LATENT LIFE OF ARTERIES.

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PLATES XL-XLIII.

I. INTRODUCTION.

A tissue is in latent life when its metabolism becomes so slight that it cannot be detected, and also when its metabolism is completely suspended. Latent life means, therefore, two different conditions, unmanifested actual life and potential life. Unmanifested actual life is a normal stage of the evolution of all organisms, when they progress from general death toward elemental death. As the metabolism is still going on, although very slowly, it is a temporary condition. Sooner or later cadaveric lesions develop which bring about the complete disintegration of the protoplasm. Potential life consists of a suspension of all actual vital processes. The metabolism being suppressed, no cadaveric changes would take place in the protoplasma. Tissues in a condition of potential life could be preserved outside of the body for an indefinite period of time.

Since the discovery by Loewenhoek, many observers have studied the conditions and the nature of latent life. Their work was of great biological and metaphysical importance, but it did not seem that their observations would ever be of practical interest. Nevertheless, it happens to-day that the evolution of surgery leads to the use of latent life in human therapeutics. In several kinds of operations on blood-vessels, nerves and even the ureter, the graft of vascular segments can be employed. Human vessels must be used, because autoplastic and homoplastic transplantations are more successful than heteroplastic transplantations. Autoplastic grafting of arteries is not possible, since every large artery is necessary to the organism. Homoplastic grafting only can be used. The graft must be taken from an amputated limb, or from

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the fresh cadaver of an executed criminal or of a man killed by accident. But such an opportunity will certainly not occur precisely when a graft is needed for an operation. The grafts, therefore, must be kept in storage and be ready for use when necessary. Such a preservation of vessels outside of the body can be obtained by placing them in a condition of latent life.

What form of latent life can be used for preserving tissues of mammals outside of the organism? Although unmanifested actual life is only a slow progress towards elemental disintegration, it would be of practical value. It would permit us to preserve the tissues for some time in such a condition that they could be revived if given back their normal physico-chemical conditions. A tissue, immediately after its extirpation from the body or after death, is still living. Its metabolism is going on slowly, while the microbian and autolytic fermentations begin which sooner or later bring about elemental death. There is a period during which the cadaveric lesions are still so slight that they can be interrupted, if the tissues are placed again in their normal condition. The length of the period intermediate between general death and protoplasmic destruction varies according to the nature of the tissues, the temperature and a great many other factors. It is well known that epidermis or periosteum can be kept for a long time outside of the body and be grafted successfully. Sixty years ago, Paul Bert (1) showed that the transplantation of rats' tails, preserved for several days in a small quantity of confined air, at a temperature not higher than +12° C., gave positive results. Lately, Jolly (2), examining a few drops of triton's blood, which had been preserved for fifteen days in cold storage and then put back at normal temperature, saw the red blood corpuscles undergoing indirect division. Ehrlich (3) has demonstrated that pieces of tumor which have been frozen for a long time grow again when transplanted. Preservation of arteries in unmanifested actual life would not be perfect, because it retards only and does not suppress the occurrence of cadaveric lesions. It may be, nevertheless, of practical value in surgery.

The ideal method would be to place the vessels in a condition of potential life. The metabolism being completely stopped, the duration of the period of preservation could be indefinite. Many
physiologists do not admit the possibility of the complete suspension of metabolism. They believe that when Doyère (4) dried a rotifer, heated it at 100° C., and revived it afterward, or when Paul Bert grafted successfully upon a rat a rat’s tail which had been dried in vacuum and heated at 100° C., the metabolism of these tissues had not been interrupted. It seems, nevertheless, that under certain conditions metabolism can be suspended. Becquerel (5) has preserved seeds for months in a vacuum, at temperatures as low as — 190° C. and — 253° C., and germination occurred afterwards. It seems reasonable to believe that protoplasm can be placed in such a condition that life does not exist actually, but merely potentially. Gautier thought that protoplasm can remain lifeless until it is given its normal physico-chemical condition, like a clock waiting in absolute immobility for the motion which starts its mechanism. Potential life can probably exist in seeds. But it is improbable, although not impossible, that tissues of mammals can be placed in a condition of potential life without undergoing a fatal disintegration.

I began in 1906 some experiments on the preservation of arteries in cold storage (6). It was found immediately that a dog’s carotid artery could be preserved outside of the body at a low temperature for several days and transplanted without suffering degeneration of its muscle fibers. Afterwards, I studied more extensively the different methods which can be used in the preservation of vessels in latent life and their biological and surgical results.

II. TECHNIQUE.

The experiments were performed on dogs. The animals were etherized not only during the operation but also during the shaving and the preparation of the skin and the post-operative examination of the vessels. They were operated on by the methods used on human patients.

Each experiment was composed of four stages: extirpation of arteries, preservation, transplantation and examination of the results.

1. Extirpation of Arteries.—Segments of common carotid artery were extirpated from medium-sized dogs during life or a short
time after death by ether or chloroform. The arteries were rapidly exposed, dissected, resected and cut in several pieces about three or four centimeters long. They were then washed in Locke's solution and placed in sterilized glass tubes. The extirpation and the handling of the vessels were made under rigid asepsis.

2. Preservation of Arteries.—In a first set of experiments, the arteries were killed in order that the evolution of dead arteries and of arteries in latent life could be compared. In a second set, it was attempted to preserve the arteries by freezing and drying. In a third set the arteries were merely preserved in cold storage.

(a) Preservation of Dead Arteries.—The arteries were killed by being placed into a sealed tube with a few drops of Locke's solution and heated at 80° C. for ten minutes, or by being immersed in glycerin, or in a 2/100 solution of formalin. Afterwards, the formalined vessel was washed in a diluted solution of ammonia and in Locke's solution.

(b) Preservation of Arteries by Freezing or by Drying.—The tubes containing the arteries were placed in a refrigerator, the temperature of which was oscillating a little above and below — 3° C. The vessels were kept at this temperature for several days. A few hours before the transplantation, the tubes were removed from the first refrigerator and put into another one about at the freezing point. Two hours after, they were taken to the laboratory at a temperature of about + 29° C.

In the preservation by drying, the arterial segments were sealed in a test tube, the bottom of which was filled with calcium chloride. After a few hours, the arteries shrank and became yellowish and, finally, hard and dried like a strand of catgut. They could be preserved in that condition for an indefinite period of time, at the temperature of the laboratory or in cold storage. In one experiment, the tube was placed for several minutes in boiling water. Two hours before the transplantation, the arteries were removed from their tubes and placed in Locke's solution. Progressively, they were imbibed by the fluid, the lumen became visible, the yellowish color disappeared, and after an hour or an hour and a half, they had regained their normal appearance. From a gross anatomical standpoint, they looked exactly like vessels freshly extirpated from an animal.
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(c) Preservation of Arteries in Cold Storage.—The arteries were placed in glass tubes filled with salt solution, Locke's solution, humid air, vaselin, serum or defibrinated blood. The tubes were sealed and deposited in cold storage. Until March, 1908, the temperature of the refrigerator was often a little above the freezing point, but was also oscillating from $-2^\circ$ or $-3^\circ$ to $+12^\circ$ or $+15^\circ$. The apparatus was unreliable. Since March, 1908, I have used an ice box, the temperature of which is constantly between $0^\circ$ and $+1^\circ$ C.

3. Transplantation of the Arteries.—The arterial segments were removed from the tubes, washed in Locke's solution, and soaked in vaselin. One of the carotid arteries of a dog was dissected and cut, and the preserved segment interposed between and sutured to its ends by the ordinary method. Rigid asepsis was employed in these operations. The degree of asepsis varied according to different changes in the organization of the laboratory. From October, 1906, to October, 1908, the degree of asepsis was generally high. From October, 1908, to January, 1909, it was lower and some non-suppurative infections were observed. After January, 1909, asepsis became excellent.

4. Examination of the Results.—The results were examined clinically and anatomically some time after the operation. After a few weeks or a few months the neck of the dog was reopened, the vessel was dissected and the conditions of the circulation were examined. It is important to observe the transplanted segment while the blood is circulating. Its morphology is often different, whether it is functioning or not. Some of the animals were examined in that manner several times. When the time which had elapsed since the operation was considered sufficient, the specimen was removed and examined from a gross anatomical standpoint. Sections of the transplanted arteries were fixed in Zenker's fluid, embedded in paraffin, sectioned and stained with hematoxylin-cosin, Weigert's elastic tissue stain, and with von Gieson's method, when necessary.

III. EXPERIMENTS.

The experiments are divided in three groups: (a) transplantation of dead vessels, (b) transplantation of frozen or dried vessels, (c) transplantation of vessels preserved in cold storage.
(a) Transplantation of Dead Vessels.—Three experiments were performed. The arterial segments were killed by formalin, glycerin and heat.

**Experiment 1. Artery Killed by Formalin.**—Small brown dog, No. 313. November 4, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on November 2, 1908, put in a 2/100 solution of formalin for two days; washed in a weak solution of ammonia, in Locke’s solution, and placed afterwards in vaselin. Microscopical examination.—Section 141. Artery normal. November 12. No pulsations. Opening of the neck. Obliteration. Marked reaction of the connective tissue. Microscopical examination.—Section 166 (Plate XL, Fig. 1). No intima. Media: elastic fibers stretched, no muscular nuclei, a few debris of nuclei in outer layers of the media. Adventitia very much thickened.


**Experiment 3. Artery Killed by Heat.**—Very young white and black male dog, No. 309. October 28, 1908. Transplantation on the right carotid artery of a segment of carotid, extirpated from a dog on October 26. The segment was placed in a sealed tube, the atmosphere of which was humidified by a few drops of Locke’s solution, and then placed in cold storage. On October 27, the tube was placed in hot water for 8 minutes, during which time the temperature went from 85° C. to 70° C. Afterwards it was deposited again in cold storage. The examination made on October 28 showed the artery small and retracted, and the muscle nuclei darkly stained and deformed. November 12, 1908. Dissection of the artery. No pulsations in the transplanted segment. Obliteration. Adhesion to the vagus. Marked reaction of the connective tissue. Resection of the artery. Microscopical examination.—Section 161. Artery dilated. No intima; lumen filled with thrombus. Media: elastic fibers stretched, broken and partly destroyed. Muscle fibers have disappeared. However, a few debris of nuclei are seen in the outer layers of the media. Adventitia very much thickened.

(b) Transplantation of Dried and Frozen Arteries.—Four transplantations of dried arteries and two transplantations of frozen arteries were performed.

**Experiment 4. Dried Artery.**—Dog No. 315. November 5, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on October 26. Immediately after extirpation, the artery was put in a
sealed tube half filled with calcium chloride. After a few hours, the artery became dry and assumed the appearance of a piece of catgut. It was then preserved at the temperature of the laboratory. On November 4, the tube was broken and the artery placed in a tube filled with slightly alkaline Locke’s solution. On November 5, the vessel was found with its normal appearance, color and consistency. It was put in normal Locke’s solution, in vaselin and grafted into the dog. November 12. Dissection of the carotid. Reaction of the connective tissue around the vessel. Obliteration. Microscopical examination.—Section 163. No intima; lumen filled with organized thrombus. Media: elastic frame-work normal. No muscular fibers. Adventitia thickened and infiltrated by leucocytes.

Experiment 5. Dried Artery.—Yellow, male, middle-aged bull, No. 320. November 11, 1908. Transplantation on the left carotid artery of a segment of carotid extirpated from a dog on November 5. The segment has been desiccated in a tube of calcium chloride and placed for one hour in Locke’s solution just before the transplantation. It resumed its normal appearance. After transplantation and re-establishment of circulation the vasa vasorum are immediately injected with blood. Normal color, caliber and consistency. Histological examination.—November 25. No pulsation on the left side. November 30. Opening of the neck. Obliteration of the transplanted segment. Microscopical examination.—Section 184. Lumen filled with thrombus. Intima present in some places and very much thickened. Media dissected in several places by infiltration of red blood corpuscles. Elastic frame-work normal. In many places, the muscular fibers have completely disappeared. In one point of the media, close to the adventitia, a number of muscular fibers are still normal. Adventitia a little thickened.

Experiment 6. Dried and Heated Artery.—Yellow, male, middle-aged bull, No. 320. November 11, 1908. Transplantation on the right carotid of a segment of carotid artery extirpated on November 5, 1908, desiccated in a tube of calcium chloride, heated for 12 minutes at 100° C., and placed for one hour in Locke’s solution, just prior to the transplantation. Microscopical examination.—Section 154. Artery apparently normal. But under high power, muscle fiber nuclei appear to be deformed. November 25. Normal pulsations. November 30. Opening of the neck. Normal pulsations. Normal appearance of the vessel. No modification of caliber of the transplanted segment. Extirpation of the upper part of the segment and re-establishment of the circulation by a segment of jugular vein. Microscopical examination.—Section 185. No intima. Media: thinner than normal, elastic fibers normal, no muscular fibers. However, a very few elongated nuclei are seen in the more external layers, close to the adventitia. Adventitia exceedingly thickened, about twice and three times thicker than the media, according to the location.

lesions to the vagus. The anastomoses are easily seen as very narrow brownish lines. Transplanted segment has same color, caliber and consistency as normal carotid. 

Microscopical examination.—Section 236. Intima as thick as the media in some points, composed of layers of connective tissue cells with elongated nuclei and infiltrated by a great many exceedingly thin elastic fibers, especially in its inner parts. Media: interna elastica normal (Plate XL, Fig. 2), no muscular fibers. However, a few elongated muscles are seen between the outer elastic layers. Adventitia a little thickened.

Experiment 8. Frozen Artery.—White and black male dog, No. 149. July 11, 1907. Transplantation on the carotid artery of a segment of carotid extirpated from a dog on July 8. The segment was put in a refrigerator at the temperature of --3° C. immediately after the extirpation. It was removed from the refrigerator on July 11 at 6 A.M., and put into a refrigerator at a temperature of +1° C. At 9:10 A.M. it was brought to the operating room at a temperature of 29° C. July 16. No pulsations. Neck opened and artery dissected. Obliteration of the lower anastomosis. Upper anastomosis normal. Wall of the transplanted segment apparently normal. Microscopical examination.—Section 51. Transverse section at the level of the middle part of the transplanted segment. No intima. Adventitia very much thickened. Around small pieces of silk, very large foci of leucocyte infiltration. Media deeply modified. In the internal portion all the muscular fibers have disappeared. Between the laminae of the elastica, a few leucocytes are seen. In the outer layers of the media, a few deformed or uniformly stained muscle cell nuclei still persist. Longitudinal section at the level of the upper anastomosis. The carotid of the host is almost normal, although its intima is infiltrated by leucocytes. Between the ends of the normal and transplanted carotids, there is a large scar. All the muscle fibers of the transplanted carotid have disappeared. A few leucocytes and debris of muscle fibers are seen between the elastic tissue fibers. Typical non-suppurative infection.


(c) Transplantation of Arteries Preserved in Cold Storage.—
The arteries kept in cold storage were preserved in different media. The experiments are divided in several classes, according to the nature of the fluid.

In three experiments the vessels were kept in normal saline solution or in Locke's solution.

Experiment 10. Salt Solution.—White bitch, No. 5. October 31, 1906. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on October 30. The segment was preserved into a tube of 10/1000 sodium chloride solution, and placed in cold storage. The temperature was irregular, a little above the freezing point. December 4. Dissection of the
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artery. Normal pulsations on and above the transplanted segment. Adhesions to the vagus. Resection of the segment. The lumen is slightly enlarged, the wall thickened and the anastomoses normal. Microscopical examination.—Section 5. Adventitia thickened. Media: muscular fibers of the inner layers of the media have completely disappeared. Between the elastic laminae, which are normal, there are a number of mononuclear leucocytes. The middle and outer layers of the media are composed of elastic laminae and of muscular fibers. In the middle part of the media, the nuclei of the muscular fibers are deformed and surrounded by a few leucocytes. No intima. A thin coat of fibrin is adherent to the interna elastica (Plate XLI, Fig. 3).


In five experiments, the arteries were kept in sealed tubes, the atmosphere of which was humidified by a small quantity of Locke's solution.

Experiment 13. Confined Humid Air.—Yellow, male cur, middle-aged, No. 208. January 14, 1908. Transplantation on the right carotid artery of a segment of carotid artery extirpated from a white bull bitch, No. 204, on January 10, 1908, and kept in a sealed glass tube, the atmosphere of which was humidified by a few drops of Locke's solution. The tube was put into a refrigerator, the temperature of which oscillated between 0° and about 10° C. February 20, 1908. Dissection of the carotid. Normal pulse. Dog sent to the farm. May 25, 1909. Dog normal. July 22, 1909. Killed in a fight with other dogs. Microscopical examination.—Section 244. Upper part of the trans-
planted segment. Intima very much thickened. In one place, it is almost as thick as the media. It consists of two coats of connective tissue cells, the internal coat being composed of transverse fibers, the external of longitudinal fibers. In the inner part of the intima, several layers of elastic fibers have developed. Media: elastic fibers normal, but more closely distributed than normally. A few nuclei of muscle fibers in the outer layers of the media. The largest part of the media is completely deprived of muscle fibers. The inter-
elastic spaces are filled with amorphous substance. Adventitia thickened.

Experiment 14. Confined Humid Air.—White and black young male fox terrier, No. 216. January 24, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on January 10, from Dog No. 204. Microscopical examination.—Section 75. Many muscular fibers, nuclei deformed and uniformly stained. November 14, 1909. Dog in excellent condition. Dissection of the right carotid. Slight adhesions to the vagus. Normal pulsations. Caliber of the carotid normal. By a close examination, the transplanted segment can be found. The location of the anastomoses can also be detected. Resection of the carotid. The transplanted segment becomes immediately very apparent because it does not contract itself, while the normal part of the carotid does. Internal surface of the transplanted segment and anastomoses are smooth and glistening. Microscopical examination.—Section 257. Intima is composed of dense connective tissue and is very much thickened. In a few places it is thicker than the media. Media is composed of its normal elastic framework and of an amorphous substance. All the muscle fibers have disappeared. Adventitia thickened.

Experiment 15. Confined humid air.—Male bull dog, No. 265. April 1, 1908. Transplantation on the right carotid of a segment of carotid extirpated from a male dog on March 10. Temperature of the refrigerator oscillating between $-2^\circ$ C. and $+10^\circ$ C. May 6, 1908. Dissection of the carotid. Artery is obliterated. Section 102. Lumen of the vessel filled with organized thrombus. Adventitia thick and well vascularized. Media has kept its normal thickness. Elastic fibers and interna elastica normal. Nuclei of muscular fibers normal. Many of them have still their vesicular appearance and their regular shape. Some others are irregular.

Experiment 16. Confined Humid Air.—White, female fox terrier, No. 267. April 2, 1908. Transplantation on the left carotid, of a segment of carotid extirpated from a male dog on March 10. April 9. Dissection of the artery. Normal pulse. Normal size and consistency of the transplanted segment. Its caliber is slightly larger than normal. October 15, 1908. Dog chloroformed. Dissection of the carotid arteries. Arteries are absolutely normal. No evidence of the previous operation. Anastomosis cannot be located. After longitudinal opening of the vessel, it is possible to locate the anastomosis and the transplanted segment, which has become absolutely identical with the other parts of the vessels (Plate XLI, Fig. 4). Section 260. Microscopical examination.—Longitudinal section at the level of the upper anastomosis. Intima is very much thickened, almost as thick as the media. It is composed of elongated cells, the nuclei of which assume the appearance of muscle cell nuclei. Media is composed of elastic frame-work, amorphous material and cells with elongated nuclei. Adventitia normal.
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Experiment 18. Confined humid air.—Long-haired, black, male dog, No. 286. May 28, 1908. Transplantation on the left carotid of a piece of dog’s carotid artery, extirpated on March 16, 1908. For several weeks, the temperature of the cold storage was irregular, going below the freezing point from time to time. October 15, 1908. Animal etherized. Carotid artery exposed. No evidence of anastomoses. By a very close dissection of the artery, two very indistinct lines are seen on the surface of the wall. The diameter and the appearance of the transplanted segment are identical with those of the carotid artery. The adaptation of the transplanted segment to the artery is absolutely perfect. The artery is extirpated. As soon as the circulation stops, the limits of the segment become evident, because the artery contracts its wall while the transplanted segment does not. Microscopical examination.—Section 122. Intima very much thickened. At one point, it is thicker than the media. No endothelium, except in a few places. Media thinner than normal. Interna elastica normal. Other laminae are indistinct. Media is composed chiefly of diffuse elastic fibers. No muscular fibers. Adventitia a little thickened (Plate XLII, Fig. 5).

Experiment 19. Confined Humid Air.—White and yellow, male fox terrier, No. 291, October 2, 1908. Graft on the left carotid artery of a segment of dog’s carotid artery, extirpated on March 16, 1908. The segment is a little modified. Its wall is softer and thinner than usual. It looks like a venous wall. Nevertheless, after reestablishment of the circulation, the vasa vasorum are immediately injected with blood. They are larger than normal without lateral hemorrhages. October 5. Neck of the dog enlarged. Incision. Hematoma. Ablation of the clots. Large hemorrhage. Slight necrosis of the wall near the upper anastomosis. Ligature of the carotid and extirpation of the transplanted segment.

Experiment 20. Confined Humid Air.—Long-haired yellow dog, No. 293. October 3, 1908. Transplantation on the carotid of a segment of dog’s carotid extirpated on March 16. October 15. Segment very adherent to the vagus. Distended and obliterated. The wall is dark, but has kept in some places its normal appearance. The wall is necrosed. The vessel is extirpated and the animal recovers.

debris of nuclei. No wandering cells. On the external side of the adventitia, connective tissue of the host forms, in some places, a coat infiltrated with leucocytes.


Experiment 25. Confined Humid Air.—Small-sized, yellow dog, No. 302. October 21, 1908. Transplantation of the left carotid of a segment of dog's carotid extirpated on September 29, 1908. Microscopical examination.—Section 125. No intima. Media: elastic fibers normal. Nuclei of the muscular fibers retracted. Many of them are uniformly stained and have lost their vascular appearance. November 25, 1908. Dissection of the artery. No adhesions. No reaction of the connective tissue. Normal circulation. Extirpation of the segment, which is harder and whiter than normal. Microscopical examination.—Section 180. Adventitia normal. Intima very much thickened and composed of elongated connective tissue cells. Media partly composed of an amorphous substance, highly refractive, which is broken in several places. This substance is disposed in two distinct bands. Between them, there are some normal muscular fibers.

Experiment 26. Confined Humid Air.—Yellow and white, young, male dog, January 5, 1909. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on February 4, 1909. The color of the segment is yellowish. It flattens more easily than normally. After reestablishment of the circulation, the vasa vasorum are immediately filled with blood. January 6, 1909. Dog is found dead. Autopsy: diffuse broncho-pneumonia.
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Experiment 27. Confined Humid Air.—Yellow, male dog, No. 326. November 25, 1908. Transplantation on the left carotid artery of a segment of carotid artery extirpated from a living young dog on November 9, 1908. January 21, 1909. Opening of the neck. Transplanted segment has same caliber and consistency as normal vessel. Its color is a little whiter. Normal pulse. Extirpation of a piece of transplanted segment and end to end suture of the artery. Microscopical examination.—Section 131. Media thinner. Adventitia and intima very much thicker. Adventitia formed of two parts: internal, composed of irregular connective and elastic fibers; external, composed of regularly disposed large connective tissue fibers. Media composed of elastic fibers of normal appearance but more closely distributed on account of the disappearance of almost all the muscular fibers, which are replaced by an amorphous substance. In the outer and inner layers of the media, a few muscular fibers can be detected. Some nuclei are normal, some others have lost their vesicular appearance, or are broken and very irregular in shape. Intima elastica normal. Intima very much thickened and covered with endothelium. Lumen of the vessel about same size as before transplantation. October, 1909. Opening of the neck. Normal circulation. Dog sent back to farm.

In ten experiments, the arteries were preserved in defibrinated blood or serum.


Experiment 30. Defibrinated Blood.—Yellow, black and white dog, No. 378. January 26, 1909. Transplantation on the left carotid of a piece of carotid preserved in defibrinated blood since January 22, 1909. Dog is sent to the farm a few days after the operation. Died of pneumonia on February 15, 1909. Macroscopical examination.—No reaction of the connective tissue. No adhesions to the vagus. Caliber, color and consistency of the transplanted segment normal. Microscopical examination.—Section 216. No intima. Media: elastic framework normal. The muscle fibers of the inner layers have disappeared. The fibers of the middle and outer layers are normal. Adventitia a little thickened.

vagus. Extirpation. Transplanted segment similar to the other parts of the carotid. Microscopical examination.—Section 265. No intima. Media: abnormally thin and composed of elastic fibers and amorphous substance. All muscle fibers have disappeared. Adventitia very much thickened.

Experiment 32. Defibrinated Blood.—Small, black and tan, male dog, No. 392. February 5, 1909. Transplantation on the left carotid of a segment of carotid preserved in defibrinated blood since January 22, 1909. Microscopical examination.—February 5, 1909. Section 212. Some muscle fiber nuclei uniformly stained and deformed. October 18, 1909. Dog died at the farm. Microscopical examination.—Dissection of the artery. Transplanted segment of normal caliber. Slight adhesions to the vagus. Consistency of the wall harder. Its color is modified by the presence of yellow and hard patches. Anastomoses smooth and glistening. The patches are located between the media and the intima. The wall is thickened, but the lumen of the transplanted segment and of the anastomoses is not modified. Microscopical examination.—Section 253. Adventitia normal. Media: elastic framework normal. In some parts it is very thin on account of the complete disappearance of the muscular fibers. It is composed only of the elastic framework and an amorphous substance. In some other parts, there are still some elongated cells between the elastic laminae. The intima is exceedingly thickened. It is, in certain parts, five or six times thicker than the media and is composed of dense connective tissue. In the middle of the adventitia, the connective tissue has undergone calcareous degeneration.


Experiment 34. Defibrinated Blood.—Brown dog, No. 408. February 17, 1909. Transplantation on the left carotid of a segment of carotid preserved for 48 hours in defibrinated blood and suprarenal. October 16, 1909. Dog killed in fighting other dogs. Microscopical examination.—No evidence of the operation. No reaction of the connective tissue. No modification of caliber, color and consistency of the transplanted segment which cannot be located. Carotid artery is extirpated and longitudinally opened. The location of the segment is detected by a slight difference in the color of the intima which is a little yellowish. Microscopical examination.—Section 226. Adventitia normal. Media: elastic framework normal. No muscular fibers. Intima thickened and composed of dense connective tissue.

marked adhesions of the transplanted segment to the vagus. Segment normal, although its caliber is slightly larger than that of the carotid. Anastomoses perfect. Microscopical examination.—Section 235. No intima. Media: normal elastic framework. No muscular fibers, although very few elongated cells are seen between the elastic laminae. Adventitia very much thickened and composed of dense connective tissue. In several places the adventitia is thicker than the media and contains a great number of small vessels.

Experiment 36. Defibrinated Blood.—Black and tan bitch, No. 419. March 2, 1909. Transplantation on the left carotid of a segment of carotid extirpated on February 4, 1909. November 14, 1909. Dissection of the left carotid. The location of the transplanted segment can hardly be determined. The carotid has everywhere the same appearance and consistency. Resection of the artery. As soon as the circulation is stopped, the location of the segment becomes apparent, because it contracts less than the normal parts of the artery (Plate XLIII, Fig. 7). Microscopical examination.—Section 259. Intima a little thickened and containing a few elongated nuclei, assuming the appearance of muscle cells nuclei. Media: elastic framework normal. No muscular cells in the outer layers of the media. Cells with elongated nuclei in the middle and inner layers. Adventitia normal.


In four experiments, the vessels were preserved in vaselin.

Experiment 38. Vaselin.—Male dog, No. 450. May 6, 1909. Transplantation on the left carotid of a segment of carotid artery preserved in vaselin and in cold storage for 24 hours. August 11, 1909. Dog killed in a fight. Microscopical examination.—Segment is obliterated by a clot of recent formation adherent to the wall at the level of its middle part. Anastomoses normal and free of clot. Microscopical examination.—Section 254. Intima slightly thickened. Media: elastic framework normal. Muscle fibers, nuclei normal. However, in a few places, between the two or three inner layers of the elastic fibers, they are missing. Adventitia normal.


IV. RESULTS.

It must be known, from these experiments, whether an artery can be preserved outside of the body in a condition of latent life, and whether a preserved vessel can play safely the rôle of an artery. The results, therefore, will be examined from both standpoints, biological and surgical.

(a) Biological Results.—It is impossible to know directly whether or not a tissue which has been preserved for a few days or a few weeks in cold storage is living. There is no chemical reagent of life. The morphology of an artery gives little indication on its biological condition. A pig’s carotid artery, which had been kept for six months in cold storage, had the same histological appearance as a fresh artery. There is no morphological difference between a living tissue and a dead tissue. What difference can be detected between the seed which is sterile and the seed which will produce a large tree? The only method of finding out the state of a tissue which is supposed to be in latent life is to replace it under normal physico-chemical conditions and to observe whether it will again manifest life. If two vascular segments which have been preserved in cold storage and assumed the same histological appearance are transplanted into a normal artery, and if after a few weeks one has retained its normal constitution while the other one has degenerated, it can be concluded that the first one was really in latent life while the second one was dead. However, the persistency of the elastic framework and of the connective tissue coats alone of the transplanted segment would not warrant the assumption that the vessel was still living at the time of the trans-
plantation. In such a case, the arterial wall might be possibly a mere outgrowth of the connective tissue from the host, the transplanted segment acting as a scaffold. But the presence of muscle fibers can demonstrate that the wall of the transplanted vessel is still present and is not merely formed by the tissues of the host.

The anatomical change undergone by dead vessels when grafted to a normal artery are shown by the experiments in which arterial segments killed by heat, formalin and glycerin were used. The dead vessels were examined just before being transplanted. The artery killed by formalin was absolutely normal. In the segments killed by heat and glycerin, the muscle cell nuclei were well stained but more or less deformed and retracted. As a whole, the vascular wall had kept an almost normal appearance. These vessels were transplanted into normal arteries and examined again a short time after the operation. In every case, a complete change had taken place. The segment, killed by formalin, spent eight days on the carotid of a dog. During this time, all the muscle fibers of the media disappeared (Fig. 1) and were replaced by amorphous material, while the elastic framework remained normal. The perivascular connective tissue of the host was very much thickened and was building a new wall around the dead structure. The artery killed by glycerin underwent similar changes. All the muscle fibers had disappeared eight days after the operation. Fourteen days after the transplantation, the media of the artery killed by heating was composed of stretched and broken elastic fibers, and of amorphous material, while the adventitia was thickened. These results are similar to those obtained previously by Levin and Larkin (7) in the transplantation of devitalized arterial segments.

These experiments show that a dead vessel undergoes, when transplanted, a rapid degeneration of all its muscle fibers (Fig. 1), while the elastic framework can remain normal. The change takes place in less than eight days. The host reacts against the dead vessel by forming around it a tube of dense connective tissue. A devitalized vessel acts as a foreign body, which can be progressively resorbed and replaced by the tissues of the host.

The arteries which were preserved by freezing behaved like dead
vessels. In Experiment 8, the transplanted segment was examined five days after the transplantation. All the muscle fibers had already disappeared and were replaced by amorphous material. The second experiment was also unsuccessful. These negative results do not mean that frozen arteries cannot be revived. It is well known, since the work of Ehrlich, that tumors, which have been frozen, can grow again normally. Other causes than freezing and especially non-suppurative infection may be responsible for the failure of my two experiments.

The results given by the arterial segments preserved by desiccation are very much better. Four experiments were performed. After having been in cold storage for a few hours in a sealed tube filled with calcium chloride, the arteries shrank and became brown and hard like a strand of catgut. In Experiment 6, the tube was then heated at 100° C. for ten minutes. Afterwards, the arteries were put in Locke’s solution and regained their normal appearance. Their anatomical condition was practically normal. Nevertheless, they behaved differently after having been transplanted. In Experiment 4, all the muscle fibers, without exception, had disappeared seven days after the operation. The anatomical changes undergone by the vessel resembled very much those of vessels killed by formalin or glycerin. In Experiment 7, where the vessel had been dried, and in Experiment 6, where it had been dried and heated at 100° C., the results were different. The media was almost entirely composed of elastic fibers and amorphous material six months after the operation in the first case (Fig. 2), and nineteen days after the operation in the second case. Nevertheless, in the outer layers of the media, a few elongated nuclei, morphologically similar to muscle cell nuclei, could be detected. In Experiment 5, nineteen days after the operation, there were, in the outer layers of the media, a large number of normal muscle cell nuclei. It shows that some muscle cells had not been killed by the desiccation. This method may perhaps be improved and give better results. It is well known that in the drying of rotifers, a very slight difference of technique modifies widely the results. When a rotifer is dried on a glass plate, it cannot be brought back to life. If it is dried in sand, the resurrection is almost constant. The
rapidity of the desiccation plays doubtless an important rôle in the production of irreparable injuries in the architecture of the tissues of the cells. The fact that nineteen days after the transplantation of an artery which has been dried, many muscle fibers are still normal, shows that desiccation does not kill necessarily the tissues of the artery.

The results of the transplantation of vessels preserved in cold storage are divided in several classes, according to the medium in which the tissues were kept.

In three experiments, the arteries were placed in Locke's solution or in a 10/1000 solution of sodium chloride. In Experiment 10 the artery, kept for twenty-four hours in saline solution, was exirpated thirty-five days after the operation. The muscle fibers of the inner layers of the media had disappeared, but elsewhere they were normal (Fig. 3). The artery used in Experiment 11 was kept six days in saline solution. Forty-four days after the transplantation, the muscle fibers were still in excellent condition. In spite of the toxic action of the sodium ions for the tissues, an artery can be preserved for twenty-four hours and six days in physiological saline solution without being killed. In Experiment 12 a segment of artery was kept for nine days in Locke's solution, and then transplanted into a dog. The result was examined fourteen months after the operation. All the muscular fibers had degenerated.

In fifteen experiments the arteries were preserved in confined humid air. After a period varying from two days to eleven months, they were removed from their sealed tubes and transplanted. When they were examined before the transplantation, their appearance was generally found normal. Even after several months, the gross anatomy of the vessels was very little modified. The histological examination showed that they were often normal.

The results of the experiments are divided in four classes. The first class is composed of Experiments, 19, 20 and 26, in which the vessels were preserved from seven to eleven months outside of the body. Necrosis occurred soon after the transplantation. The second class consists of Experiments 14, 16, 18, 21 and 25 in which the muscle fibers degenerated completely, and were replaced by an
amorphous material (Fig. 5), while the elastic framework remained normal. Compensating changes took place in the adventitia and the intima. Therefore, these vessels behaved very much like vessels killed by heat, formalin or glycerin. The period of preservation of the vessels outside of the body had lasted from fourteen to seventy-two days. The examination of the transplanted vessel was performed from six days to six months after the operation. The third class is composed of Experiments 13, 15, 24 and 27. The arteries were partially killed during the period of preservation, which lasted from two days to twenty-one days. The results of the transplantation were examined from eight days to eighteen months after the operation. Some muscle fibers were still normal even after eighteen months. In the fourth class, composed of Experiments 17, 22 and 23, the media was found to be normal. The arteries had been preserved for two days, seven days, and seventeen days outside of the body. The results of the transplantation were examined twenty-one days and fifteen days after the operation. The muscle fibers had remained normal (Fig. 6).

The marked differences observed in the results are due to several different causes. It is probable that the changes of temperature, the slight infection occurring during the period of preservation and after the transplantation caused many of the lesions found in the vessels. Several of the experiments were made when the temperature of the refrigerator was very inconstant and when the asepsis of the laboratory was doubtful. The preservation in confined humid air is not alone responsible for the observed degenerative changes of the arterial wall.

The preservation of arteries in serum or defibrinated blood was used in ten experiments. The period of preservation lasted from two to thirty-one days. The results were examined from fifteen days to nine months after the operation. In Experiment 28 the histological examination was not made. In Experiment 37 the transplanted vessel was resorbed. The results of the eight remaining experiments can be divided in two classes according to the conditions of the muscle fibers. In two experiments, 29 and 30, where the vessels were preserved for two days and four days, the muscle fibers remained normal. In the six other experiments
all the muscular fibers had disappeared. The elastic framework was normal and the muscular fibers were replaced by amorphous material. In the conditions of my experiments, serum or defibrinated blood acted as defective preservative media and their results were inferior to those of confined humid air.

The results of the four experiments of preservation of vessels in vaselin were very much better, although in Experiment 39, the artery became occluded and atrophied. The accident was due probably to a fault in the technique of the anastomoses and not to the method of preservation. In Experiments 38, 40 and 41, the vessels were kept in vaselin for twenty-four hours, fourteen days, and twenty-three days. In Experiment 38, the artery was examined and the specimen removed three months after the operation. A great many muscular fibers were normal. In the intima there were elongated nuclei which resembled muscle cells nuclei. The results of Experiment 40 were examined five months after the operation. A great many normal muscle cells were found in the media and in the intima. The muscle cells of the outer layers of the media had degenerated (Fig. 8). In Experiment 41, almost all the muscle fibers of the media had disappeared, six months after the operation. A few only were seen in the inner layers of the media. But the intima is thickened and contains many muscle fibers. In these experiments the preservation of the muscle fibers depends on the duration of the period of preservation outside of the body. It must also be noticed that the degenerated fibers are located in the outer part of the media, while they are in its inner part in the other methods, and also that they proliferated into the thickened intima.

All preservative media, salt solution, Locke's solution, confined humid air, defibrinated blood and vaselin permit the vessel to live in latent life under certain conditions, but vaselin appears to be the best medium.

(b) Surgical Results.—The practical value of a vessel preserved in a condition of latent life depends on its ability to act as a canal for the arterial blood. The only complication which interfered occasionally with the function of the transplanted vessel was thrombosis. The method used in the preservation of the vessel
has a marked influence on the occurrence of obliteration. When
the arteries were killed by heat, formalin and glycerin, thrombosis
occurred always. The results were a little better when the arteries
were frozen or dried. In six operations, two positive results only
were observed. In the experiments where the arteries were pre-
served in salt solution, Locke’s solution or confined humid air, the
results were very much better. If we eliminate three experiments
in which the vessels were kept for more than six months in cold
storage, we find ten positive results for fifteen operations. The
vessels preserved in defibrinated blood gave eight positive results
in ten experiments, and the vessels preserved in vaselin, three posi-
tive results for four experiments.

Negative results were observed after the transplantation of
devitalized arteries. In every case, the segment was found after
a few days, occluded by a thrombus. Levin and Larkin (7) also
observed frequently the same accident. Nevertheless, thrombosis
is by no means a necessary consequence of the graft of a dead
artery. It is certain that the circulation can take place normally
through an inert structure for some time at least. Once I per-
formed the patching of the abdominal aorta of a dog with a piece
of rubber, and the circulation went on normally. Guthrie (8)
claims to have observed a normal circulation through a formalined
vessel. Levin and Larkin found that ten days after an aortic
graft, the circulation was still normal. The artery acts as a foreign
body around which the organism builds a new wall of connective
tissue (Fig. 1). On the intima of the dead vessels, the blood de-
posits a thin layer of fibrin, and it is possible that the dead structure
is progressively resorbed and replaced by the connective tissue of
the host. The devitalized artery is a resorbable foreign body.
Nevertheless, it must be emphasized that it is a dangerous method.
It can yield occasionally some good results. But immediate or
secondary thrombosis occurs very much more often than in the
case of transplantation of a living structure.

The results of the transplantation of frozen or dried vessels are
better. Frozen vessels were transplanted twice and twice throm-
bosis occurred a short time after the operation. In four experi-
ments of graft of dried vessels, thrombosis took place twice. The
two positive results were found nineteen days and six months after the operation. In Experiment 6, an artery which had been dried and then heated at 100° C. for twelve minutes was transplanted. Nineteen days after the operation, the neck was opened and the transplanted segment was found to have the same appearance, caliber and consistency as the carotid. The circulation was normal. As the muscular fibers of the media had degenerated, the vessel had adapted its wall to the pressure by increasing its adventitia which was two or three times thicker than the media.

In Experiment 7, the result of the transplantation of a dried vessel was examined six months and eighteen days after the operation. The grafted segment assumed the same color, caliber and consistency as the other parts of the carotid. The morphologic and functional adaptation of the vessel was excellent. The media was weakened by the almost complete disappearance of the muscle fibers. But the elastic framework of the media was still normal, while a great many small elastic fibers had developed in the intima, which was very much thickened (Fig. 2). These experiments show that perfect functional results can be obtained by transplanting dried arteries. But the method is not safe, since thrombosis occurred twice in four experiments. It is possibly due to the technique I used. It would be necessary to repeat the experiments with a better method, and to try to preserve the dried vessels at a very low temperature, as Becquerel did with seeds. If tissues of mammals could be placed in potential life, it would be the ideal condition for the preservation of vessels outside of the body.

The results of the transplantation of arteries preserved in cold storage were examined from a few days to eighteen months after the operation. The proportion of positive results was 66 per cent. when the vessels were kept in Locke's solution and confined humid air, the experiments in which they were preserved outside of the body for more than six months not being counted. The proportion became 75 and 80 per cent. when the arteries were preserved in defibrinated blood or in vaselin.

The only complication which occurred was thrombosis. There were no aneurysms nor dilatation of the transplanted segment. In Experiment 19 a hemorrhage due to necrosis of the segment was
observed. The artery had been preserved for seven months in cold storage. The method cannot be expected to prevent the disintegration of a tissue after such a long time. Thrombosis is really the only complication to be feared. It is difficult to know its causes. Certainly, in several cases, thrombosis was not due to the method of preservation but merely to a fault in the technique of the suture, or to non-suppurative infection. It must be noticed that thrombosis occurred mainly during the period where the asepsis of the laboratory was doubtful. The percentage of positive results can certainly be increased. Most of the dogs were medium and small sized animals. By using larger vessels, for instance, vessels like the human humeral artery, better results could be obtained. Nevertheless, the method of preservation has certainly a marked influence. It seems that vaselin or defibrinated blood are the best media.

The function of the transplanted segment remained normal, even in the cases where the wall has undergone marked histological changes. The new artery adapted itself exactly to the vessel on which it was grafted. The union became so perfect that after a few months, it was difficult to find the location of the transplanted segment. In several experiments, the dissection of the carotid artery did not permit the locating of the graft (Figs. 4 and 7). It was necessary to open the vessel longitudinally in order to see the small linear scars or the faint change of color which were the only evidences of the operation. As soon as the vessel was empty of blood, the transplanted segment could be located easily. When the circulation stopped, the normal wall contracted itself, while the transplanted wall generally did not. The new segment appeared then as a slightly dilated portion of the vessel. In Experiment 16 a segment of carotid had been transplanted on a dog, after having been preserved in confined humid aid and in cold storage for twenty-two days. Six months after the operation, both carotid arteries were dissected. No evidence of the operation was seen. Both carotids had the same color, caliber and consistency. The transplanted segment had become absolutely identical to the normal parts of the carotid. Nevertheless, after the opening of the vessels, it became a little larger than the normal parts of the artery
and could be exactly located. The new arterial segment adapts itself, from a morphological standpoint, to the functioning artery.

In two experiments only the arterial wall underwent pathological modifications. Calcification and atheromatous patches, almost identical to the lesions observed in human arteriosclerosis, developed in its wall. But compensatory changes occurred and the caliber of the vessel was not appreciably modified.

The functions and the macroscopical appearance of the transplanted vessels did not depend at all on its histological architecture. For instance, in Experiment 18 (Fig. 5) the artery, five months after the transplantation, did not show any evidence of the operation. In Experiment 40 (Fig. 8) the artery, five months after the operation, was also normal. The only difference was that in Experiment 40 the wall of the transplanted segment contracted itself slightly after the stopping of the circulation, while in Experiment 18 it did not contract at all. Nevertheless, from a histological standpoint, the differences between these vessels were exceedingly marked. In Experiment 40, the wall was almost normal, although the muscle fibers of the outer layers of the media had disappeared. Compensatory change had taken place in the intima which was thickened and infiltrated by muscle fibers. In Experiment 18, the muscle fibers had been destroyed. The wall was composed only of an almost normal elastic framework and of connective tissue. But compensatory changes, such as thickening of the intima and of the adventitia had taken place, and the resistance and consequently the caliber of the vessel was not modified.

In many other cases the same modifications occurred. When the media was weakened by partial or complete degeneration of the muscular fibers, the wall increased its strength by thickening its intima, or its adventitia, or both media and adventitia. When the lesions of the media were more extensive in one point, the compensatory changes of the intima were marked at the same point. The compensatory changes consisted mainly of a sclerosis of the intima or of the adventitia. Very seldom, new elastic fibers developed in the intima. In the arteries preserved in vaselin, the muscle cells invaded the intima. These changes are adaptive changes which enable the weakened wall to resist the blood pres-
It is the experimental demonstration of the theory advocated by Adami (9) in the development of arteriosclerosis. The thickening of the intima or of the adventitia was surely a compensatory change due to the weakening of the media. When the media was normal, no sclerosis of the other coats took place. The degree of sclerosis was proportional to the extent of the lesions of the media.

Not a single instance of arterial dilatation was observed. If the wall is composed of normal tissue, it reacts against the blood pressure by thickening itself. The function redintegrates quickly the weakened vessel. But its morphology is reestablished only in the measure necessary to the function.

V. CONCLUSION.

When a segment of artery, killed by heat, formalin or glycerin is transplanted, it undergoes a rapid degeneration. Its muscle fibers disappear while the tissue of the host reacts by building a new wall of connective tissue. When the transplanted vessel has been preserved in a condition of latent life, no degeneration of the wall occurs, or the wall undergoes only partial degeneration. The muscle fibers can keep their normal appearance, even for a long time after the operation. It is, therefore, demonstrated that arteries can be preserved outside of the body in a condition of unmanifested actual life.

The best method of preservation consists of placing the vessels, immersed in vaselin, in an ice box, the temperature of which is slightly above the freezing point.

From a surgical standpoint, the transplantation of preserved vessels can be used with some safety. When the arteries were kept in defibrinated blood or vaselin and in cold storage, the proportion of positive results was 75 and 80 per cent., and this can probably be increased.

EXPLANATION OF PLATES.

PLATE XL

Fig. 1. Formalinized carotid artery. Eight days after transplantation. Experiment 1. Section 166. Hematoxylin-cosin.

Fig. 2. Carotid artery preserved by drying. Six months after transplantation. Experiment 7. Section 236. Weigert's elastic tissue stain.
Latent Life of Arteries.

PLATE XLI.

Fig. 3. Carotid artery preserved for 24 hours in salt solution and in cold storage. Thirty-five days after transplantation. Experiment 10. Section 5. Hematoxylin-eosin.

Fig. 4. Carotid artery preserved for 22 days in confined humid air and in cold storage. Six months after transplantation. Experiment 16.

PLATE XLII.

Fig. 5. Carotid artery preserved for 72 days in confined humid air and in cold storage. Five months after transplantation. Experiment 18. Section 122. Hematoxylin-eosin.

Fig. 6. Carotid artery preserved for 2 days in confined humid air and in cold storage. Fifteen days after transplantation. Experiment 23. Section 162. Hematoxylin-eosin.

PLATE XLIII.

Fig. 7. Carotid artery preserved for 26 days in defibrinated blood and cold storage. Eight months after transplantation. Experiment 36.

Fig. 8. Carotid artery preserved for 14 days in vaselin and in cold storage. Five months after transplantation. Experiment 40. Section 252. Hematoxylin-eosin.

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