

SKIN GRAFTING AND MUSCLE REGENERATION
IN MUS MUSCULUS

by Michael S. Davis

Honors Thesis

in fulfillment of the requirements

of I D 499

Dr. Gordon L. Rosene, advisor

August 11, 1969

The Library
Ball State University
Muncie, Indiana

Sp0011
7/10/59
24-9
12/11
1959
.D88

SKIN GRAFTING AND MUSCLE REGENERATION
IN MUS MUSCULUS

Purpose: To determine by the use of skin grafting whether a strain of white mice is a pure strain.

At Ball State University, cancer research is being carried out by Dr. Gordon Rosene on a strain of white mice in which 95% of the females that have litters also develop mammary carcinomas. Dr. Rosene received this strain of mice from the University of Illinois in 1959, and it was claimed to be a pure strain of white mice. The term pure strain means that all of the mice have a common heredity and identical genetic background.

During the course of experimentation, rejection of transplanted tumors was noticed when none should have occurred. This led Dr. Rosene to believe that the strain was not completely pure. The possibility of impurity was also supported by the fact that the University of Illinois had rather sloppy animal handlers at the time the mice were obtained. If even one mouse from another strain had gotten mixed in with this strain then the purity would be marred.

We decided to check the purity of this strain by performing skin grafts. If the strain were pure, a piece of skin removed from one mouse and grafted onto another mouse should be accepted by the other mouse and grow as part of its own skin.

Two types of grafts were performed, a syngraft and an autograft. A syngraft is a graft from one mouse to another. This was the graft which was actually used to determine the purity of the strain.

An autograft is a graft from one place on a mouse to another place on the same mouse. This was done as a control to make certain that rejection was not caused by the technique of grafting



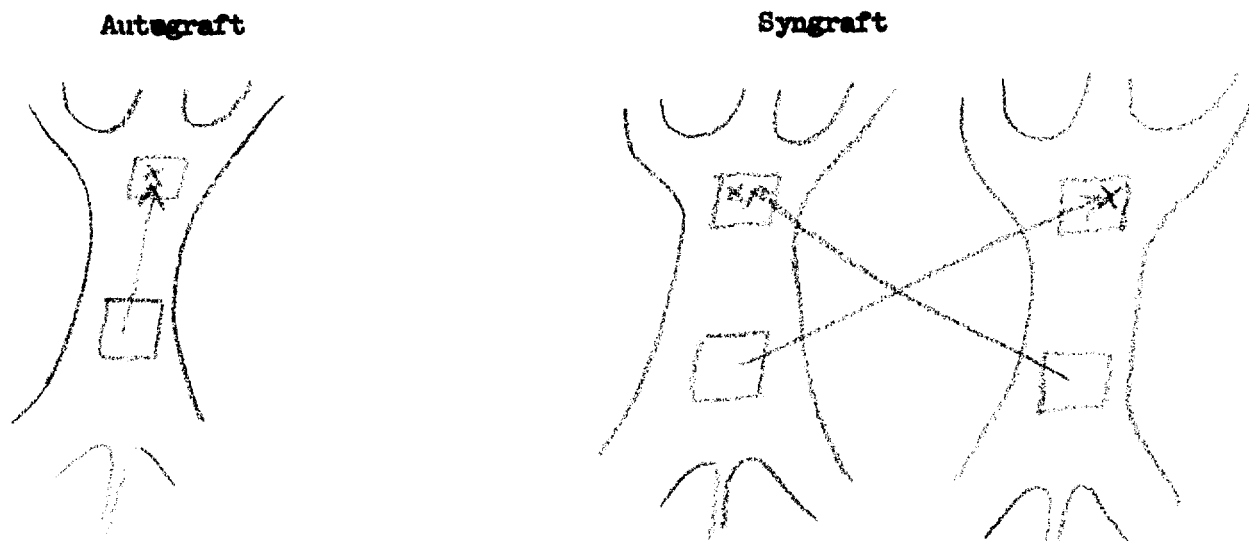
Picture 1. Skin Graft

Some grafts were removed after 3 days and some after 6 days for the purpose of making slides and photographs. The amount of rejection could be compared by counting the number of white blood corpuscles present in a field of given size. The outward sign of rejection was the formation of scablike material over the graft.

Syngrafts were always carried out between members of the same sex in order to eliminate the possibilities of critical genetic differences between the sexes.

It later became apparant that the working out of a good grafting technique was the critical factor in the experiment. The inability to establish a technique which could be relied upon not to cause rejection prevented the completion of the experiment and the establishment of conclusive results.

The value of the research came in the knowledge acquired about lab techniques and research procedures.



The original plans were to take 20 males and 20 females for syngrafts and 10 males and 10 females for autografts as a control. Our success or failure with these would determine how many additional grafts would be required.

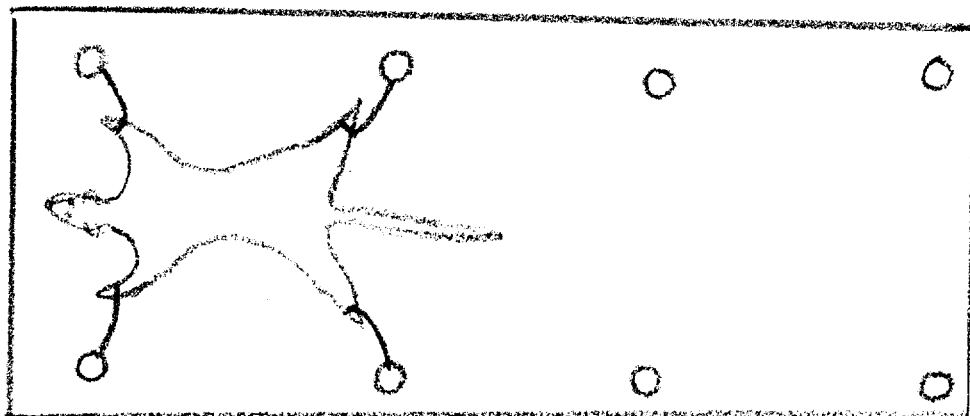
Before any of this could be done, however, a good technique for performing these skin grafts was needed. Autografts were used to develop this technique.

Our first step in all the techniques we tried was to anaesthetize the mice and prepare them for the operation. The anaesthetic used was chloral hydrate, which was administered by a hypodermic injection into the peritoneal cavity. The dosage was determined by reference to a chart which was based on the weight of the mouse in grams. The mice were weighed to the nearest tenth of a gram on a double pan balance.

After the drug had sufficient time to work , the mouse was tied onto an operating platform which consisted of a board with pegs sticking up from it. One end of a rubber band, lubricated with soapy water, was tied around each of the mouse's legs and the other end was looped around a peg. This spread the mouse's limbs out so that he remained motionless and there was easy access to the back or abdomen.

All surgical equipment was sterilized in a boiling water bath and alcohol was kept near the operating board for storage of instruments not actually in use.

The above procedures remained constant through all the operations on the mice.



Operating platform

The first technique was to try autografts on 5 female mice, using black surgical sutures to secure the graft in place. The backs of the mice were shaved using barber's soap for lubrication and a razor blade held by a hemostat for shaving. A piece of skin was removed from a place on the back near the neck, put back in the same place, and then sutured in place.

After every operation the mice were put in a special box for recovery from the anaesthetic. The box was lined with soft cloth and had a light bulb for added warmth.

After 6 days the mice were again anaesthetized , the grafts removed and the wounds sewn up. The mice were then returned to the colony, but no mouse was used more than once for skin grafting. The grafts showed outward signs of rejection and the scabs were about ready to be lost. Microscopic examination showed large numbers of white blood corpuscles. The same procedure was carried out again to check the validity of the results and the same situation occurred.

It was decided to try next to perform the same procedure again, but this time under sterile conditions. All surgical equipment was sterilized as before and sterile plastic gloves were worn. A face mask was also used to keep the graft free from breathe borne bacteria. Extreme care was taken to not contaminate any of the equipment or the person performing the operation. Again, 5 female mice were chosen for the operation.

After 3 days, signs of rejection began to show and after 6 days the rejection was well advanced. It was thought that perhaps the sutures were being treated as a foreign protein by the mouse and causing the rejection. In order to test this hypothesis we purchased pre-sterilized needles with gut sutures attached.

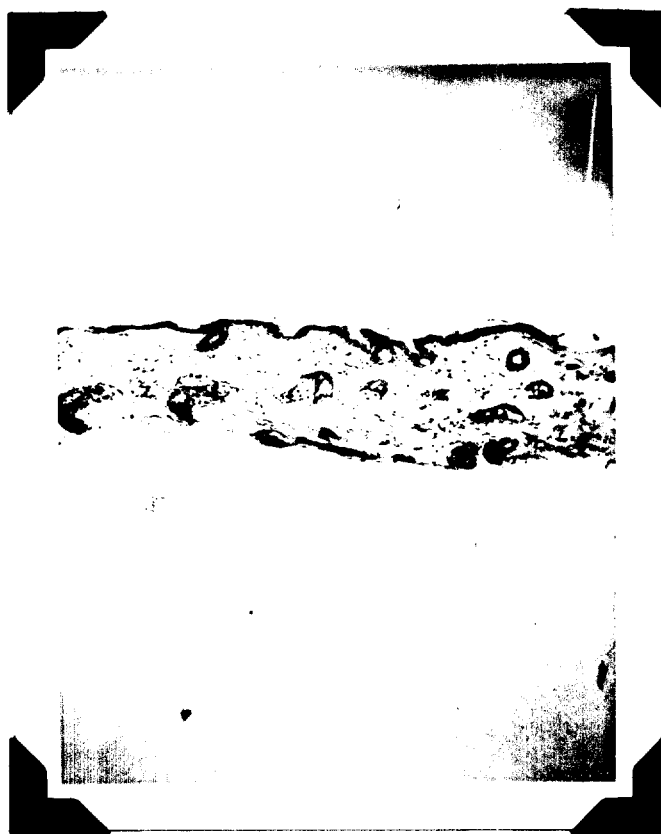
Following the same sterile procedures, 5 males and 5 females received autografts with the gut sutures. The results were quite disappointing because rejection still occurred.

Shortly after this Dr. Rosene received a letter from Dr. Dale Bockman of Ohio whom he had written to for advice. Dr. Bockman had used skin grafting in previous experiments and had some valuable information for us.

In mice and other rodents there is a muscle under the skin named the panniculus carnosus which is responsible for the movement of the skin. Dr. Bockman discovered that in order to obtain a successful graft the panniculus carnosus must be scraped from the graft tissue itself, but must remain behind in the area where the graft is to be placed.



Picture 2. Skin graft with panniculus carnosus intact



Picture 3. Skin graft with
panniculus carnosus removed

He also recommended the use of plaster casts to hold the grafts in place rather than sutures. However, since we were more familiar with sutures we decided to try them with the new technique before resorting to plaster casts.

We also decided to perform the autografts differently with this new technique. The graft was removed from the abdomen where the skin is thinnest and was placed on the back. The graft tissue was stored in sterile saline solution during the time between the removal of the graft and the preparation of the back to receive it.

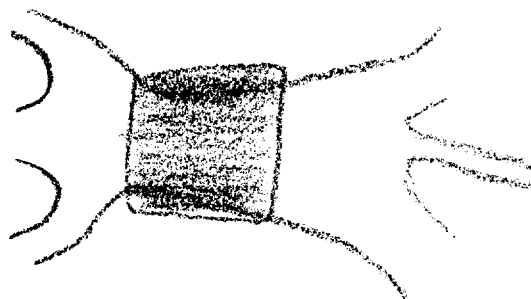
The graft was cut as thinly as possible and before being placed on the mouse it was spread out on a glass plate, which was covered with sterile saline, and scraped with a scalpel. The panniculus carnis was known to have been removed when the graft had a transparent appearance and no longer constricted. After a few attempts this technique was perfected fairly well.

The difficulty came in trying to prepare a position on the back of the mouse for placement of the graft without removing the panniculus carnis. It was extremely hard to determine whether the muscle had been left behind or not. If the removed piece of skin did not badly shrivel up, then the panniculus carnis was assumed left in place.

This method was tried on 20 female mice and rejection occurred in all cases. The gut sutures could have been causing the rejection, so we decided to try Dr. Bockman's suggestion of plaster casts

Plaster of Paris was mixed up immediately before each operation, and the same conditions existed as in the previous attempt except for the use of the casts in place of the sutures. After the graft had been placed on the back, merthiolate was added to keep the wound sterile and petroleum jelly was added for protection.

Gauze was dipped in the Plaster of Paris and wrapped around the mouse's body to hold the graft in place



Plaster cast

This technique looked promising, but turned out to be a failure. The casts weighed down the mice so much that they couldn't walk and hence were unable to eat or drink. The mice would have died before anything of value could have been determined, so the mice were cut loose from the cast after 1 day.

We then decided to try wrapping gauze around the mice without dipping the gauze in the Plaster of Paris. We followed the same procedure as previously, except that the gauze was merely tied in place. The operation was performed on ten male mice. Seven of the mice wiggled out of the gauze and the grafts were lost. The other 3 grafts produced rejection.

This was the last technique tried. Time for the experiment was running out and our techniques were exhausted. The fact that we were unable to leave the panniculus carnosus behind in the area which was to receive the graft appeared to be the greatest problem. Another possible cause of rejection is the introduction of bacteria into the wound, although great precautions were taken to maintain sterile conditions.

An interesting phenomenon was observed while looking for signs of rejection on microscope slides. It appeared that the skeletal muscle tissue in our slides was regenerating. Skeletal muscle regeneration is quite common in some lower vertebrates, such as a few amphibians and reptiles, and is largely responsible for replacement of lost limbs, but this is highly unusual behavior in mammals.

There was no time to investigate further, but this is a subject which is worthy of further study at a later date.



Picture 4. Regenerating skeletal muscle - low power magnification

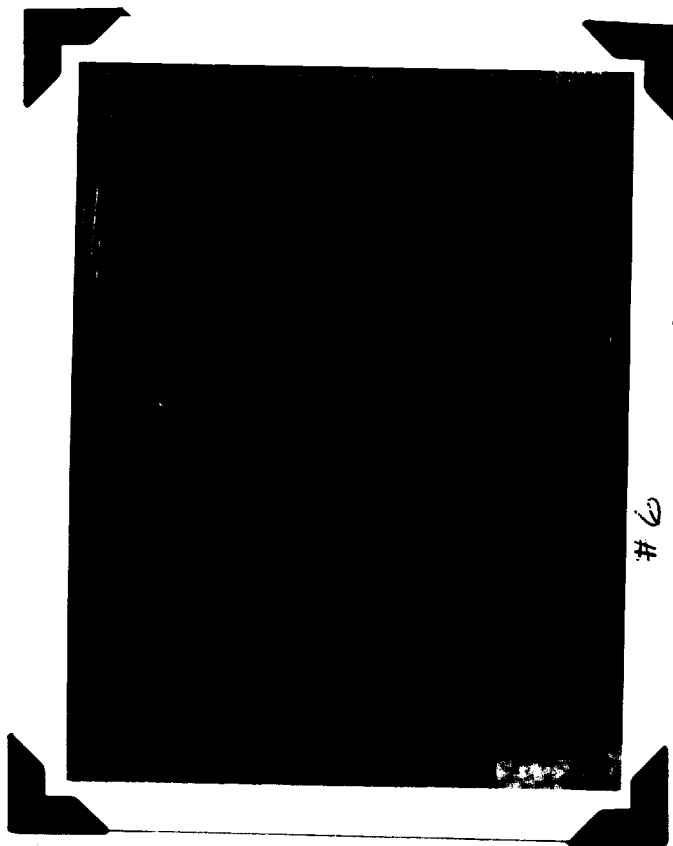
Conclusions

Due to the inability to establish a useful technique, the actual experiment which had been planned was not carried out. A few syngrafts were performed for comparison, but nearly all the grafts were autografts. Even though the original intent of the research was not carried out, much value was received.

Perhaps someone working on a similar experiment can learn from the mistakes and failures of this researcher and start at a place farther along toward his goal.

The interesting regeneration of the skeletal muscle tissue is very exciting and might provide another area of study and research for this student in the future.

This researcher gained much valuable experience in lab techniques, procedures, and equipment, which will be of great use later upon entry to veterinary school.



Picture 5. Regenerating skeletal muscle- high power magnification

