THE INFLUENCE OF PENICillin AND TRANSFUSIONS ON
THE LIFE SPAN OF MAMMALIAN HEART TRANSPLANTS

A Thesis
Presented to the Faculty of the Graduate School of the
University of Toronto in Partial Fulfilment of the
Requirements for the Degree of

Master of Veterinary Science

By
Harry Glendinning Downie

April, 1952
BIOGRAPHICAL SKETCH

The author was born on June 11, 1926, in Toronto, Canada. His primary education was at Hodgson Public School, and he received his senior matriculation in the summer of 1944, from the North Toronto Collegiate Institute.

In September, 1944, he enrolled at the Ontario Veterinary College, Guelph, Ontario, Canada. He graduated in May, 1948, with the degrees D.V.M. and V.S.

During his undergraduate years, the author worked as a student assistant in Physiology and Bacteriology, and on completion of his senior year, received the Helen Duncan McGilvray Memorial Award.

From May, 1948, to September, 1949, the author lectured in the Department of Physiology at the Ontario Veterinary College under Dr. H. T. Batt.

In September, 1949, he enrolled in the Graduate School of Cornell University with a major subject of Physiology and a minor subject of Physiology of Reproduction. He received the Master of Science degree in January, 1951.

In September of 1950, he enrolled in the School of Graduate Studies at the University of Toronto and for two years worked under the guidance of Dr. J. Markowitz of the Department of Physiology, in Experimental Surgery.
ACKNOWLEDGEMENTS

Sincere thanks are due to Dr. H. T. Batt and Dr. J. Markowitz for their enthusiasm, helpful advice, and constructive criticism of the manuscript.

Thanks are due to Dr. J. D. Schroder of the Department of Pathology for his suggestions and aid in the production of the histological sections, and to Dr. H. Neely for his aid in the photography.

The Department of Small Animals assisted greatly by allowing the use of a portion of their wards for the housing of the experimental animals. Members of the same department also aided in the post-operative care.
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF THE LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURE</td>
<td>24</td>
</tr>
<tr>
<td>Selection and handling of experimental animals</td>
<td>24</td>
</tr>
<tr>
<td>Preoperative care</td>
<td>25</td>
</tr>
<tr>
<td>Anaesthesia</td>
<td>27</td>
</tr>
<tr>
<td>Preparation and disinfection of the skin</td>
<td>28</td>
</tr>
<tr>
<td>Operating table restraint, intubation</td>
<td>29</td>
</tr>
<tr>
<td>Drape and instrument preparation</td>
<td>31</td>
</tr>
<tr>
<td>Disinfection of the skin and draping</td>
<td>33</td>
</tr>
<tr>
<td>Surgical technique</td>
<td>34</td>
</tr>
<tr>
<td>Post-operative care</td>
<td>41</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>42</td>
</tr>
<tr>
<td>Protocol number 1</td>
<td>51</td>
</tr>
<tr>
<td>Protocol number 2</td>
<td>53</td>
</tr>
<tr>
<td>Protocol number 3</td>
<td>55</td>
</tr>
<tr>
<td>Protocol number 4</td>
<td>57</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>59</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>60</td>
</tr>
<tr>
<td>PHOTOGRAPHIC PLATES</td>
<td>64</td>
</tr>
</tbody>
</table>
INTRODUCTION

It is not likely that in the near future we will be able to transplant tissues from one unrelated individual, or one species to another and have them survive indefinitely.

There are still great gaps in our knowledge regarding the relationship and make-up of cells and organs that prevents a completely successful homoiotransplantation of tissues. Hundreds of workers using all types of vertebrate and invertebrate animals and tissues are, however, painfully and slowly adding building stones of information to our foundation of knowledge. We are assembling data about the individuality of tissues.

The organ used in this investigation was the heart of a pup. The heart was transplanted by vascular anastomosis into the neck of an adult dog. The heart was used because its viability could be readily estimated after transplantation, by noting the character of the pulsations. The dog also, according to the work of Olson (1940), has isoemagglutinins present in blood plasma but not in the serum and due to the weakness of the natural isoemagglutinins, a transfusion of incompatible blood will produce no symptoms so the transplantation of the hearts could be performed in the dog and the typing of blood dispensed with.

Other workers have transplanted mammalian hearts but their work was performed before the discovery of antibiotics. In this investigation I hoped to show that the use of penicillin preoperatively
to the adult and pup, and post-operatively to the adult, would increase the active life span of transplanted hearts. Other workers have attempted to modify the differentials between a transplanted tissue and its host. Some of the methods used included Roentgen rays, graded exposure to heat, immunization with homoiogenous tissue and freezing.

Since the body fluids appear to influence the rate of deterioration of a transplant, it was thought that transfusions from the adult to the donor pup would perhaps attenuate the differentials. In a number of animals transfusions were performed.

It was hoped that improvements could be made in the surgical technique and that by the use of penicillin and transfusions a mammalian heart could be kept active and beating for a longer period of time than previously.
REVIEW OF THE LITERATURE

Tissue transplantation from one individual to another with survival has been the dream of many investigators. Paul Bert (1866) used parabiosis in an attempt to unite the tissues of two individuals. The union of blood vessels was recognized as the fastest and most normal method of re-establishing the blood supply to an organ or tissue and if tissues were to be transplanted, improved methods in blood vessel suturing would have to be developed.

Stephen H. Watts (1907) reviewed historically the suturing of blood vessels and in his paper mentions the first successful venous suture by Schede in 1882, with the aid of ivory clips, pins and thread. Carrel in 1902 is stated to have introduced a method of circular suturing that, although differing very little from the methods employed at that time, greatly facilitated the operation.

Mention is made of Jensen (1903) who also spent considerable time attempting to improve the methods of vascular anastomosis.

With the improvement in the methods of vascular anastomosis attempts were immediately made to use the techniques.

Hoepfner (1903) first carried out the retransplantation of an amputated leg in a dog. He united the blood vessels, the skin and muscles. Later Carrel (1907) again improved the methods of vascular anastomosis with rigid asepsis, and by approximating the ends of the vessels with three threads applied in three equidistant points of the circumference of the vessels. Traction on
each thread transformed the circumference into a triangle. While the sides of the triangle were stretched, a continuous suture was made along each of them. He found that the end-to-end suturing of vessels could be performed in four or five minutes and that stenosis seldom occurred. The way was now open for successful organ transplantation.

Guthrie and Carrel (1905) transplanted organs singularly and in mass.

Heteroplastic transplantation of organs from species to species proved unsuccessful. Carrel concluded that the transplanted organ must be prepared to support the serum of the animal on which it was to be grafted for a successful operation. Carrel was enthusiastic regarding the possibilities of organ transplants and stated that, "If I were a veterinary surgeon, and had to treat a myxoedematous dog, I would not hesitate to transplant into its neck a thyroid gland from another dog. In the case of a dog presenting Bright's disease, it would be a rational procedure to substitute for one of his kidneys a sound kidney extirpated from some normal dog. This operation would probably give good results if performed on large sized dogs and in a proper operating room. But the question of the transplantation of organs in man is difficult and very far from settled."

Carrel (1908) extended his transplantation experiments. He transplanted kidneys, thyroids, adrenal glands and ovaries and believed that auto- and homoiotransplantations of blood vessels would succeed. He thought that structures such as skin, muscle,
vessels and bone were not very sensitive to slight modifications in the composition of the serum, and that after transplantation the blood of their new owner would not be toxic for them. He therefore considered that there was no apparent reason why the leg or the arm of an animal or of a human being could not be transplanted successfully on another animal of the same species or another human being.

In a single experiment he was apparently successful in transplanting the limb from one terrier to another with healing by first intention.

Other workers were not as successful and it became apparent that there was a cellular antagonism present that prevented the establishment of all but autotransplants.

Halsted (1909) found that autotransplantation of parathyroid tissue in dogs was successful only if a deficiency 'greater than one-half' had been previously created in the recipient, and put forward the general hypothesis that a necessary condition for the success of any transplant of endocrine tissue is the prior existence in the recipient of a deficiency of the corresponding tissue.

Carrel and Ebeling (1921)(1922) recognized that in the aging of the body as in transplantation, there is a change in the fibroblasts and they studied their multiplication and growth in heterogenic serum.

Fleisher (1921) recognized that there were three subjects to consider: (1) the general reaction of biological relationship between tissues and host; (2) individual reactions of the species acting as the host; and (3) reactions and activities of the specific
tissue transplanted. His experiments were subcutaneous transplants of iris and cornea in guinea-pigs, rats and rabbits, without direct vascular anastomosis. He saw that there was a difference in both the lymphocytic and fibroblastic reaction of the host to different tissues. In some animals the leucocytic reaction may be sluggish while in others rapid.

He also noted that the reactions to heterotransplantation was entirely different to that encountered from an auto- or a homoiotransplant. He concluded that at least in part different factors are concerned in the reaction occurring in transplantation of tissue into homologous and into heterologous animals. He thought that the factors active in the one type of transplantation played little or no part in the other.

Crossen (1928) estimated in a quantitative manner the similarity or lack of similarity between individuality differentials of host and donor, of a transplant, by estimating the intensity and the time of appearance of lymphocytic infiltration, connective tissue invasion, and fibrous tissue production. He noted that a portion of autotransplanted cartilage when placed in close proximity to an autotransplanted lymph node failed to elicit any action from the lymphocytes because the individuality differentials were identical in both tissues. He then combined an autotransplanted lymph node and a piece of homoiotransplanted cartilage and observed a definite migration of the lymphocytes from the lymph gland toward the cartilage. He concluded that homoiotoxins were developed which attracted the cells from the lymph node in the direction of the
cartilage and that the organismal differentials act on the cells of the host as simple toxins.

Leo Leob (1926) from a group of experiments commencing in 1901 drew the conclusion that various mammalian tissues had potential immortality. He found that cartilage of the rat can be transplanted serially to other rats at least for a period of three years. This low susceptibility of cartilage to death following serial transplantation was due to the fact that cartilage elicits a less intense homoioreaction; that the cartilage resists more effectively the invasion of lymphocytes and connective tissue; and that after a period of time the lymphocyte reaction on the part of the host seems to give way to a gradual adaption to the reaction, decreasing progressively the longer the cartilage is kept in the host.

Loeb (1930) thought that it was conceivable through certain chemical or physical alterations of a tissue previous to transplantation, to change not only the reaction of the host against a strange organismal differential but that the organismal differential may undergo a certain modification. He attempted to modify the differential by selecting different sites for transplantation. He recognized that in an inadequate environment the individuality differentials assume injurious properties either directly or after interaction with the body fluids of the host, the differentials thus becoming transformed according to the relationship between the host and donor into syngenesio-, homoio-, or heterotoxins. It is towards these toxins that the host tissue responds. The toxins inhibit directly or indirectly regenerative processes, and also growth
processes caused by specific agencies.

Attempts were made by several workers to change transplanted tissues' capacity for the production of these toxins. Murphy (1914) with the aid of the Roentgen ray attempted to affect heteroplastic tissue grafts by destroying lymphoid tissue. Siebert (1928) intended to change tissue before transplantation to stop homoiotoxin and heterotoxin formation. He used graded exposure to moderate degrees of heat, which allowed the tissue to live but prevented them from attracting lymphocytes. He found that the homoiotoxin, lymphocyte, connective tissue reaction depended on the metabolism of living tissue and that the heterotoxins were active in necrotic tissue. From this he concluded that secondary or immune substances play a role in homoiotransplantation.

Fleisher (1921) found that previous immunization of an animal of the same species by homoiogenous tissue did not alter the kind and the course of reactions which develop around a homoiotransplant.

Blumenthal (1939; 1941) saw that following transplantation changes occurred in the absolute and relative number of various types of leucocytes in peripheral blood and using these changes as an index, he attempted to change the organismal differentials in transplanted tissues. Temperatures of 54 to 56° C. he found destroyed the organismal differentials but that freezing the tissues did not. He also noted that chemicals which affected proteins injured the differentials. Successive transplantation of a tissue accelerated the breakdown of the tissue, indicating to him
the development of immune substances.

Parkinson and Woodworth (1947) observed changes in the organismal differentials of kidneys and blood vessels transplanted in goats. They stored the tissues at a temperature of minus 40° F. and concluded that freezing and storage of tissues will not alter characteristics of survivability.

All homoiotransplanted tissues are not always rapidly destroyed. Transplantation between animals of a closely inbred strain is often successful. Ingle and his associates (1938) have reported the survival of homoiotransplanted adrenal gland in an inbred strain of rats. Certain sites, notably the brain and the anterior chamber of the eye, appear to be favourable for homoiotransplantation. Murphy and Sturm (1923) report successful intracerebral homoi- and heterotransplantation of tumour tissue. Markee (1932) has reported successful intra-ocular homoiotransplants of endometrium. Billingham (1951) and his associates have indicated that cortisone may enhance the survival time of skin homografts in rabbits.

Leo Leob (1945) reviews in detail the work in the transplantation of tissues to that time and includes the findings of his own associates. He states that in individualities separate constituents can be distinguished. One is the mosaic type which represents the sum of the particular organ and tissue characteristics (organ and tissue differentials) which determine structure, metabolism, motor and psychical activities and the component parts of which differ in different individuals. These multiple characteristics
are combined into a composite or mosaic which is peculiar to each individual.

Another type of individuality which may be designated as the essential individuality is characterized by the presence of a chemical factor—the individuality differential—which is common to the different organs and tissues of each individual and which differs from the corresponding chemical characteristics of the organs and tissues of every other individual.

In the same sense in which individuality differentials characterize individuals, there are species, order and class differentials each possessing a specific chemical constitution which characterizes the larger groups of organisms. All these various differentials may be grouped together as organismal differentials in contrast to the organ and tissue differentials which constitute the mosaic individuality. Various kinds of interactions take place between the organismal and organ differentials and these interactions are required to make of the individual an integrated whole. The term 'individual' is applied to human beings to emphasize the distinctive unique features which such a person possesses. We accentuate in general, our impression that the different persons we meet are more or less distinct from one another.

Individuality may be conceived of as the original physical and psychical state of an organism, which has developed in accordance with the genetic constitution of this organism with the cooperation of a sequence of more or less fixed physical-chemical environmental conditions.
The term 'individual' is extended from man to other living organisms which also show distinctive features, and it is applied even to non-living things. In a literal sense, it signifies that an organism or a thing is an integrated whole, which cannot be further divided without ceasing to be this particular organism or thing, without losing its identity.

In the phylogenetically higher organisms the differentiation between the various parts, together with their functions, is greater, and likewise the integration of the parts into one organism is more fixed and rigid.

All these individual characteristics in living organisms which have been mentioned so far, are localized in certain areas of the organism, in special organs or tissues, and they are either functional or structural peculiarities of the latter. If we conceive of the individual as a mosaic of many tissues and organs, each one functioning and metabolizing in its own peculiar way, we may consider this mosaic of separate parts as the biological basis of individuality.

The individuality differential is a particular characteristic distinguishing one individual from another. It is common to all the tissues and organs of an individual. It can be discovered by observing the reaction of certain cells and tissues belonging to one individual towards the tissues and cells of another individual of the same species. These reactions are indicative of a characteristic common to all the parts of one organism, which differs from the analogous characteristic of all the parts in a different
organisms of the same species. The cells and tissues of one individual recognize different individuals as such and they recognize the degree of difference between two individuals in accordance with their genetic constitution.

There are two methods by means of which the organismal differentials in general can be analyzed: (1) by transplantation and (2) by serological methods.

In transplantation where various organs or tissues are transplanted from one individual to another of the same species (homoiotransplantation), the reaction of the host of the transplant differs in accordance with the degree of genetic relationship between the host and the donor. These reactions consist in the activity of the lymphocytes, the connective tissue cells and the blood vessels of the host, towards the grafts. In addition, tissues, especially the more sensitive ones, are also influenced by the degree of their compatibility with certain constituents of the blood of the host. Tissues are injured by the body fluids of a strange host, playing a greater role in some species than in others.

It appears, however, that it is the lymphocytes which sense or recognize the finest degrees of similarity or difference in the constitution of the individuality differentials between host and transplant. The distinctive reaction of the connective tissue cells becomes noticeable if there is a slightly greater difference between these differentials. Different tissues have an unequal power to call forth a tissue reaction; for instance, thyroid gland usually induces a stronger reaction than cartilage or perichondrium.
This is due to the fact that a certain substance responsible for the reaction, "the individuality differential", is given off in sufficient quantities more readily by thyroid than by cartilage.

The individuality differential can be further studied by introducing variations in the relationship between host and transplant, which are expressed by the terms auto-, syngenesio-, homoio- and heterotransplantation.

Autotransplantation is the transplantation of various kinds of tissues and organ pieces into the same animal from which they were taken. Lymphocytes are practically lacking around such grafts and connective tissue cells are attracted in only a moderate number, forming a loose embryonal stroma around the transplanted cells. The blood vessel supply is rich and in the course of a relatively short time, the transplant assumes about the condition of the normal tissue or organ in the host.

Syngenesiotransplantation is the transplantation of tissue from brother to brother and in this case the individuality differentials are not as great as in the case of a homoio transplant, and the reaction is not as severe. This is especially true if the parents belong to closely inbred strains.

Heterotransplantation is the transplantation of tissue from one animal to another which is genetically still further removed than in cases of homoio transplantation. In this case animals from different species serve as host and graft reactions are more severe. The transplants die in a relatively short time, depending on the degree of resistance of the particular tissue. The connective
tissue reaction is very strong and the polymorphonuclear leucocytes are attracted first, rather than the lymphocytes, due to the presence of a strong heterotoxin. The reaction of the lymphocytes is the test for a milder toxin, namely, a homoio- or syngenesiotoxin.

Experiments show that the similarity or difference between two individuality differentials corresponds to the similarity or difference in the composition of the gene sets in the host and donor. It is the similarity or difference in the gene sets in two individuals which primarily determines the kind of reaction which takes place between host and transplant. Through close inbreeding we render their gene composition more similar, the individuality differentials correspondingly become more and more similar in successive generations and the severity of the reaction of the host against the graft is correspondingly diminished.

In the fertilized ovum the chromosomes and gene sets are the same as in the cells of the adult individual yet in the fertilized egg the individuality differential is not yet fully formed, but develops from a precursor substance in the course of embryonal life. In young animals before the age of sexual maturity, these mechanisms of defense against a strange individuality differential are not yet fully developed, as indicated by transplantation experiments of tissues into hosts of various ages. The connective tissue reaction is diminished in intensity and the lymphocytes may have, therefore, a better chance to become active in these young animals.

It is improbable that one will ever obtain an autogenous reaction after homoiotransplantation in non-inbred strains.
The number of genes entering into the individuality differential is great.

Gene hormones may mediate the effects of the genes on the organismal differentials; also, other factors which form part of the environment in which the organism develops may modify the development of the individuality differential. There are indications that adaptive processes which take place in the interaction between host and transplant may modify these differentials. Adaptive processes are prominent in the serial transplantation of tumours.

It is not possible to determine the organismal or individuality differentials directly. One must determine the consequences of the interaction of the organismal differentials of host and transplant.

Their manifestation depends on the nature and the amount of organismal differences produced by the host and especially by the transplant. We observe (1) the mode of attack on the part of the host; (2) the degree of reactivity of the host against strange differentials; and (3) the ability of the graft to resist these injurious conditions.

Not all substances produced by tissues or accumulating in certain organs possess an individuality differential; for example, many hormones and vitamins, and there are some tissues in which the finer organismal differentials have apparently disappeared and only some of the coarser ones have remained, such as in the lens fibres of the vertebrate eye.

Tissues give off substances which differ in their effect,
depending on the genetic relationship of the host and the donor. In their own habitat these substances are of an autogenous character and do not incite any reaction; but with genetic strangeness they assume the character of toxic substances. In near relatives these organismal differentials act as syngeneisotoxins; and in another unrelated individual of the same species, they act as homeoitoxins; and in different species as heterotoxins. These substances diffuse into the area surrounding the transplanted piece and they enter the circulation and are carried by the blood to more distant organs. They may in addition stimulate the formation of immune substances.

In his own work Loeb has observed the following general facts:

1. Effect of body fluids of host on the transplanted tissues.

2. Effect of connective tissue and blood vessels of the host on the state of the graft.

3. The significance of the lymphocytes and polymorphonuclear leucocytes on the fate of the transplant.

4. Distant reactions exerted on the host after the first two weeks.

From these observations he concludes that:

Lymphocytes are indicative of finer differences between the individuality differentials. They are not found in any considerable numbers if there is complete compatibility between host and transplant, and they do not as a rule appear in very large masses if the incompatibility between host and graft is so great that the
metabolism of the latter is seriously affected within seven to 10 days following transplantation. Polymorphonuclear leucocytes are seen in small numbers soon after the grafting of a piece of tissue, owing to circulatory disturbances and also to the presence of necrotic tissue.

During his work he noted differences in the mode of reaction against strange individuality differentials exhibited by different species.

Rat, mouse, and the guinea-pig show varying quantitative differences in reaction to a transplant.

In the mouse the lymphocytic and connective tissue reactions are in many cases less prominent and consequently, the direct injurious action of the body fluids becomes more prominent.

There are also differences in the reactions of different strains belonging to the same species, against strange individuality differentials. There are indications that certain individuals exhibit a stronger reaction to the tissues of various other individuals than do other animals of the same strain.

Differences in reactions to different tissues occur.

Tissues differ in respect to their resistance to injurious conditions. Adult ganglion cells are very difficult to transplant. Adrenal cortex, anterior hypophysis, cartilage and perichondrium are very resistant.

He found intermediate behaviour with kidney, fat tissue, salivary glands, ovary, and thyroid.

Striated muscle he found to be fairly resistant and that
it could be easily transplanted.

Bone marrow and testes he found very sensitive.

Different tissues produce different quantities of individuality differential substances. Higher metabolism in a tissue appears to produce more. It can be assumed that the accumulation of lymphocytes is an indicator of the amount of active individuality differential substances.

Place of transplantation is important. It must not interfere with the health of the animal, the transplant must be easily recovered at termination of the experiment. Evaluation of results may be complicated accidentally. Bacterial infections occur and are recognized by localized masses of polymorphonuclear leucocytes. They may also accumulate due to sterile necrotic tissue in area.

He decided that there are three phases following homoiotransplantation:

(1) Phase where reaction to all types of transplants would be the same, dominated by injury due to process of transplantation.

(2) Regeneration of damaged tissue occurs. Transplant subjected to injuries by the host. Initiate activity of the host's connective tissue. Host exerts injurious effects under the influence of homoiogenous individuality differentials.

(3) The initial damage of transplantation and secondary differential damage prevents complete recovery and the transplant dies in the third phase.

Loeb intimates that the reasons for the changes in phase
1. Action of the homoiotoxins of the host.

2. Activities of the host cells, especially the reactions of the lymphocytes, but also the connective tissue and blood vessels. He found that the age of the host influenced the action of the connective tissue and it became diminished if the host was very young.

Loeb felt that the success or failure in transplantation of tissues and organs depended on the antigens present in erythrocytes. He considered that future analyses would show that they are either identical with, or are part of, the factors which call forth the individuality differential reactions against all kinds of strange tissues.

Landsteiner (1931) shows that blood has some analogies to tissues and Loeb felt that the compatibility of the blood may be taken as an indication of the compatibility of the tissues comprising an individual.

Loeb also reviews the effect of blood vessel anastomosis and transplantation on the tissue reaction. He mentions that with the anastomosis the homologous individuality differentials act on tissues which are well provided with food and should be better able to resist the reaction of the host. The individuality differential substances produced in the transplant are carried directly into the general circulation of the host and do not need to diffuse slowly. They are also very much diluted. Loeb then expects that the local reaction around the transplant is not so prominent in those joined to the host by means of blood vessels.
Theoretically he believed that transplantation by blood vessel anastomosis makes possible a separation of the effects of the body fluids on transplanted tissues from those of the host cells.

Loeb also found that the individuality differentials are fully developed in young donors and that a lack of the differentials is not one of the factors that cause the difference in the results of homoiotransplantation in animals of different ages. The great tendency to the formation of fibrous tissue in older homio-genous hosts and the greater growth energy of younger tissues may explain at least some of these differences.

Loeb concludes that in all the experiments designed to change the organismal differentials there is no evidence that in higher organisms an actual change in the constitution of the differentials occurs. He maintains that changes may take place in the quantity of differentials which are produced and in the character of the reaction against these differentials.

Fischer (1924) attempted to cultivate chicken heart tissue in vitro. Portions of developing heart tissue from eight to 10 day old embryos were grown in chicken plasma diluted with 20 per cent Ringer's solution. After a period of time the tissue started to beat or pulsate. Fischer attempted then to get two separate fragments of heart tissue to beat synchronously by anastomosing the autonomic contractile fibres. He used portions from the same heart and portions from two different hearts. He concluded that fragments of heart from the same species when cultivated in vitro are capable of uniting and pulsating synchronously. He found that cell
contact was necessary for the development of physiological identity. No physiological union was shown between embryo duck heart and a fragment of embryo chicken heart.

Since that time Weinstein (1946) and his associates have used somewhat the same principle in vivo for the grafting of free striated muscle transplants upon the myocardium which aided in the reconstitution of the myocardium following damage.

The fibroblastic union of the damaged myocardium was replaced by parallel muscle fibre growth from the area at the edge of the graft.

Internal oblique muscle from the abdominal wall was in each case attached to the myocardium with cotton sutures. The pericardium was sewn over the top and after 15 weeks the cardiac transplants were examined. The grafts appeared well fixed, no mobility or shrinkage was apparent. In all cases the connective tissue in the area had increased and the epicardium had thickened. Mann, Markowitz and associates (1933) performed a series of transplants with the intact mammalian heart. They used a pup as the donor of the heart. A left intercostal incision was made in the chest. Loose ligatures were placed on the vessels of the heart. The pericardium was entered and the pulmonary artery branch was tied. The left pulmonary vein was ligated and the right pulmonary arterial branch and right pulmonary vein exposed. All the ligatures were then tied and the heart was removed. The left branch of the pulmonary artery was then anastomosed to the central end of the adult's jugular vein.
A second method was used later in their experiments where the pup's thorax was opened by an incision along the midline of the sternum. One hundred milligrams of heparin in 50 cc. of saline was injected into the inferior vena cava. The vessels were tied and cut as before. The heart was then approximated in the neck of the adult dog and the pulmonary artery of the pup was sewn to the central end of the jugular vein, and the brachiocephalic artery of the heart was sewn to the peripheral end of the adult's carotid artery. When their serrefines were removed from the vessels following the anastomosis, with the restoration of the coronary circulation the hearts in three cases out of four started to beat once again.

They attributed their failures to cardiac distention. From their experiments they made the following observations. When the animals recovered from anaesthesia, if the heart had survived, it gained a regular and vigorous pulsation. The average rate of action for transplanted hearts was 100 to 130 beats per minute and the rate was constant. In one series of transplants the pericardium was removed from each heart but its removal did not appear to influence the survival time. Irregularities appeared in the pulsations of the transplanted hearts anywhere from the first to the eighth day. These irregularities were either followed by an absence of a beat or by fibrillation. The average survival time for their hearts was four days. They had one heart that survived for eight days.

They noticed that if the host struggled the pulsations of the transplanted heart increased and that if the host was exercised the rate of the transplanted heart's pulsations increased 15 beats
per minute. They showed that thyroxine when injected into the host, caused an acceleration of the heart rate of transplant before this acceleration was manifested in the heart of the host. Their experiment indicated that the tachycardia of hyperthyroid states is independent of the central nervous system and is of a peripheral nature.

On post mortem they state that the left auricle usually contained a blood clot and that the right auricle and ventricle were invariably distended. Ecchymosis were present on the surface of the heart and on sectioning the muscle tissue was friable. Extensive oedema was present around many of the hearts in the neck region of the adults.

Microscopic sections of the hearts showed that the areas were completely infiltrated with lymphocytes, large mononuclear cells and polymorphonuclear leucocytes. Few normal muscle fibres were apparent.

They attempted to transplant the heart of a pup into the neck of its mother. The heart survived but showed no signs of increased viability.

Many of their hearts stopped suddenly for no apparent reason except that the biological differences between the host and the transplant were incompatible.

They concluded that the heart is not different from any other homoiotransplant with regard to the reactions instituted by the host.

Sinitsin Nikolai (1945) has, according to his paper, been successful in transplanting hearts in frogs and fishes with their survival for up to 190 days.
**Experimental Procedure**

Selection and handling of experimental animals. The animals that were to be used had to be in good health. The adult dogs and pups for the experiment were obtained from a reliable source and were housed immediately in an isolation ward of the Small Animal Clinic at the Ontario Veterinary College. The temperatures were taken and the dogs were given a routine physical examination. Anti-distemper serum was administered to each dog and pup on admittance and again in 10 days if they remained in the hospital wards.

The experiment required large adult dogs averaging approximately 35 pounds in weight. Dogs having long necks and heavy jowls were better specimens because of the increased area present for the transplantation of the heart in the neck region and because the loose skin allowed the heart to be covered without undue pressure.

Attempts were made to obtain mature dogs that were not aged; however, the source of supply could not always provide these animals and some aged dogs were used.

The pups that were required for the transplantation weighed approximately 2700 grams and were six to eight weeks of age.

Any animals which showed a temperature of over 102.0°F Fahrenheit were isolated and kept under surveillance until the temperature declined.
Preoperative care. The animals that were to be used in the experiment were observed for at least two days before the operation and in most cases they were in the isolation ward for a week. Careful note was taken of any signs of canine distemper such as mucus-filled eyes, rhinitis, loss of appetite or rises in temperature.

The animals selected were given a final thorough physical examination the day before the operation. Any animals showing a rise in temperature were deferred until a future date. They were not given any food on the evening before the operation. In the cases where penicillin was being used, the therapy commenced three days preoperative. Three hundred thousand International Units of procaine penicillin-G in oil were given each day into the muscles of the hip. The penicillin therapy was carried on following the operation until the transplanted heart ceased beating.

The pups, received similar treatment. They were each confined to a small cage three days before the operation and a dose of 300,000 International Units of penicillin-G in oil was administered daily preoperative. The pups were fasted for 24 hours before the anaesthesia was administered.

One series of experimental animals were donors and recipients for transfusions. The blood was drawn from the adult who was to receive the transplant, and given to the pup who was to be the donor of the heart. The daily transfusions were begun four to six days before the operation.

The forearm of the donor for the transfusion was clipped,
over the cephalic vein and the area thoroughly disinfected with alcohol. The vein was raised by an assistant and approximately 30 cc. of blood were drawn by means of a sterile 50-cc. syringe equipped with an 18 gauge needle. In some cases approximately 60 cc. of blood were drawn and 30 cc. of this amount were stored in a sterile, cotton-sealed bottle in a refrigerator and used in 24 hours. In order to prevent coagulation during withdrawal and storage, 0.5 cc. of heparin (500 units in physiological saline) was drawn into the syringe before attempting to draw any blood.

Following the withdrawal of the blood from the donor a sterile 20 gauge needle was substituted on the syringe and the blood was injected into the peritoneal cavity of the pup. The skin over the abdomen was thoroughly cleansed with alcohol previous to the administration. Ravenel, as quoted by Weiner (1948), states that the intraperitoneal route is recommended when there are technical difficulties in the intravenous route. He also mentions that the blood is rapidly absorbed from the peritoneal cavity into the general circulation and that this mode of administering blood may be considered almost equivalent to a transfusion by the intravenous route. The pups in this experiment were too small to administer the blood intravenously without difficulty and so the intraperitoneal method was chosen.

The amount given varied from 20 to 30 cc. according to the size of the pup. The pup usually showed no distress at the amount administered. Occasionally when the entire dose was given, the pup, for a few minutes, showed a slight distress from distention. At this
time it is necessary to be especially precautions in order to avoid pushing the needle through into the lumen of the gut. If this occurred and the transfusion was given, the pup showed intense pain from the distention and immediately evacuated the blood from the bowel.

The pups which received the transfusions appeared to gain weight, and looked healthier than their litter mates. The transfusions were continued until the day of the operation.

During the summer the operations were performed as early as possible in the morning in order to escape the heat of the day. Immediately before the operation the experimental animals selected were given a brief exercise period to facilitate evacuation of the bowels and bladder. They were then brought to the operating room.

Anaesthesia. The animals were weighed immediately and their temperatures were recorded. An increase in temperature in either the pup or the adult indicated a possibility of poor health and the operation was delayed or another set of animals was selected. The adults, in the majority of cases, were exposed to ether in a chamber especially designed for that purpose. To allow time for vaporization and ensure rapid absorption by the patient, the chamber floor was flooded with ether half an hour before the animals were introduced. The chamber was illuminated by a set of lights in the sides and a full view of the animal could be obtained through windows in the top. In all cases the adults passed through the excitement stage rapidly. They were removed from the chamber when the
proper stage of surgical anaesthesia appeared.

The adult was then placed on its back on a preparation table where the mouth was held open with tapes and a special intratracheal cannula was inserted. A soft rubber balloon was attached near the tip of the cannula so that it could be blown up from the outside. When expanded it closed off the area about the cannula and the trachea, eliminating the necessity of packing the throat. The cannula was then attached to a Joseph Becker anaesthesia jar of the to-and-fro type. A valve allowed the air-ether mixture to be controlled and the dog was maintained in a stage of surgical anaesthesia with the aid of this modified auto-inhalation method as described by Hardenbergh and Mann (1927).

On occasions when no anaesthetist was present the adults were given intravenously 30 milligrams per kilogram of pentobarbital sodium; however, this was not the routine practice. It was found that, upon recovery from the anaesthetic following the operation, a short excitement stage reduced the danger of the animal damaging the transplant by uncontrolled movements. With pentobarbital sodium the stage of excitement during recovery was prolonged.

The pup was weighed and given intraperitoneally 15 milligrams of pentobarbital sodium per kilogram of body weight. A deep anaesthesia resulted in five minutes.

Preparation and disinfection of the skin. The adult dog when anaesthetised was clipped with an Oster clipper on the ventral neck region from the cariniform cartilage of the sternum to the top
of the larynx. The clipped area extended well down on the sides. The use of a razor to shave the area was dispensed with when a size 40 surgical blade set was used. The area was then brushed to remove loose hair, washed with germicidal soap, dried thoroughly, and then painted with a two and a half per cent Tincture of Iodine.

The pups were clipped in the same manner over their entire thoracic, abdominal and neck regions. They were washed with germicidal soap and painted over the area with weak Tincture of Iodine.

**Operating table restraint, intubation.** The adult dogs when properly clipped, washed, and painted with iodine were then wheeled into the operating room where they were placed, back down, on a modified Brodie table.

Restraining ropes were slipped over the limbs and tightened by means of slip-knots. The forelimbs were then drawn down beside the body. The rope from each forelimb was slipped under the animal's body to the opposite side where it was passed over the other limb and fastened securely to the operating table clips. The hind limbs were then secured by ropes to the nearest clip so that the animal was extended on the table. The Joseph Becker anaesthesia bottle was now checked to see that sufficient room had been left at the head of the table for it to rest upon, to prevent unnecessary pressure on the intratracheal tube.

The anaesthetist then took up a position on a stool at the head of the Brodie table.

The anaesthetised donor pup was then placed upon a small
Joseph Becker operating table with a V-shaped top. The four limbs were tied to clips at the sides so that the pup's head hung down slightly over the end of the table. A glass tube with an inside diameter of 5 mm., an outside diameter of 8 mm., and 8 3/4 inches long, was then inserted into the trachea. The tube was passed down until it extended approximately one inch below the tip of the cariniform cartilage. This procedure prevented the end of the tube from being blocked by distortion of the line of the trachea when the extra weight of the tubes connecting it to the artificial respiration pump were applied. When the tube was forced farther down into the thoracic cavity, the tip interfered, during the operation, with the manipulation of the blood vessels and the thymus gland at the entrance to the thoracic cavity. After the glass cannula was inserted the throat was carefully and completely packed with 3" x 3" gauze squares to prevent the escape of air around the sides of the glass tube.

A Harvard Apparatus artificial respiration pump was then attached by a rubber tube to the glass cannula. A special valve was prepared in the rubber tube which allowed the air to leak from the system on expiration. This valve was used to control the extent of inflation of the lungs of the pup during the operation. The stroke of the pump was set at 16 per minute. The pump was then connected to an electrical circuit and switched on. The valve was closed momentarily to verify that the lungs were being inflated.
Drape and instrument preparation. The instruments and solutions to be used for the operation were sterilized by steam and boiling water in operating room sterilizers immediately before the preparation of the animals. The drapes, gowns, sponges, masks and gloves were in all cases sterilized in a steam autoclave the evening before and stored for use in a closed cupboard until morning. The packs were made up as follows:

Pack #1 and #2 each contained 15 towels 30" x 16" and 1 small pack of gauze containing 10, 2" x 2" sponges and 30, 3" x 3" sponges.

Pack #3 contained 3 gowns, 3 caps and 3 masks.

Pack #4 contained 1 stainless steel tray on which were placed, separately wrapped, 2 rolls of Deknatel surgical silk U.S.P., the one size 2-0 (25 yards), the other size 2 (25 yards); the tray also contained 2 long, pointed, fine, rubber-bulb minum pipettes and 1, 250-cc. beaker (pyrex); and 1 large enamel container to hold sterile sponges. The bottom of the tray was lined with 3 Fisher gauze laboratory sponges, 8" x 4", six layers.

Pack #5 contained 2 pairs of surgical rubber gloves and paper packages of talc.

The instruments used in the operation consisted of the following:

1. 4" pair of iris scissors, straight sharp points.
2. 5\(\frac{1}{2}\)" straight hospital scissors, counter-sunk screw.
3. 5\(\frac{1}{2}\)" curved, Kelly's haemostats.
4. 5\(\frac{1}{2}\)" straight, Kelly's haemostats.
<table>
<thead>
<tr>
<th>Count</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6&quot;, 4 x 5 teeth, Allis's intestinal forceps</td>
</tr>
<tr>
<td>8</td>
<td>5&quot; Halstead's mosquito forceps</td>
</tr>
<tr>
<td>4</td>
<td>5&quot; Halstead's mosquito forceps, curved</td>
</tr>
<tr>
<td>25</td>
<td>5½&quot; Backhaus towel forceps</td>
</tr>
<tr>
<td>1</td>
<td>4&quot; thumb forcep, serrated handle, straight</td>
</tr>
<tr>
<td>1</td>
<td>4&quot; thumb forcep, serrated handle, curved</td>
</tr>
<tr>
<td>1</td>
<td>5&quot; tissue forcep, serrated handle, 1 x 2 teeth</td>
</tr>
<tr>
<td>1</td>
<td>cilia forcep, plain, narrow points</td>
</tr>
<tr>
<td>6</td>
<td>serrefines, narrow width, light spring</td>
</tr>
<tr>
<td>1</td>
<td>Beckman goitre retractor, self-retaining, hinged arms</td>
</tr>
<tr>
<td>1</td>
<td>Bard-Parker scalpel handle No. 3</td>
</tr>
<tr>
<td>1</td>
<td>Bard-Parker scalpel handle No. 4</td>
</tr>
<tr>
<td>2</td>
<td>Bard-Parker blades No. 10</td>
</tr>
<tr>
<td>2</td>
<td>Bard-Parker blades No. 21</td>
</tr>
<tr>
<td>1</td>
<td>needle, regular, half curved, plain eye, triangular cutting edge, size No. 7</td>
</tr>
<tr>
<td>1</td>
<td>needle, regular, half circle, round body, size No. 7</td>
</tr>
</tbody>
</table>

Five hundred cc. of physiological saline were sterilized in a Fenwal Pour-O-Vac bottle immediately before the operation.

Twenty cc. of mineral oil were sterilized and made ready.

The suture material consisted of the Deknatel surgical silk in pack #4 and 8 tubes of Davis & Geck Inc. sterile, 5-0, Anacap silk, 18" long, with a B-8 swaged-on atraumatic needle. This suture was used in the later operations. At the beginning, Surgilon braided nylon, sterile, 5-0, 18" long, with a B-8 swaged-on atraumatic needle was used. It became impossible to obtain and the suture was
changed to the Anacap silk. The nylon suture was stronger and showed less resistance to passage through tissues and would have been used throughout if possible.

Disinfection of the skin and draping. When the animals were safely restrained and the anaesthesia was progressing, the surgeon and the assistant-surgeon scrubbed up. The method was similar to that described by Walter (1948).

Twenty cubic centimetres of phisoderm, a synthetic detergent containing Herachlorophene (G-11) three per cent, was scrubbed into the hands and arms for 20 seconds after a rinse in tap water to wet the arms. Five cc. of phisoderm were then applied and water was added until suds were apparent, the addition of water and scrubbing were continued for three minutes. The suds were then rinsed off and 70 per cent alcohol was rubbed into the skin for one minute. Precautions were taken at the end of the washing period to prevent contamination of the skin.

The assistant-surgeon then arranged the gowns, masks, gloves, instruments and the packs which had been opened and removed from the autoclave by the anaesthetist. The operating and instrument tables were arranged so that one set of instruments was used for the donor pup and the adult with the least inconvenience.

Masks and gowns were put on with the anaesthetist's assistance. Gloves were put on by the dry glove technique.

The animals were then draped completely, using the 30" x 16" towels. The operative area on the neck of the adult was kept clear
of towels. The clear area was from the lower edge of the arch of the cricoid cartilage to the tip of the cariniform cartilage and down to the jugular groove on the sides. The towels were held in place with Backhaus towel forceps. The adult dog was then draped completely in towels so that no area of the body was exposed except the operative sight. The towels hung over the edge of the operating table and prevented contact with it.

The donor pup was then draped in a similar manner. The area left exposed ran from a point halfway between the umbilicus and the xiphoid cartilage to a point two inches above the cariniform cartilage and extended far enough on the sides to allow an adequate view of any skin incision along the sternum. Care was taken then to drape the entire table and animal.

The instruments were then checked for arrangement. When the anaesthetist considered the depth of anaesthesia complete, the operation was commenced on the adult dog.

**Surgical technique.** A skin incision was made from one inch above the cariniform cartilage to half an inch below the cricoid cartilage, on the midline. The subcutaneous fascia was then incised to the muscle layer. Any bleeding vessels on the skin edge were picked up with haemostats and tied. Skin towels were then applied and secured with Backhaus towel clamps.

With the aid of a pair of curved Kelly's haemostats the sternohyoideus muscles were parted in the midline and by blunt dissection a length of the trachea was exposed. The index finger was
then slipped around the side of the trachea and down, on the side nearest the surgeon, until it contacted the right common carotid artery. A curved Kelly's haemostat was then slipped under the vessel and the adherent vagus nerve, and the vessel and nerve were drawn to the surface where the vagus nerve was separated from the artery for a length of three or four inches and allowed to drop back into the area beside the trachea. A ligature of 2-0 silk was then tied at the extreme cephalic end of the carotid artery. The blood was milked out of the exposed area with the fingers towards the heart, and a serrefine was then applied at the cardiac end. The carotid artery was then transected with a pair of sharp scissors half an inch below the ligature. The tied end was allowed to slip back to its original position in the neck. The remaining open end and serrefine were covered with a warm gauze sponge soaked in normal saline.

The skin edge towards the surgeon was then pulled away from the neck with Allis's forceps and the subcutaneous fascia was broken down to the jugular vein. A three to four inch length of the vein was exposed and brought to the surface by traction. A ligature of 2-0 silk was placed on the cephalic end and the blood was milked to the cardiac end with the fingers. A serrefine was then applied to the cardiac end. The vessel was then severed with a pair of sharp scissors half an inch below the cephalic ligature. The ligated end was then allowed to return to its original position in the neck.

The artery and vein with serrefines attached were then exposed on the surface of the neck and the ends were prepared for vascular anastomosis as described by Markowitz (1949). Moist gauze
sponges were used to isolate the vessels from the other tissues of
the neck. The adventitia was carefully stripped from the immediate
dge of the cut extremities with the aid of cilia forceps. The ad-
ventitia was pulled over the end of the vessels and cut off with a
pair of fine iris scissors. The lumen of the vessels was then
thoroughly syringed out with warm saline in order to remove any
clots or loose tissue. Oil was not used. Warm saline soaked sponges
were then placed over the vessels.

The anaesthesia on the adult recipient was lightened when
the manipulation of the tissues in the neck area was complete.

The skin of the pup was incised along the midline from a
point halfway between the umbilicus and the xiphoid cartilage to a
point one inch above the cariniform cartilage. Skin towels were
then applied.

The xiphoid cartilage was elevated and quickly cleared of
fascia and a Kelly's haemostat applied to each edge so that a scissor
cut could be made between them. A scalpel was used to begin the in-
cision through the sternum, and a pair of sharp, heavy scissors was
used to complete the incision.

The artificial respiration pump was not switched on until
most of the cutting was completed, because if it were operating,
the lungs would rise out of the thoracic cavity and interfere with
the incision. As the cariniform cartilage was approached special
care was taken to keep above and to avoid cutting the internal
thoracic arteries and veins. The sternum was then completely divided,
and artificial respiration was commenced. A Beckman goitre retractor
was set in place in the thoracic incision so that the chest cavity was opened to its fullest extent. The internal thoracic vessels were then tied off on the right and left side and cut between ligatures. The thymus gland was pulled up and forward out of the thoracic cavity. Its dorsal border was dissected carefully by blunt dissection from the great vessels in the area. A Kelly's haemostat was fastened to its tip as a weight to keep it from returning to the thoracic cavity.

The mediastinum was then gently broken down to allow entrance to both sides of the thoracic cavity. The vena azygos was found, tied close to the anterior vena cava, and cut between ligatures. The anterior vena cava was then separated gently from surrounding tissues, tied and cut between ligatures. The ligatures in this case were placed as close to the heart as possible.

The brachiocephalic artery was found and, if it appeared too large to be sewn to the carotid of the recipient, was tied close to its emergence from the base of the heart and cut between ligatures. If it appeared to be the correct size for sewing it was tied as far forward as possible and cut between ligatures. The ends of the ligature on the portion attached to the heart were left long for future handling.

Where the brachiocephalic artery proved too large, the subclavian artery was selected and tied as far forward as possible and cut between ligatures. The ends of the ligature on the portion closest to the heart were not removed.

The aorta was then uncovered and a loose ligature was
slipped through the arch. The posterior vena cava was found and a loose ligature was placed around it close to the heart. With the aid of a sterile syringe and a 25-gauge needle, 0.5 cc. of Heparin (500 units) was then injected into the posterior vena cava between the diaphragm and the ligature. Sufficient time was allowed for the Heparin to make its way through the heart and then the posterior vena cava was tied and cut between ligatures. The heart usually continued to beat for some time. After 30 or 40 seconds the aorta was tied tightly and cut between ligatures.

The heart, with the lungs attached, could then be removed from the thoracic cavity. It was placed on a towel moistened with warm, sterile, 0.85 per cent saline. The lung lobes were then tied firmly and cut off individually. With the aid of curved Halstead's mosquito forceps the pulmonary artery was then dissected free and a serrefine was placed over its cardiac end. With sharp, fine, iris scissors the vessel was severed at right angles to its length, as close to the lung stump as possible.

The brachiocephalic artery or the subclavian artery was stretched slightly with the aid of its attached ligature and a serrefine was placed as close to its cardiac end as possible. The vessel was then stretched and cut across at right angles to its length by the iris scissors. The cut should be made so that a sufficient length of the artery remains for sewing.

The pulmonary artery and the subclavian artery were prepared for sewing. The adventitia was removed with the aid of the cilia forceps and the iris scissors and the vessels were thoroughly
washed free of clots and debris with the aid of the sterile saline. The heart was then carried to the recipient dog and placed on its neck. The moist gauze packs were removed and the vessels to be sewn were approximated.

Six 5-0 Anacap braided silk sutures with a swaged-on atraumatic needle were then oiled. The pulmonary artery was then sewn to the jugular vein of the adult and the subclavian artery of the pup to the carotid artery of the adult. The method used was similar to that described by Markowitz (1949) for an end-to-end anastomosis of a transected vessel. The vessels were brought into apposition by three guy sutures and tied in position. The dependent guy suture was weighted so that the bottom row was not included in the process of suturing. Care was taken in the laying of the guy sutures to see that the edges of the vessels were everted. The assistant exerted gentle traction on the guy thread closest to him. The surgeon then picked up his needle in a mosquito forcep and, exerting traction with the remaining guy thread, rapidly sewed one side of the triangle with over-and-over sutures one millimeter apart. When the next corner of the triangle was reached, a tie was made to the guy suture. The vessel was then rotated. The dependent suture was taken by the surgeon and his guy thread was handed to the assistant. The assistant's guy thread then became dependent. When the suturing of the second side was completed the entire vessel was rotated through an arc of 240 degrees so that the remaining incompletely side was brought uppermost for suturing. When the suturing was completed the guy threads were cut and the ends with needles were set aside as
emergency sutures.

Any towels that were pressing on the vessels were then removed. The serrefines on the veins were removed and the serrefine on the carotid artery on the cardiac side of the anastomosis was released. The increase in pressure usually caused a slight amount of hemorrhage at the suture line. Air in the vessel next to the second arterial serrefine was then quickly squeezed out through the suture line. When this had been accomplished the second serrefine on the artery was released. The bleeding at the suture line was stopped immediately in most cases and the heart started to swell slightly with the entrance of the arterial blood. Atrial and ventricular contractions were initiated usually in 30 or 40 seconds. The beat was fast and regular and the expulsion of blood occurred through the jugular vein.

When the heart had gained a regular beat and the hemorrhage had stopped, a suture was passed, with the aid of a round half-circle needle, through the pericardium at the apex of the heart. A stitch was then passed through the skin and tied so that the heart was held forward towards the chin of the adult. The artery and vein leaving the transplant were thus not twisted or compressed by the weight of the heart itself. Three hundred thousand I.U. of crystalline penicillin were then dusted into the neck incision and onto the heart.

The skin edges were freed of all towels and with the aid of Allis forceps were brought into apposition over the heart. If the area were not sufficient subcutaneously to facilitate the burial,
the subcutaneous tissue had to be broken down by blunt dissection and the area enlarged.

The wound was then quickly closed by a number of interrupted sutures. The ether anaesthesia was stopped and the cannula removed. Three hundred thousand I. U. of procaine penicillin-G in oil were administered intramuscularly at this time.

The dog was then released from the table and carried to a kennel where it could recover from the anaesthesia. Recovery was usually rapid and uneventful.

Post-operative care. Three hundred thousand I. U. of procaine penicillin-G in oil were administered intramuscularly, daily, to the patient. A small amount of water was given three or four hours after recovery but food was withheld for 24 hours. After this time water was placed in the kennel where the dog could take as much as he desired. Routine temperature checks were taken daily and the dog's own and the transplanted heart's rhythm and rate were checked routinely as long as the transplanted heart functioned. Stitches were removed on the third or fourth day. When the transplant ceased to beat the adult was anaesthetised with the aid of sodium pentobarbital and the heart removed. Care was taken to preserve the suture line on both the artery and vein. A gross examination was then performed and the cavities were opened and examined along with the suture line for signs of thrombosis or clotting. The heart was then fixed in formaldehyde and sectioned at a later date.
RESULTS AND DISCUSSION

Following a series of operations in which the technique of the operation was perfected, 30 hearts were transplanted into the necks of adult dogs by vascular anastomosis. The subclavian artery from the aorta of the heart was connected to the central end of the carotid in the adult. The pulmonary artery of the heart was sewn to the jugular vein of the adult. When the circulation was restored, the blood passed from the carotid, through the subclavian artery into the aorta of the heart. The pressure closed the semilunar valves and the blood passed into the coronary artery of the transplanted heart. The blood supplied the musculature of the heart through the coronary system, and was emptied from the coronary sinus into the right side of the heart. The contractile force of the right ventricle then emptied the chamber through the pulmonary artery into the jugular vein of the host (Figure 1).

Of this number 23 survived and were active. The hearts that failed to manifest a controlled beat when the circulation was renewed went into ventricular fibrillation almost immediately. It was noted that the older the pup and the larger the heart, the more likely ventricular fibrillation would be an end result of the operation. When the weight of the pup was used as an indication of the relative size of the heart, the tendency was for the larger hearts to beat for a shorter period of time than the smaller hearts (Figure 2). The smaller the heart became, however, the more difficult
Diagramatic representation of the heart. The red arrows indicate the flow of blood through the vessels of the adult and the vessels in the musculature of the heart.

FIGURE 1.
Graph showing:

(1) the correlation between the size of the heart (estimated from the weight of the pup) and the length of life of the transplant;

(2) the effect of transfusions on the life span of the transplanted hearts.

**Figure 2:**
was the vascular anastomosis.

The vessels were often friable and too small for a strong anastomosis and as a result, with the extremely small hearts, mechanical difficulties shortened their active life span after transplantation.

A number of the hosts under ether anaesthesia showed a low blood pressure and when the circulation was restored to the transplanted hearts in these animals fibrillation and finally overextension of the transplanted hearts resulted. In two cases ether anaesthesia was administered again, following the successful transplantation of the hearts and in both cases the hearts stopped during the primary stages of anaesthesia. It was noted that one of the prime essentials for the resuscitation of the transplanted hearts was an adequate coronary blood pressure. This was supplied by anastomosing the central end of the carotid to the subclavian artery of the heart. Markowitz (1949) states that other workers have used the cephalic or distal end of the cut carotid, intending to reduce the pressure of the blood entering the coronary system, in the belief that since the heart performs only a fraction of the work demanded of it in the intact condition, the coronary flow need not be so great as when the heart is in the chest. If the transplanted heart functions poorly it is possible that the contractile power of the right ventricle might be insufficient to expel the normal amount of blood reaching it from the coronary sinus. No such condition was apparent in our transplants. Once they had gained a regular beat the systole of the heart was powerful and the flow of blood into the jugular
Penicillin was administered to all of the animals except three. When compared with those of Mann and Markowitz (1933) and the three animals of our experiment, the administration of penicillin appeared to have beneficial effects. Mann and his associates performed their work before the introduction of antibiotics and penicillin, using the best surgical asepsis available and excellent surgical technique. Their transplanted hearts beat on an average of four days (96 hours) and they had one heart that functioned for eight days (192 hours). They reported that sections of these hearts were completely infiltrated with lymphocytes, large mononuclears and polymorphonuclear leucocytes and that there were but few normal muscle fibres left. Extensive oedema was present in the region of their transplants.

The dogs of my experiment, which did not receive penicillin, had more stitch abscesses and maintained high temperatures for longer periods of time. In one case a large abscess developed below the transplanted heart. A protocol sheet shows the variations in temperature in this case (page 51).

Bacterial invasion was limited in all of our cases that received penicillin. Pathological sections showed a reduction in polymorphonuclear leucocyte numbers and the oedema that presented itself about the wound was not as extensive when penicillin was used.

The temperatures of the animals dropped more rapidly to normal when penicillin was used.
The muscle fibres in the sections of our hearts were in
good repair in most cases. Necrosis was reduced.

The transplants with penicillin beat on an average for
129 hours (5 days, 9 hours). We had two hearts that beat for 245
and 240 hours respectively, after removal from the pup.

In five of the transplants blood transfusions were admin-
istered preoperatively for a period of five or six days from the
adult to the pup. The blood was administered intraperitoneally.
These hearts survived for 153 hours (6 days, 9 hours), on the aver-
age. This may indicate that the differences between the host and
the donor were attenuated somewhat by the transfusions (Figure 2).

The transplanted hearts beat on the average 135 times per
minute. Terminally the rates became slower and irregular, skipped
beats, and premature contractions appeared and, in one case, periods
of tachycardia alternated with periods of bradycardia. In a few
cases the hearts gave no indication that they were about to stop.
A regular, rhythmical beat was maintained till the end. The heart's
force usually decreased as the end approached, the contractions be-
coming soft and less powerful.

A series of pups' hearts were transplanted before the
actual experiment was initiated, in order to master the technique.
During these operations a number of errors were made, observed and
corrected. Changes were also made in certain portions of the pro-
cedure, that improved the operation. Ether and nembutal were used
in a number of cases and because of the lengthened period of excite-
ment during recovery the use of nembutal was discontinued with the
adult dogs. The ether anaesthesia was stopped as the skin was sutured at the termination of the operation and the animal usually recovered rapidly following removal from the table. If nembutal was used, the animal could not readily damage the transplant if it was placed in a small cage where its movements during recovery were restricted. Following removal of the pup’s heart, care was taken to properly align the vessels before anastomosis. If due to an error, one of the vessels became rotated on its axis and was anastomosed in this position; when the serrefines were removed, the blood usually had difficulty passing the twist. When the twist was on the arterial side the heart would not receive enough blood. The pressure on the suture line in some cases was increased and caused it to break or leak excessively.

If the twist was on the venous side, over-distention of the heart rapidly occurred and fibrillation invariably resulted. In some cases the suture lines leaked excessively due to the improper placing of sutures or due to the friable nature of the vessels being sewn. Emergency sutures, if used promptly, stopped the hemorrhage in most cases. The emergency sutures had to be inserted without disturbing the blood flow through the vessel. If the vessel was compressed, rapid clotting occurred at the suture line and the clotting appeared to extend into the area of anastomosis. When the flow resumed, portions of the clot were invariably carried into the coronary vessels, and fibrillation often resulted. Hemorrhage on the venous side was not as serious and usually slowed and stopped as clots formed.
Hemorrhage was most common where the vessels to be sewn varied greatly in diameter. This was noticed most often in the case of the anastomosis of the jugular vein and pulmonary artery. Triangulation in these cases was often difficult. Excessive tension could not be applied because the pulmonary vessel was invariably non-elastic and weak. The jugular vein was thus allowed to hang somewhat loosely between the guy threads and on occasions the opposite wall was included in the suture line. Upon release of the serrefines, the blood had difficulty passing the sewn area and over-distention of the heart followed because of the resistance. If the area of the opposite wall included in the suture line was not too great, manipulation between the fingers often tore the suture free.

At times the ligatures about the lung roots slipped or the tissue about them ruptured as the heart over-distended. The pulmonary vessels leaked and to reapply a satisfactory ligature was almost impossible. In order to prevent this from occurring, it was found that tying the lung roots off individually, with thick, strong silk, was more likely to hold than a single ligature around the base of each lung.

In some cases the atria distended to such an extent that they ruptured. When this occurred the entire atria was tied off. Attempts to sew the opening closed with an emergency suture were in most cases hopeless because of the friable nature of the structure. One heart beat for 123 hours following this procedure. In order to tie off the atria, the pericardium has to be opened. The heart is quickly incapacitated because the blood is released from the atria.
into the pericardial sac and unless the pericardium is opened, the pressure rises rapidly and the heart will stop. When the sac is opened the fluid and blood escape and the ruptured area can be quickly tied with heavy silk.

The heart in a number of cases when the circulation had been restored, showed a change in rate and rhythm due to an excessive pressure in the pericardial sac. Sharp iris scissors were quickly used to make a slight opening in the structure through which the fluid could escape. With the release in pressure, the heart invariably regained a stronger, more regular rhythm. The pericardial sac was removed entirely in two cases, but no advantage was gained by this procedure and in the rest of the transplants the pericardial sac was left intact.

This was perhaps an advantage. After the heart had been in the neck of the adult for a number of days adhesions occurred from the tissues of the neck to the pericardial sac. The heart itself continued to beat without the encumbrance of adhesions in the pericardium. Very few adhesions were apparent on necropsy from the pericardium to the epicardium.

When the pericardium was removed the adhesions were directly on to the musculature and would likely act as pathways for white cell, or connective tissue invasions.

Surgilon, nylon artery sutures were used in the first five operations. The material then became unobtainable and Anacap silk artery sutures were used instead. Both suture materials had
swaged-on needles which reduced the trauma when they were forced through the vessel walls. Nylon proved to be the strongest suture and offered less resistance in passage through the vessel walls than did the silk.

It was observed that if the adult hosts were very old, their vessels were brittle and non-elastic. The wall of the artery in these cases was thick and the sutures had a tendency to cut through. The anastomosis were often more patent if the walls of the vessels showed some elasticity.

If the length of carotid artery or jugular vein to which the vessels of the heart were anastomosed proved to be too long, they had a tendency to fold or kink when the heart was buried under the skin in the neck. This kinking reduced the blood supply to the heart and endangered the suture line. In order to keep the vessels straight and keep the heart from moving about under the skin, a heavy suture was passed through the apex of the pericardium and the skin in such a manner that when the suture was tied the heart was held in place and the suture prevented it from dropping back on the vessels.

Gelfoam (sterile blocks of gelatin foam) were used in the preliminary operations in order to reduce hemorrhage at the suture lines and induce clotting. It was found that the material was effective, but when the clots were induced to form, they formed on the inside of the vessels also and thrombosis resulted. Gelfoam was dispensed with and fine suturing proved later to stop all hemorrhage.

Heparin (500 units) was injected into the posterior vena
cava immediately before the final vessels were tied and the heart removed from the thorax of the pup. This substance prevented clotting intravascularly. Sufficient time had to be given before tying the posterior vena cava for the heparin to make its way into the lungs, the chambers of the heart, and the coronary vessels. Heparin was invaluable as an anticoagulant. With its use apparently no intravascular clotting took place while the heart was without a coronary circulation.

The following are some examples of the protocol sheets used during the experiment.

Protocol number 1 shows the observations on a dog with a transplanted heart, that received no medication.

Protocol number 2 shows the observations on a dog with a transplanted heart, that received penicillin therapy.

Protocol number 3 shows the observations on a dog with a transplanted heart. The pup received blood transfusions and penicillin preoperative; the adult received penicillin post-operative.

Protocol number 4 shows the observations on a dog with a transplanted heart that beat for 245 hours.
Protocol number 1.

Date: May 11, 1951.

**ADULT**

Breed or Description: Mongrel black Spaniel, female, young.
Initial Weight: 8555 grams.
Condition: Fair.
Anaesthesia: Ether.

**PUP**

Breed or Description: Mongrel Collie, male, age - nine weeks.
Initial Weight: 2850 grams.
Condition: Good.
Anaesthesia: Nembutal intraperitoneally.

**MEDICATION**

Adult: None.
Pup: None.

Operation commenced at 9:30 a.m. Operation complete at 12 noon.
Impressions of operation: Proceeded well, heart started almost immediately.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult Heart Rate</th>
<th>Transplanted Heart Rate</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/11/51</td>
<td>12:00 noon</td>
<td>196</td>
<td>140</td>
<td>103</td>
<td>Dog out of anaesthesia.</td>
</tr>
<tr>
<td></td>
<td>1:00 p.m.</td>
<td>200</td>
<td>136</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>133</td>
<td>113</td>
<td>103.2</td>
<td></td>
</tr>
<tr>
<td>5/12/51</td>
<td>8:00 a.m.</td>
<td>172</td>
<td>124</td>
<td>105.4</td>
<td>Neck swollen. Two lower skin stitches removed.</td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>172</td>
<td>138</td>
<td>105</td>
<td>Neck swollen.</td>
</tr>
<tr>
<td></td>
<td>2:00 p.m.</td>
<td>150</td>
<td>150</td>
<td>105.5</td>
<td>Neck swollen.</td>
</tr>
<tr>
<td></td>
<td>4:30 p.m.</td>
<td>148</td>
<td>168</td>
<td>105.2</td>
<td>Neck swollen.</td>
</tr>
<tr>
<td>5/13/51</td>
<td>10:00 a.m.</td>
<td>150</td>
<td>148</td>
<td>105.5</td>
<td>Heart regular, neck swollen.</td>
</tr>
<tr>
<td></td>
<td>1:00 p.m.</td>
<td>150</td>
<td>140</td>
<td>104.2</td>
<td>Transplant regular.</td>
</tr>
<tr>
<td></td>
<td>3:45 p.m.</td>
<td>130</td>
<td>140</td>
<td>105.0</td>
<td>Transplant regular.</td>
</tr>
<tr>
<td>5/14/51</td>
<td>8:00 a.m.</td>
<td>114</td>
<td>104</td>
<td>104.6</td>
<td>Neck swollen.</td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>116</td>
<td>96</td>
<td>104.5</td>
<td>Neck swollen.</td>
</tr>
<tr>
<td></td>
<td>1:00 p.m.</td>
<td>116</td>
<td>100</td>
<td>104.8</td>
<td>Transplant irregular. Abscess forming below transplant. Incised. 50 cc of sero-sanguinous fluid removed. Stitches removed. Transplant regular.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>129</td>
<td>140</td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>
POST MORTEM EXAMINATION

Gross: No clots in vessels or chambers of the heart. Arterial and venous anastomosis intact. Pericardial sac distended with fluid. Petechial hemorrhages on the wall of ventricles.

Pathology Laboratory Report: Marked proliferated pericarditis. Myocarditis. The inflammatory cells were chronic in nature and there was a beginning degeneration of the muscle fibres. The degeneration and cell invasion appeared to be due to the pericarditis.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult Heart Rate</th>
<th>Transplanted Heart Rate</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/15/51</td>
<td>8:00 a.m.</td>
<td>108</td>
<td>136</td>
<td>102.8</td>
<td>Transplant regular.</td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>104</td>
<td>140</td>
<td>102.6</td>
<td>Transplant regular.</td>
</tr>
<tr>
<td></td>
<td>1:00 p.m.</td>
<td>92</td>
<td>136</td>
<td>102.2</td>
<td>Abscess cleaned, drained.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>96</td>
<td>140</td>
<td>103.0</td>
<td></td>
</tr>
<tr>
<td>5/16/51</td>
<td>8:00 a.m.</td>
<td>96</td>
<td>144</td>
<td>104.0</td>
<td>Swollen neck.</td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>98</td>
<td>132</td>
<td>104.0</td>
<td>Transplant irregular.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td></td>
<td></td>
<td></td>
<td>Heart stopped. Removed. Heart beat 5 days, 120 hours.</td>
</tr>
</tbody>
</table>
Protocol number 2.

Date: June 29, 1951.

**ADULT**
Breed or Description: Mongrel Terrier, female, mature.
Initial Weight: 45 pounds.
Anaesthesia: Ether.

**PUP**
Breed or Description: Collie, male, age - nine weeks.
Initial Weight: 2500 grams.
Anaesthesia: Nembutal intraperitoneally.

MEDICATION
Adult: 300,000 I.U. penicillin daily for 3 days before operation.
Pup: 300,000 I.U. penicillin daily for 3 days before operation.
Post-operatively: 300,000 I.U. penicillin, crystalline, in wound.
300,000 I.U. penicillin-G in oil daily.

Operation commenced at 9:00 a.m. Operation complete at 11:45 a.m.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult Heart Rate</th>
<th>Transplanted Heart Rate</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/29/51</td>
<td>1:00 p.m.</td>
<td>148</td>
<td>144</td>
<td>103.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3:00 p.m.</td>
<td>148</td>
<td>144</td>
<td>101.8</td>
<td></td>
</tr>
<tr>
<td>6/30/51</td>
<td>9:00 a.m.</td>
<td>160</td>
<td>148</td>
<td>102.1</td>
<td>Transplant soft, regular.</td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>160</td>
<td>142</td>
<td>102.0</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>150</td>
<td>160</td>
<td>102.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11:30 p.m.</td>
<td>155</td>
<td>160</td>
<td>102.8</td>
<td>Transplant regular.</td>
</tr>
<tr>
<td>7/1/51</td>
<td>10:00 a.m.</td>
<td>162</td>
<td>165</td>
<td>102.5</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>3:00 p.m.</td>
<td>155</td>
<td>160</td>
<td>102.5</td>
<td>Transplant fast, soft.</td>
</tr>
<tr>
<td></td>
<td>9:00 p.m.</td>
<td>160</td>
<td>160</td>
<td>102.3</td>
<td></td>
</tr>
<tr>
<td>7/2/51</td>
<td>10:00 a.m.</td>
<td>160</td>
<td>160</td>
<td>104.0</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>3:00 p.m.</td>
<td>165</td>
<td>164</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:00 p.m.</td>
<td>160</td>
<td>158</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>7/3/51</td>
<td>9:00 a.m.</td>
<td>160</td>
<td>150</td>
<td>102.4</td>
<td>Regular.</td>
</tr>
<tr>
<td></td>
<td>11:00 a.m.</td>
<td>160</td>
<td>172</td>
<td>102.2</td>
<td>Fast, regular.</td>
</tr>
<tr>
<td></td>
<td>3:00 p.m.</td>
<td>130</td>
<td>165</td>
<td>102.6</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>128</td>
<td>144</td>
<td>102.8</td>
<td>Region swollen, oedematous.</td>
</tr>
</tbody>
</table>
### Post Mortem Examination

Gross: Heart appeared normal, no clots in vessels or heart cavities. Adhesions from surrounding tissues to the pericardium.

Pathology Laboratory Report: Degeneration, necrosis and inflammatory cell infiltration, especially in the myocardium.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult Heart Rate</th>
<th>Transplanted Heart Rate</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/4/51</td>
<td>10:00 a.m.</td>
<td>140</td>
<td>150</td>
<td>104.0</td>
<td>Stitches removed. Serum drainage.</td>
</tr>
<tr>
<td></td>
<td>11:00 a.m.</td>
<td>140</td>
<td>150</td>
<td>104</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>3:00 p.m.</td>
<td>120</td>
<td>160</td>
<td>103.6</td>
<td>Fast, feeble, regular. Heart stopped.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>128</td>
<td>160</td>
<td>103.2</td>
<td>Heart beat 124 hours (5 days, 4 hours).</td>
</tr>
</tbody>
</table>
Protocol number 3.

Date: June 25, 1951.

**ADULT**

Breed or Description: Hound, male, young.
Initial Weight: 50 pounds.
Condition: Good. Temperature: 102.4°.
Anaesthesia: Ether.

**PUP**

Breed or Description: Mongrel Collie, female, age - eight weeks.
Initial Weight: 2495 grams.
Anaesthesia: Nembutal intraperitoneally.

**MEDICATION**

Adult: 300,000 units of penicillin daily for 3 days before operation.
Pup: 300,000 units of penicillin daily for 3 days before operation.
Post-operatively: 300,000 I.U. of crystalline penicillin in wound.
300,000 I.U. of penicillin-G in oil daily.

Pup was given blood transfusions from the adult in 30 cc. amounts intraperitoneally daily, for 6 days, before the operation.

Operation commenced at 9:30 a.m. Operation complete at 12 noon.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult Trans-</th>
<th>Heart Rate</th>
<th>Heart Rate</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/25/51</td>
<td>12:00 noon</td>
<td>170</td>
<td>164</td>
<td>100</td>
<td>Under anaesthesia.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>160</td>
<td>132</td>
<td>104.4</td>
<td>Panting.</td>
<td></td>
</tr>
<tr>
<td>6/26/51</td>
<td>8:00 a.m.</td>
<td>120</td>
<td>160</td>
<td>102.8</td>
<td>Regular.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>100</td>
<td>160</td>
<td>102.2</td>
<td>Penicillin administered.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2:00 p.m.</td>
<td>92</td>
<td>144</td>
<td>102.2</td>
<td>Slightly swollen.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>88</td>
<td>144</td>
<td>102.8</td>
<td>Slightly swollen.</td>
<td></td>
</tr>
<tr>
<td>6/27/51</td>
<td>8:00 a.m.</td>
<td>120</td>
<td>140</td>
<td>104.2</td>
<td>Swollen.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>132</td>
<td>138</td>
<td>104.0</td>
<td>Penicillin administered.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2:00 p.m.</td>
<td>144</td>
<td>132</td>
<td>103.8</td>
<td>Refused food.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>132</td>
<td>144</td>
<td>103.5</td>
<td>Refused food.</td>
<td></td>
</tr>
<tr>
<td>6/28/51</td>
<td>8:00 a.m.</td>
<td>120</td>
<td>120</td>
<td>103.4</td>
<td>Diarrhoea. Penicillin administered.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12:00 noon</td>
<td>88</td>
<td>100</td>
<td>103.2</td>
<td>Refused food.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>108</td>
<td>88</td>
<td>102.8</td>
<td>Refused food.</td>
<td></td>
</tr>
<tr>
<td>6/29/51</td>
<td>8:00 a.m.</td>
<td>120</td>
<td>94</td>
<td>103.0</td>
<td>Penicillin administered.</td>
<td></td>
</tr>
</tbody>
</table>
POST MORTEM EXAMINATION

Gross: Left ventricle contracted. Extensive adhesions of pericardium to surrounding tissues. Venous and arterial anastomosis patent.

Pathology Laboratory Report:
Right Ventricle: There were inflammatory changes in the epicardium with quite marked changes in the myocardium. There was degeneration, necrosis and inflammatory cell infiltration with round cell neutrophiles and fibrous tissue proliferation.

Left Ventricle: Some inflammatory cell infiltration between the muscle fibres. The muscle fibres appeared essentially normal.
Protocol number 4.

Date: August 24, 1951.

**ADULT**
Breed or Description: Mongrel Collie, male, old.
Initial Weight: 36 pounds.
Condition: Good. Temperature: 101.5°.
Anaesthesia: Ether.

**PUP**
Breed or Description: Mongrel Collie, female, age - six weeks.
Initial Weight: 1700 grams.
Condition: Good. Temperature: 100.5°.
Anaesthesia: Nembutal intraperitoneally.

**MEDICATION**
Adult: 300,000 I.U. penicillin daily for 3 days before operation.
Pup: 300,000 I.U. penicillin daily for 3 days before operation.
Post-operatively: 300,000 I.U. penicillin, crystalline, in wound.
300,000 I.U. penicillin-G in oil daily.

Pup was given blood transfusions from adult, daily, in 30 cc. amounts for six days preoperatively.

Operation commenced at 9:30 a.m. Operation completed by 11:45 a.m.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult Heart Rate</th>
<th>Transplanted Heart Rate</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/24/51</td>
<td>1:00 p.m.</td>
<td>200</td>
<td>160</td>
<td>103.5</td>
<td>Under anaesthesia.</td>
</tr>
<tr>
<td>3:00 p.m.</td>
<td></td>
<td>168</td>
<td>152</td>
<td>104.0</td>
<td>Recovering.</td>
</tr>
<tr>
<td>5:00 p.m.</td>
<td></td>
<td>156</td>
<td>156</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>8/25/51</td>
<td>8:00 a.m.</td>
<td>128</td>
<td>160</td>
<td>102.0</td>
<td>Heart regular, strong.</td>
</tr>
<tr>
<td>11:30 a.m.</td>
<td></td>
<td>128</td>
<td>160</td>
<td>102.0</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td></td>
<td>128</td>
<td>160</td>
<td>102.2</td>
<td></td>
</tr>
<tr>
<td>8/26/51</td>
<td>10:00 a.m.</td>
<td>108</td>
<td>108</td>
<td>102.5</td>
<td>Slight swelling, refused food.</td>
</tr>
<tr>
<td>4:00 a.m.</td>
<td></td>
<td>88</td>
<td>88</td>
<td>103.0</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td>8/27/51</td>
<td>8:00 a.m.</td>
<td>100</td>
<td>60</td>
<td>102.0</td>
<td>Skips beats occasionally.</td>
</tr>
<tr>
<td>12:00 noon</td>
<td></td>
<td>100</td>
<td>92</td>
<td>102.2</td>
<td>Skips beats occasionally.</td>
</tr>
<tr>
<td>2:00 p.m.</td>
<td></td>
<td>104</td>
<td>88</td>
<td>102.0</td>
<td>Skips beats occasionally.</td>
</tr>
<tr>
<td>3:00 p.m.</td>
<td></td>
<td>100</td>
<td>92</td>
<td></td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td>5:00 p.m.</td>
<td></td>
<td>100</td>
<td>80</td>
<td></td>
<td>Skips beats occasionally.</td>
</tr>
</tbody>
</table>
Protocol number 4. (continued)

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Adult Heart Rate</th>
<th>Transplanted Heart Rate</th>
<th>Temperature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/28/51</td>
<td>8:00 a.m.</td>
<td>100</td>
<td>80</td>
<td>--</td>
<td>Slight swelling.</td>
</tr>
<tr>
<td></td>
<td>11:00 a.m.</td>
<td>100</td>
<td>56</td>
<td>101.8</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>2:00 p.m.</td>
<td>100</td>
<td>68</td>
<td>101.5</td>
<td>Stitches removed.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>100</td>
<td>68</td>
<td>101.0</td>
<td>Heart slow, strong, irregular.</td>
</tr>
<tr>
<td>8/29/51</td>
<td>9:00 a.m.</td>
<td>85</td>
<td>85</td>
<td>101.5</td>
<td>Skipped beats.</td>
</tr>
<tr>
<td></td>
<td>2:00 p.m.</td>
<td>104</td>
<td>74</td>
<td>--</td>
<td>Dog weak, not eating.</td>
</tr>
<tr>
<td></td>
<td>4:00 p.m.</td>
<td>108</td>
<td>64</td>
<td>--</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td>8/30/51</td>
<td>3:00 p.m.</td>
<td>102</td>
<td>88</td>
<td>101.5</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td>8/31/51</td>
<td>9:00 a.m.</td>
<td>92</td>
<td>84</td>
<td>101.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2:00 p.m.</td>
<td>104</td>
<td>82</td>
<td>--</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>4:30 p.m.</td>
<td>96</td>
<td>92</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>9/1/51</td>
<td>9:00 a.m.</td>
<td>108</td>
<td>88</td>
<td>--</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>3:00 p.m.</td>
<td>110</td>
<td>84</td>
<td>101.5</td>
<td></td>
</tr>
<tr>
<td>9/2/51</td>
<td>9:00 a.m.</td>
<td>101</td>
<td>82</td>
<td>101.7</td>
<td>Strong beat.</td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>110</td>
<td>88</td>
<td>--</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td>9/3/51</td>
<td>9:00 a.m.</td>
<td>120</td>
<td>78</td>
<td>101.6</td>
<td>Penicillin administered.</td>
</tr>
<tr>
<td></td>
<td>5:00 p.m.</td>
<td>130</td>
<td>70</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>9/4/51</td>
<td>8:00 a.m.</td>
<td></td>
<td></td>
<td></td>
<td>Heart stopped.</td>
</tr>
</tbody>
</table>

POST MORTEM EXAMINATION
Heart excellent attachment. Recent hemorrhage into the pericardial sac. Anastomosis intact. No clots in vessels or in cavities of heart. Musculature appeared normal.

Pathology Laboratory Report:
Myocardium: The lesions were chiefly those of cell infiltration, the nature of which was both mononuclear and neutrophilic.
SUMMARY AND CONCLUSIONS

1. This thesis presents the technique for the transplantation of a mammalian heart by a direct vascular anastomosis.

2. Reduction in the bacterial invasion, and in the proliferation of bacteria already present in the transplanted hearts' tissue by the use of adequate amounts of penicillin preoperative to the adult and the pup, and post-operative to the adult, appears to increase the active life span of the transplant.

3. Establishment of an adequate coronary blood pressure appears to be of prime importance in cardiac resuscitation.

4. The administration of transfusions preoperative from the host to the donor of the heart appears to have increased the life span of the transplanted hearts.

5. Due to improved surgical techniques and penicillin, transplanted hearts have been kept actively beating for up to 245 hours, with the average remaining active for 129 hours.

6. The average rate of pulsation of the transplanted hearts was 135 times per minute.

7. There may be a correlation between the size of the heart and the tendency for fibrillation to occur when the circulation is restored.
BIBLIOGRAPHY


(3) Blumenthal, H. T. Organismal differentials and leukocytes in circulating blood. Arch. of Path., 1939, 27, 510.


(10) Crossen, R. V. The attraction of lymphocytes by homeotoxins. Arch. of Path., 1928, 6, 396.


Figures 3 to 10 include a series of photographs that were prepared during and following the operations, and are reproduced on the following pages with an explanation.

A series of photomicrographs were also made and a set of these plates are reproduced, showing the extent of cellular invasion and degeneration of muscular tissue in a number of transplanted hearts.

Figures 11 and 12 are photomicrographs of tissue under high and low power from the normal heart of an eight week old pup. These figures can be used for comparison with Figures 13 to 24.
FIG. 3: Shows the chest of the pup opened and retracted. The heart appears in the central with lungs enveloping it. The thymus is retracted to the right and some of the vessels have been ligated.

FIG. 4: Shows the heart upon removal from the chest of the pup with the lungs still attached. The anterior vena cava, posterior vena cava, the vena azygos veins, the aorta, brachiocephalic and subclavian arteries have all been ligated. The ligature on the subclavian artery was not cut and can be seen extending to the left in the photograph.
FIG. 5: Shows the heart with pericardial sac intact. The lungs' roots have been tied individually and removed. A ligature is attached to the subclavian artery in the photograph.

FIG. 6: Shows the same heart anastomosed to the carotid and jugular of the adult dog. The carotid, subclavian anastomosis is in the foreground. The pulmonary artery, jugular anastomosis is in the background.
FIG. 7: Shows a transplanted heart immediately after the serrefines were removed from the vessels connecting it to the host. The jugular vein in centre background can be seen to be full of blood and the heart itself is swollen and turgid. Immediately after this picture was taken the ventricles commenced to beat.

FIG. 8: Shows the same heart 240 hours after transplantation. The heart had ceased to beat, the host was anaesthetised and the skin incised over the transplant. The fibrous tissue adhesions were broken away from the pericardial sac and the organ was removed. In the photograph, the fibrous tissue can be seen on the pericardial sac and in the neck region of the dog. The arterial anastomosis is intact and can be seen above the heart in the region of the incision.
FIG. 9: Shows a dog with a heart transplanted into its neck region. There is evidence of slight oedema at the base of the heart. This particular heart beat for 98 hours and the photograph was taken at the end of the third day.

FIG. 10: Shows a dog with a heart transplanted into its neck region. There is evidence of slight oedema at the base of the heart. This particular heart beat for 123 hours and the photograph was taken at the end of the second day.
FIG. 11 shows a low power section (X250) and FIG. 12 shows a high power section (X500) of the right ventricle of a normal eight-week old healthy pup.

Cross and transverse striations are visible. The muscle is seen to be composed of parallel-coursing main fibres, anastomosing with each other by somewhat thinner oblique-running branches. The large ovoid granular nuclei are situated in the centre of the fibre.
FIG. 13 shows a low power section (X250) and FIG. 14 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 123 hours.

The pup and the adult received penicillin therapy before transplantation and the penicillin was given daily to the host until the transplanted heart ceased to beat.

Pathology. The essential lesions in the sections were areas of degeneration and necrosis with an inflammatory cell infiltration centred mainly in the epicardium. There appeared to be a proliferation of connective tissue and an increased number of macrophages was noticed.
FIG. 15 shows a low power section (X250) and FIG. 16 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 124 hours. The pup and the adult host received no medication, either before or after the operation. The operation was performed aseptically.

Pathology. On gross examination, the pericardium was greatly thickened and the myocardium of both ventricles was pale. At the apex of the left ventricle there were two nodules and at this point the pericardium was adherent. The nodules may have been abscesses. Microscopically there was a marked proliferative pericarditis. There was myocarditis, the inflammatory cells were chronic in nature and there was beginning degeneration of the muscle fibres.
FIG. 17 shows a low power section (X250) and FIG. 18 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 154 hours.

The pup before transplantation received transfusions of blood from the adult to which its heart was to be transplanted. The pup and the host received penicillin preoperative and the host following transplantation received penicillin daily.

**Pathology.** There were inflammatory changes in the epicardium with quite marked changes in the myocardium. There were areas of degeneration, necrosis and inflammatory cell infiltration, with round cell, neutrophile and fibrous tissue proliferation. The left ventricle showed some inflammatory cell infiltration between the muscle fibres but the muscle fibres themselves appeared essentially normal.
FIG. 19 shows a low power section (X250) and FIG. 20 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 240 hours. The pup and the adult host received penicillin preoperative and the host following transplantation received penicillin daily.

Pathology. There was a fibrinous pericarditis with an infiltration of lymphocytes and neutrophils. The myocardium was diffusely affected. The muscle fibres in some areas were widely separated with an exudate containing both lymphocytes and neutrophils. In other areas muscle fibres had undergone degeneration and necrosis.
FIG. 21 shows a low power section (X250) and FIG. 22 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 245 hours.

The pup before transplantation received transfusions of blood from the adult to which its heart was to be transplanted. The host following transplantation received penicillin daily.

Pathology. The lesions were chiefly those of cell infiltration, the nature of which was both mononuclear and neutrophilic. The nuclei of the muscles appear healthy. The longitudinal and cross-striations in the muscle fibres are still quite apparent.
FIG. 13 shows a low power section (X250) and FIG. 14 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 123 hours.

The pup and the adult received penicillin therapy before transplantation and the penicillin was given daily to the host until the transplanted heart ceased to beat.

Pathology. The essential lesions in the sections were areas of degeneration and necrosis with an inflammatory cell infiltration centred mainly in the epicardium. There appeared to be a proliferation of connective tissue and an increased number of macrophages was noticed.
FIG. 15 shows a low power section (X250) and FIG. 16 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 124 hours. The pup and the adult host received no medication, either before or after the operation. The operation was performed aseptically.

Pathology. On gross examination, the pericardium was greatly thickened and the myocardium of both ventricles was pale. At the apex of the left ventricle there were two nodules and at this point the pericardium was adherent. The nodules may have been abscesses. Microscopically there was a marked proliferative pericarditis. There was myocarditis, the inflammatory cells were chronic in nature and there was beginning degeneration of the muscle fibres.
FIG. 17 shows a low power section (X250) and FIG. 18 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 15.4 hours.

The pup before transplantation received transfusions of blood from the adult to which its heart was to be transplanted. The pup and the host received penicillin preoperative and the host following transplantation received penicillin daily.

**Pathology.** There were inflammatory changes in the epicardium with quite marked changes in the myocardium. There were areas of degeneration, necrosis and inflammatory cell infiltration, with round cell, neutrophile and fibrous tissue proliferation. The left ventricle showed some inflammatory cell infiltration between the muscle fibres but the muscle fibres themselves appeared essentially normal.
FIG. 19 shows a low power section (X250) and FIG. 20 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 240 hours. The pup and the adult host received penicillin preoperative and the host following transplantation received penicillin daily.

Pathology. There was a fibrinous pericarditis with an infiltration of lymphocytes and neutrophils. The myocardium was diffusely affected. The muscle fibres in some areas were widely separated with an exudate containing both lymphocytes and neutrophils. In other areas muscle fibres had undergone degeneration and necrosis.
FIG. 21 shows a low power section (X250) and FIG. 22 shows a high power section (X500) of the right ventricle of a heart that had beat following transplantation for 245 hours.

The pup before transplantation received transfusions of blood from the adult to which its heart was to be transplanted. The host following transplantation received penicillin daily.

Pathology. The lesions were chiefly those of cell infiltration, the nature of which was both mononuclear and neutrophilic. The nuclei of the muscles appear healthy. The longitudinal and cross-striations in the muscle fibres are still quite apparent.
FIG. 23: Shows a low power section (X250) at the point of anastomosis of the subclavian artery to the carotid artery of the host. The vessels were united and the heart beat for 245 hours.

Pathology. The anastomosis was intact and the silk fibres from the suture material did not appear to be producing an extensive cellular reaction. The surrounding tissues showed areas of degeneration and necrosis with an increase in the number of connective tissue cells.

FIG. 24: Shows a low power section (X250) of the right ventricle of a transplanted heart that beat for 96 hours. The section showed degeneration and necrosis of muscle fibres in some areas with inflammatory cell infiltration of both neutrophiles and lymphocytes.