar, in 1961, has

brought about the "stem cell theory" (29). The theory states that sensitization occurs in mature cells, while there is an induction of tolerance in the immature or primitive cells. This is in complete opposition to Rostenberg's theory. Brent's more recent work has given support to the "stem cell theory" (3).

The work done by Ørskov (31), Haxthausen (19), and Rabinovici (33) has shown that the lymphocytes which circulate in the body do not play an active role in transplant rejection by the skin as formerly thought. These works concur with the hypothesis of Cannon (4). It is the purpose of this study to attempt to demonstrate that transplant rejection by the skin is greatly influenced by the subcutaneous tissue. That is to say, the latter tissue is an independant producer of a factor or group of factors which render transplant immunity.

METHODS

A rat neoplasm, the Walker 256 carcinoma (8), was chosen as my material for implantation. Malignant tissue was chosen because such tissue normally has a tendency to increase in size. It was determined to use this latter property as the governing criterion for a successful transplant. In this way, the confusion inherent in determining the success or failure of a transplant (17) was avoided. The material for each test group and its controls is provided by a single tumor. This eliminates problems arising from

variation in tumor viability.

The donor and recipient animals of the Walker carcinoma were Sprague-Dawley male rats weighing approximately 150 grams each. Males were chosen over females due to the former group's having an increased susceptibility to neoplastic growth. The site of implantation for the test animals was the subcutaneous tissue on the left side of the back, and that for the control animals was the retroperitoneal area behind the left kidney. The retroperitoneal area was chosen for the control series because it had similar embryonic origin and vascularity.

The details of the procedure are as follows: A portion of the growing neoplasm (7-8 days after transplantation) is surgically removed from the donor rat and placed in a tissue press. The material is passed through the press five times and then transferred to a tissue grinder (Fisher, pyrex glass, 16mm. x 150 mm.) in order to obtain as much homogenization as possible. To the mince is added .4 gms. of penicillin-streptolysin and the volume adjusted with 85% saline to produce the desired concentration of tumor cells in a volume of .1 ml. A portion of skin on the back of the test rats is dissected on three sides and the resultant flap turned back so as to expose the subcutaneous tissue directly. The tumor homograft having been introduced by means of a #18 hypodermic needle, the flap is sutured back in place. A midline incision is

made on the control rats and the viscera moved aside so as to allow the hypodermic needle to insert the transplant into the retroperitoneal area. The incision is closed and sutured in the standard surgical manner. Sterile technique is maintained throughout the procedure. Each rat is then placed in an individual cage. This latter precaution is taken so as to insure that rats weakened by the carcinoma will not be molested by others in the same cage.

1. Test vs Control

The first series of experiments was performed in order to establish the relationship of the test injection site to the control injection site. Each group, containing 18 rats, received .1 ml. of a 10% Walker carcinoma mince. The rats were checked daily and the day of death recorded.

2. Position Effect

An experiment was run in order to determine whether the longer survival rate of the subcutaneously implanted series was due to some factor inherent in the tissue itself, or merely a position effect. The implantation site for this experiment was the rat testis and the control site was the retroperitoneal area. The testis was chosen not only because it possessed physical properties found in subcutaneous tissue, but also because the vascularity and embryonic origin of the testis was homologous to both the retroperitoneal and subcutaneous tissues. Each of the 12 test and 18 control animals received a .1 ml. of a 10%

carcinoma mince. The rats were checked daily and the day of death recorded.

3. Dosage Effect

To ascertain the effect of dosage upon the results obtained from the test and control sites, several series of experiments were run varying the Walker carcinoma mince concentration from 5% to .1% per .1 ml. of implant. The number of rats used for each series of experiments varied. The rats were checked daily and the day of death recorded.

4. Transferral of Resistance

In order to determine the transferability of the "resistance to implantation factor (RIF)" encountered in the subcutaneous tissue in the previous experiments, three groups of rats, with 36 rats in each group, were handled in the following manner: All groups were implanted retroperitoneally with a 1% mince of Walker 256 carcinoma. The cellular concentration of the implant was 1%/.1ml. The implant of the control group was adjusted to the proper concentration with 85% saline; the second group had its implant concentration adjusted with a 1% subcutaneous tissue extract. The extract is made by obtaining a mince of the subcutaneous tissue and then extracting cells and fluid with 85% saline. Lastly, the third group received implants adjusted to the proper concentration with the resultant supernatant obtained by centrifuging the extract of the subcutaneous tissue at 3000 r.p.m. for 15 minutes. All the rats were

checked daily and the day of death recorded.

RESULTS

1. Test vs Control

The differential response to identical homograft implants by the test and control sites is clearly evident in Fig. 1. No overlap of the mean day of survival for each group is observed, and the results obtained fall well within the range of normal distribution.

2. Position Effect

The curve obtained from the testis injected rats closely approximates that of the control (Fig. 2), with the mean days of survival overlapping one another. This is the type of result that one would obtain when the location of the homograft implant is not a determining factor.

3. Dosage Effect

The various dosages of the Walker carcinoma mince are observed to alter the response of the subcutaneous tissue to the homograft implant (Figs. 1,3,4,5,6,7). A given concentration in the subcutaneously implanted groups brought about a shorter survival rate as the dosage was increased (Fig. 8). The group which was subcutaneously implanted with a concentration of .1% had 100% non-takes (Fig. 3).

4. Transferral of Resistance

In Fig. 9, it is shown that a saline extract of subcutaneous tissue is capable of imparting homograft resistance to a given area. The graph also shows that when

compared with Group I (control group), Group II had a mean survival differential of 2.3 days, and Group III had a mean survival differential of 12.2 days

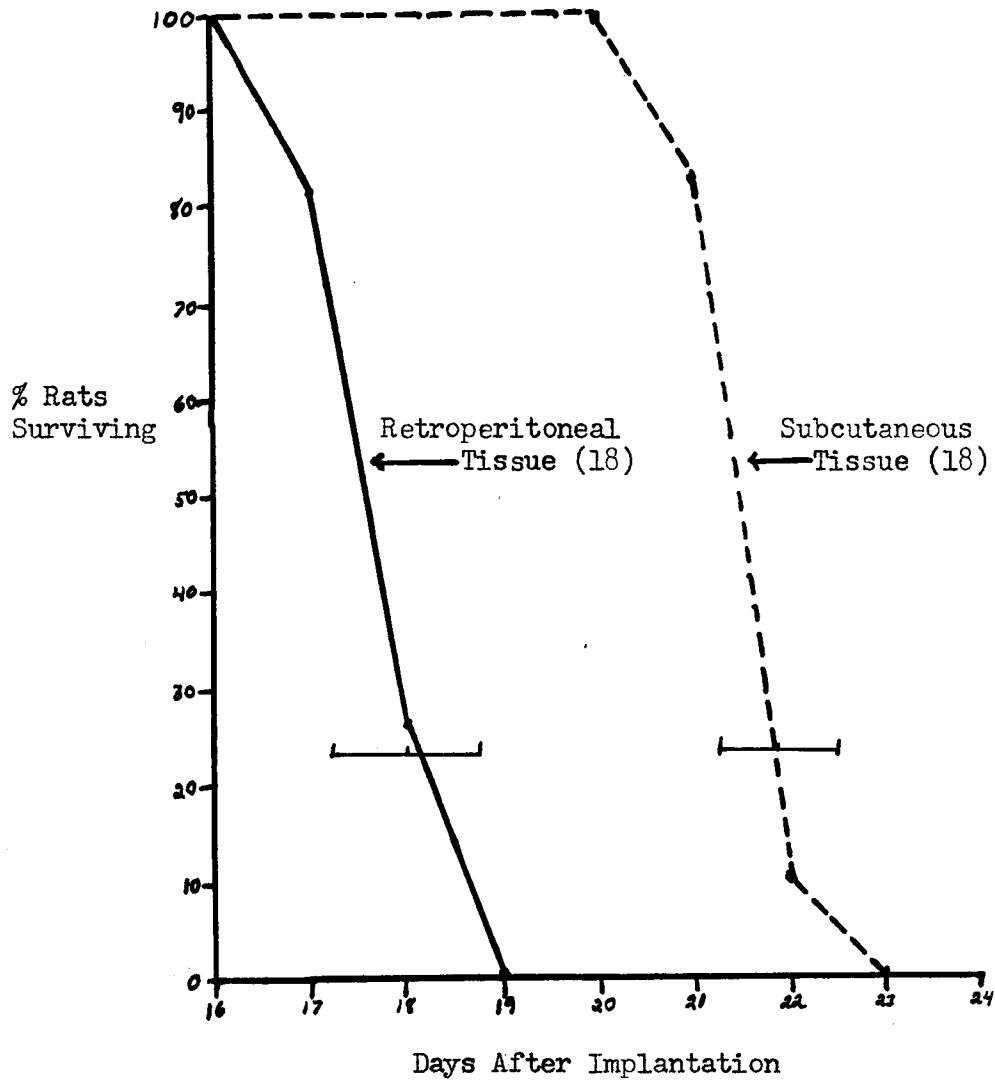


Fig. 1 Survival curves after a 1% carcinoma mince implant to retroperitoneal and subcutaneous areas.

Brackets indicate the standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

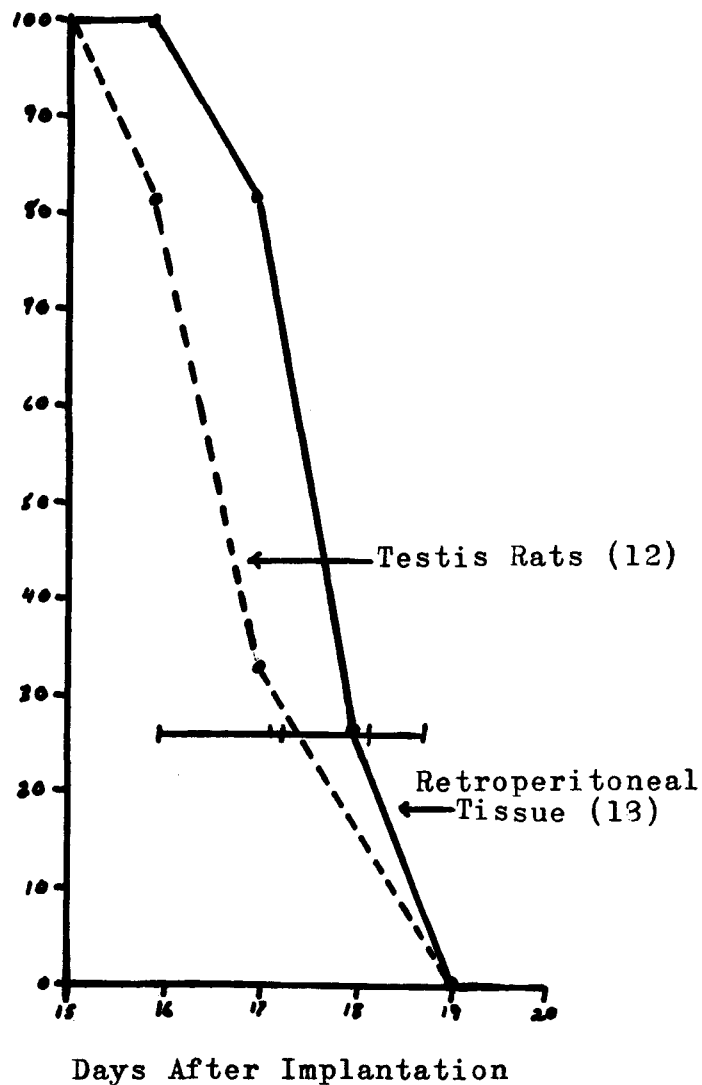


Fig. 2 Survival curves after a 1% carcinoma mince implant to the testis and retroperitoneal area.

Brackets indicate the standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

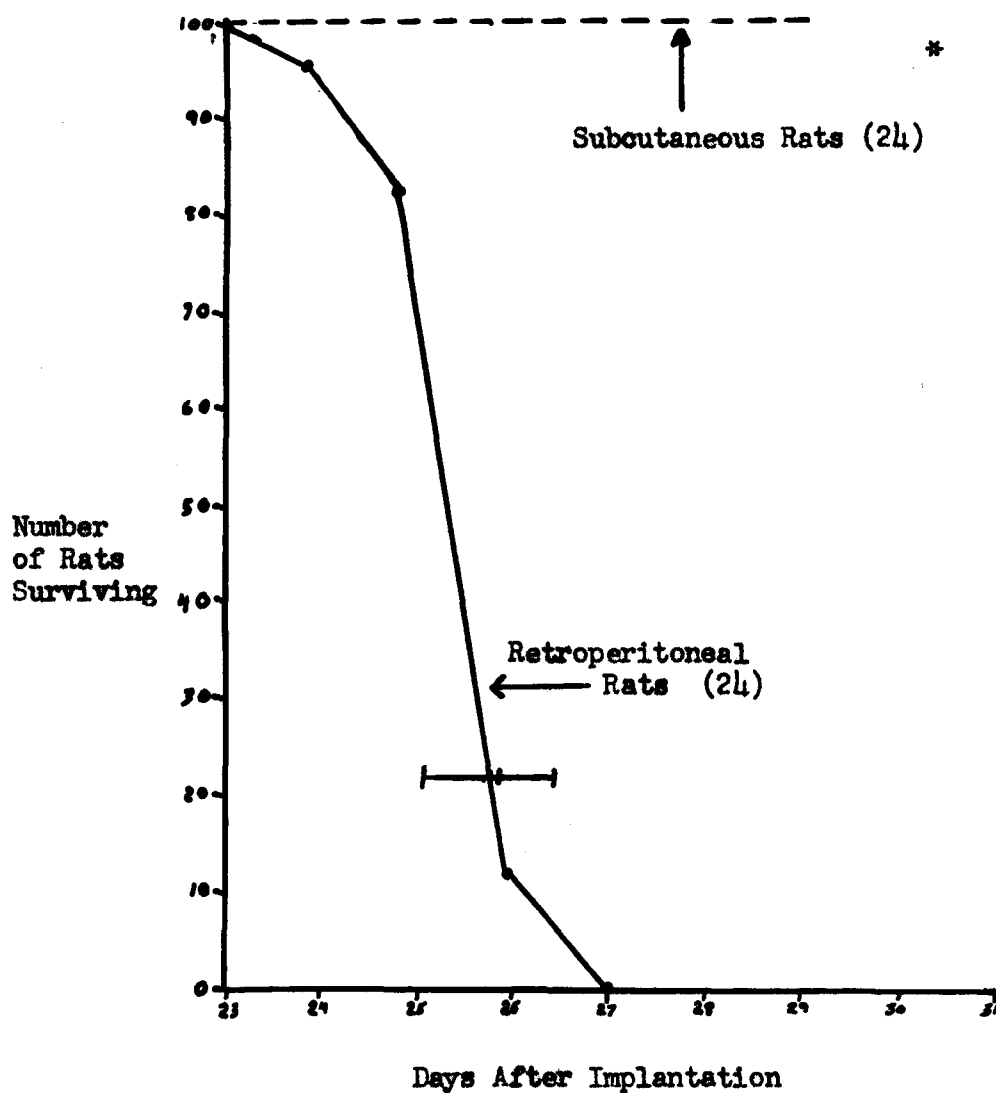


Fig. 3 Survival curves after a .1% carcinoma mince implant to the retroperitoneal and subcutaneous areas.

Brackets indicate the standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

* Asterisk indicates no takes in the entire group.

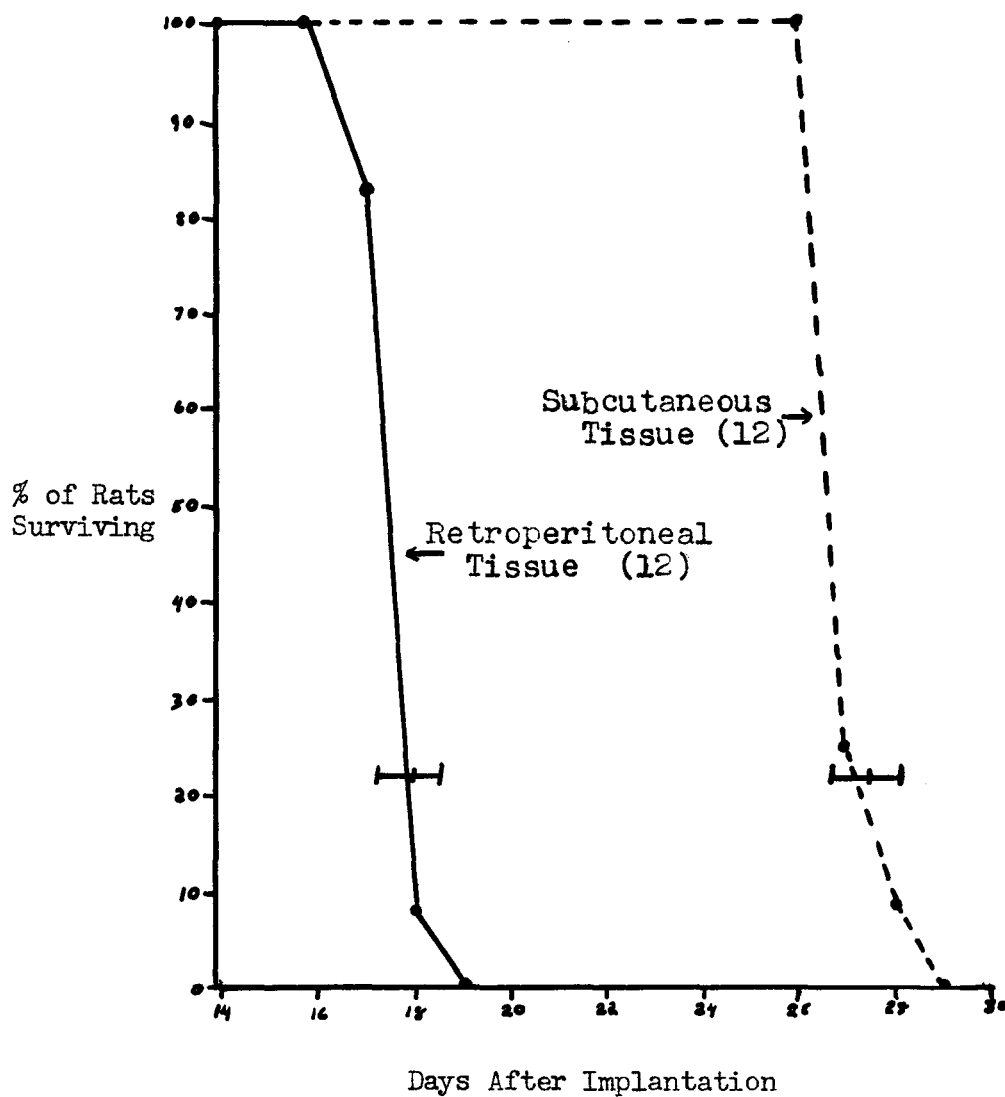


Fig. 4 Survival curves after .25% carcinoma mince implant to the retroperitoneal and subcutaneous areas.

Brackets indicate the standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

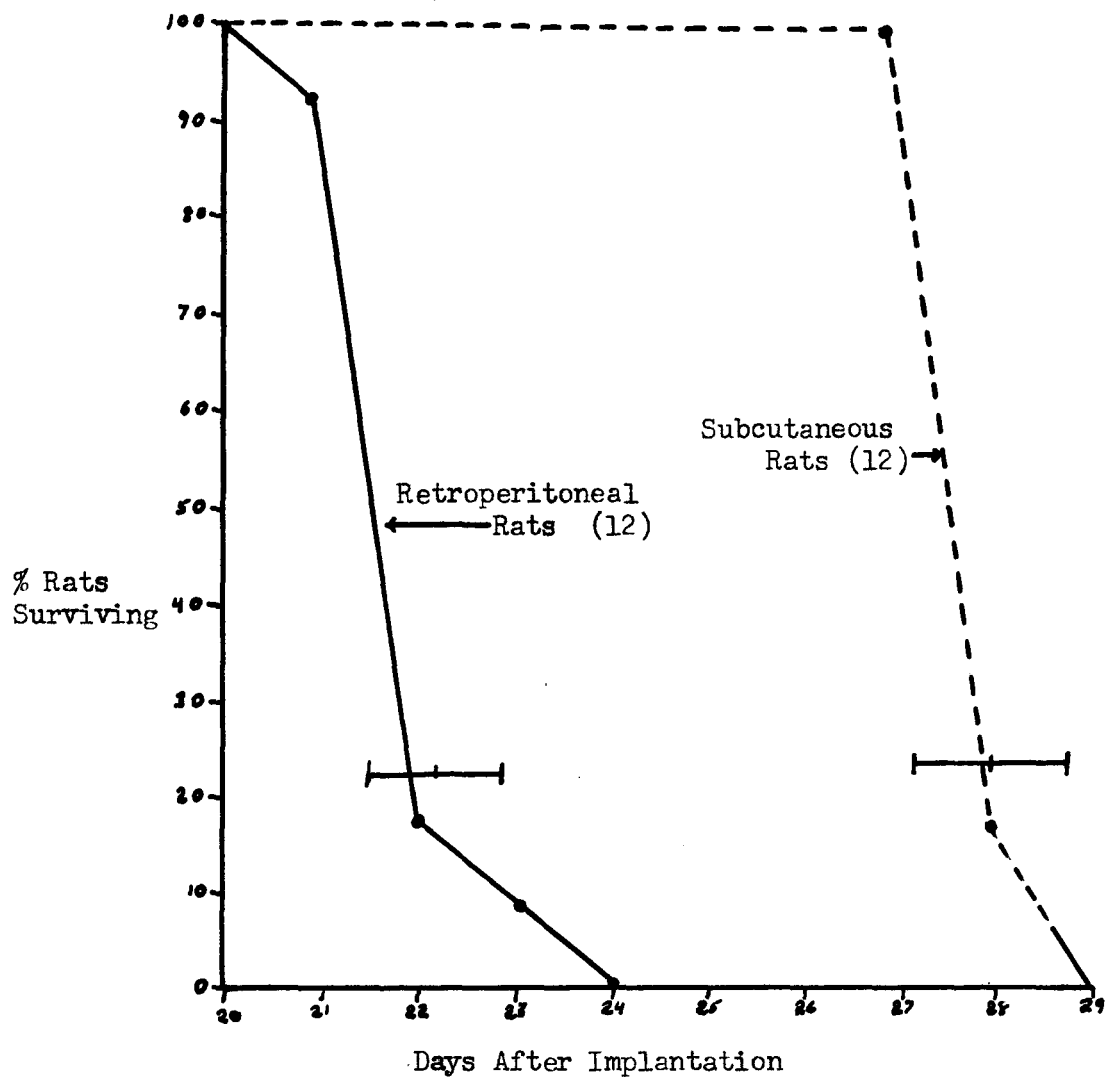


Fig. 5 Survival curves after a .5% carcinoma mince implant to the retroperitoneal and subcutaneous areas.

Brackets indicate the standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

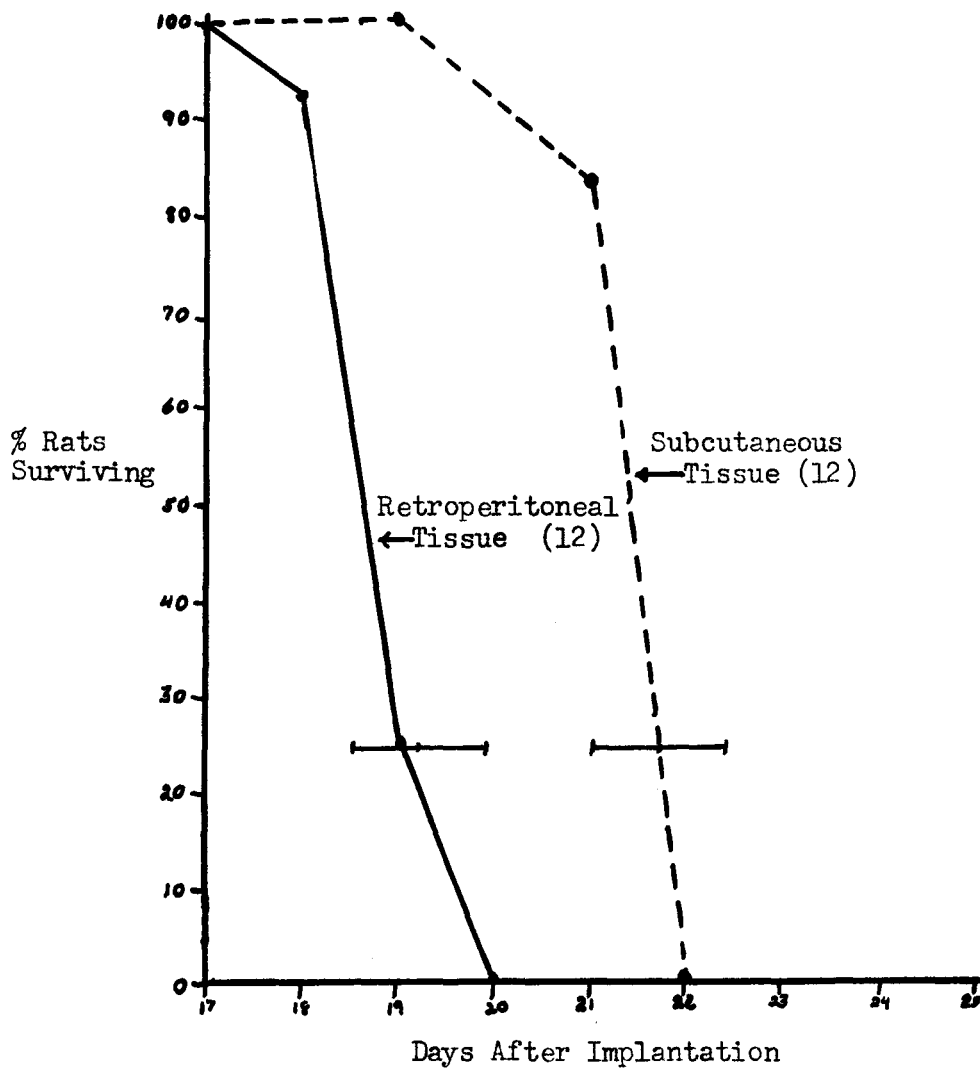


Fig. 6 Survival curves after a 2.5% carcinoma mince implant to the retroperitoneal and subcutaneous areas.

Brackets indicate the standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

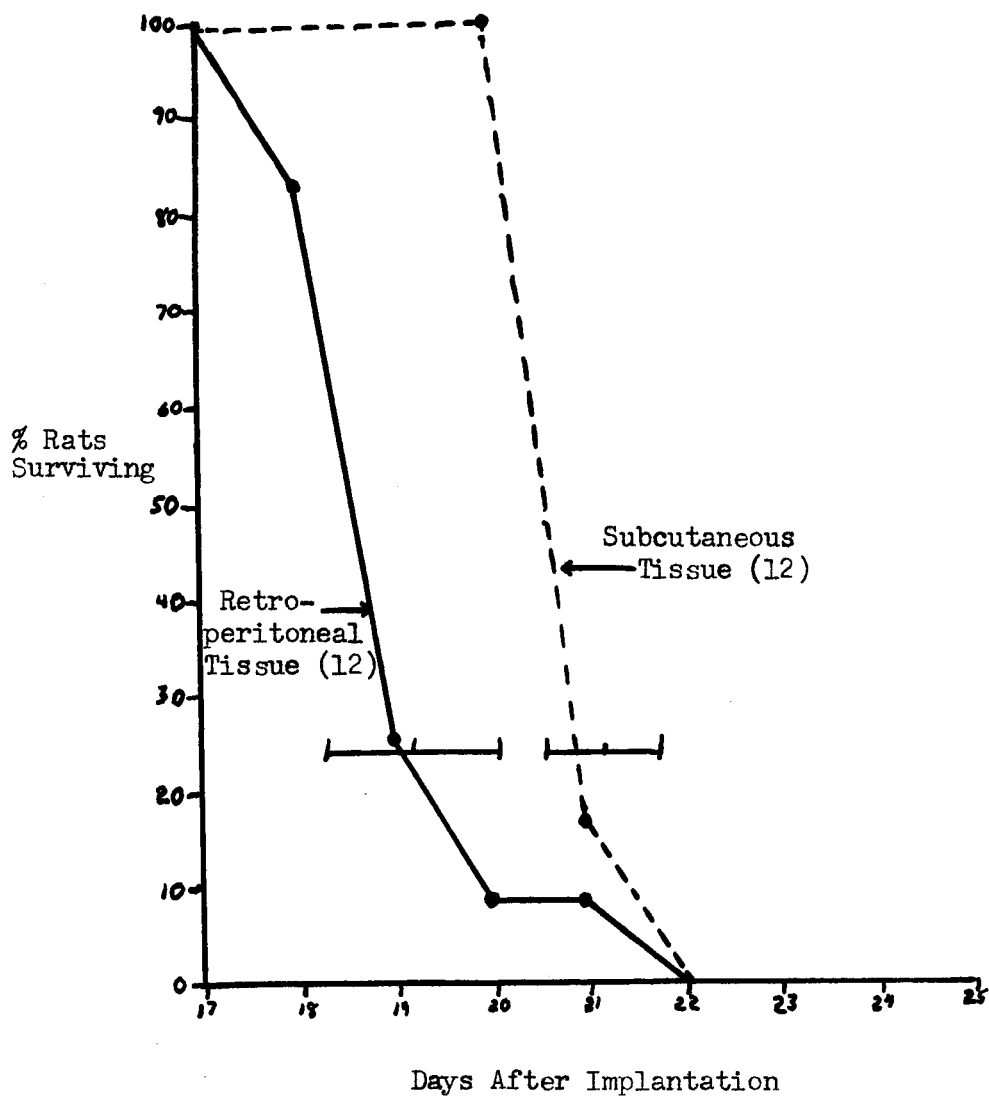


Fig. 7 Survival curves after a 5% carcinoma mince implant into the retroperitoneal and subcutaneous areas.

Brackets indicate standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

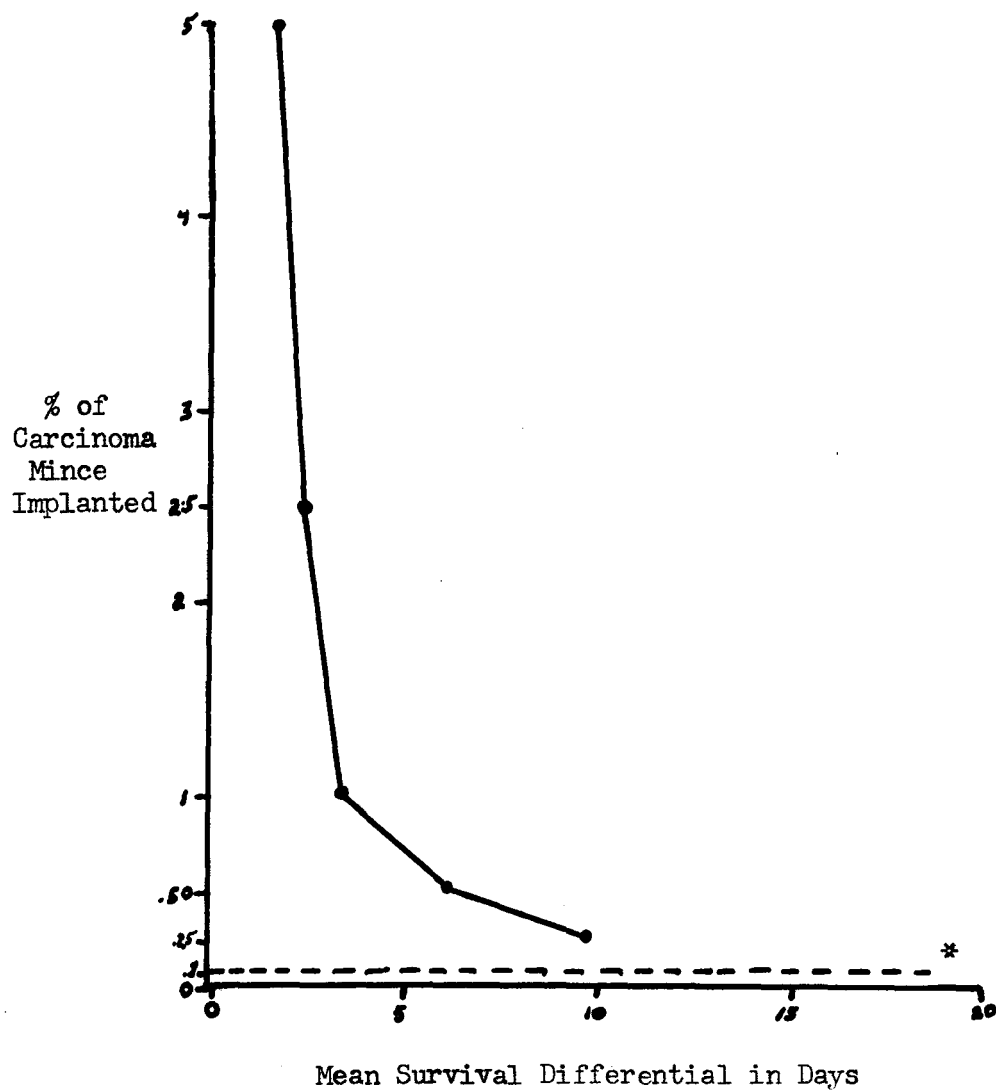


Fig. 8 Curve obtained by plotting the mean survival differential (mean survival day of test group—mean survival day of the control group) against the concentration of tumor in the implant.

* Asterisk indicates that the survival differential is unable to be obtained for that group.

Brackets indicate standard deviation of the mean.

The figure in parenthesis indicates the number of animals in that group.

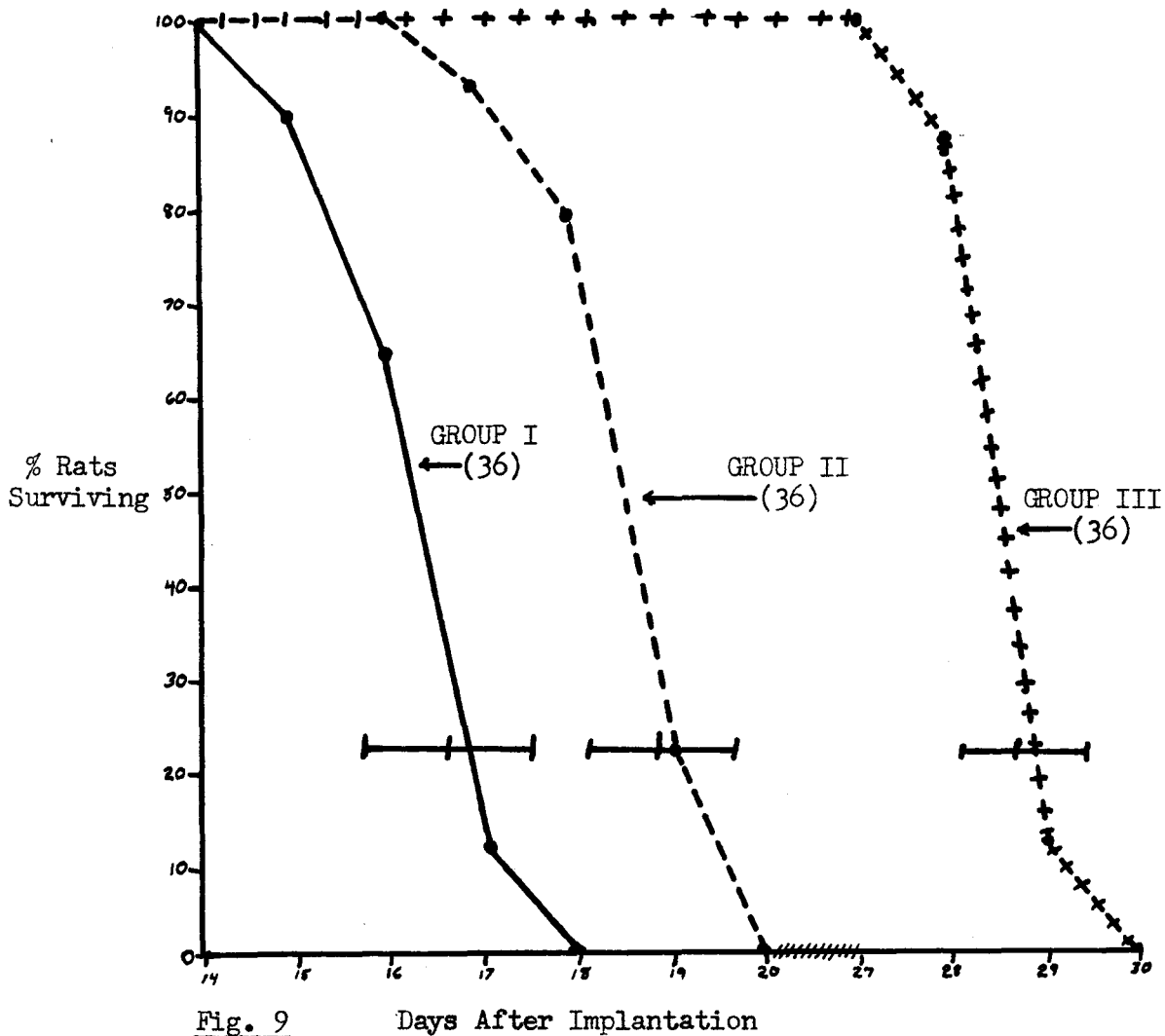


Fig. 9 Days After Implantation

Survival curves for 3 groups of rats implanted retroperitoneally with a carcinoma mince whose concentration was adjusted to 1% with the following:

- GROUP I - physiological saline (control group)
- GROUP II - extract of subcutaneous tissue containing both cells and fluid of said tissue
- GROUP III - supernatant fluid obtained by centrifuging subcutaneous tissue extract

DISCUSSION

The determination of a differential response to a homograft implant by two homologous tissues has been presented. The results obtained seem to show that subcutaneous tissue is an independent producer of transplant resistance. This is consistent with the theory of local immunization as proposed by Cannon (4) and others. The position occupied by the implant has been shown to exert no influence upon the results obtained. That is to say, a tissue which is similar to the subcutaneous tissue and homologous to both it and retroperitoneal tissue behaves in a manner similar to the latter in its reaction to implants. This appears to indicate that the transplant resistance encountered in the subcutaneous tissue is not due to physical factors, but possibly to chemical factors. Williams (40) has stated in his work that subcutaneous tumor implants are ultimately rejected. However, my studies indicate that tumor implants in subcutaneous tissue are not ultimately rejected, but rather that rejection is a function of the concentration of tumor material implanted. Tumor mince concentrations below .25% had a high percentage of non-takes in the test groups. This may indicate the possible existence of a minimum subcutaneous transplant dosage below which, due to a transplant resistance factor (TRF) inherent in the subcutaneous tissue, rejection takes place. It has also been shown that tumor mince concentrations ranging from .25% to 5% yielded a differential

response (mean survival day of the test group - mean survival day of the control group) which approached zero as the concentration increased. Such results are not consistent with transplant resistance imparted by lymphocytes or antigen-antibody reactions. Therefore, my studies agree with the work done by Ørskov (31), Haxthausen (19), and Rabinovici (33). Results of this nature are only capable of explanation if one adopts the hypothesis of a constant amount of transplant resistance factor (TRF) in a given volume of subcutaneous tissue, with no buildup or mobilization of the factor in response to increased dosages of implant material. It has been shown that a saline extract of subcutaneous tissue is capable of imparting to other areas a form of transplant resistance. This resistance has been found to be more evident in the supernatant fluid of a centrifuged subcutaneous tissue extract than in the non-centrifuged extract containing both cells and fluid of the tissue. Evidence of this nature seems to point to the fluid or humoral portion of the cell as the major source of subcutaneous transplant resistance and/or rejection. The ability to impart transplant resistance to other areas is important from a number of aspects. If the TRF is in some way responsible for the high degree of non-takes in skin transplantation, then blockage of this factor would greatly enhance such transplantations. Also, it was observed that the TRF was capable of rejecting tumor mince concentrations below .25%,

while above this concentration it was unable to do so. This, it was hypothesized, was due to the TRF being constant in a given volume of subcutaneous tissue. If the factor were capable of being extracted and concentrated, then it would be quite conceivable that large doses of the TRF might be capable of handling greater amounts of tumor tissue and either inhibiting or destroying the latter. Such a substance might, in effect, be a good chemotherapeutic agent in the management and treatment of malignant tumors.

SUMMARY

The determination of a differential response to a homograft implant by two homologous tissues has been presented. The homologous tissues used were those of the retroperitoneal and subcutaneous areas. The homograft material was the Walker 256 carcinoma. There is a decrease in the ability of the transplant resistance factor as the concentration of the implant increases. Transplant resistance is able to be imparted to other areas by means of a subcutaneous tissue extract. The major source of said resistance appears to be contained within the fluid portion of the tissue cells.

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