CONCERNING VISCERAL ORGANISMS.*

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In previous articles[1] it has been shown that a fragment of heart pulsates normally for more than 100 days after its extirpation from the animal, and that connective tissue can be kept indefinitely proliferating *in vitro*. We have now observed strains of connective tissue cells that are still multiplying actively after more than sixteen months of life outside the organism. But up to the present only fragments of tissue have survived *in vitro*. Since the survival of entire organs outside of the body would undoubtedly have important physiological uses, I began in June, 1912, to develop a technique by means of which a system of organs could be made to live and functionate when separated from the other organs. Within a few months this technique has been sufficiently perfected to permit the separated organs being put to practical use for the study of several problems.

The method consists in removing aseptically the abdominal and thoracic organs of an animal and in preserving the organs in an incubator at a temperature of 38° C., while the lungs are being artificially ventilated. The operations were performed on both cats and dogs, but more often on cats. The animals were etherized and killed while they were still under the influence of the ether. Aseptic technique was employed and the same general rules were observed as in complicated thoracic or abdominal operations.

Two animals were used for each operation; the first one was prepared for transfusion, and from the second the viscera were taken.

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[1] Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516; Carrel, A., and Burrows, M. T., in Abderhalden, E., Handbuch der biochemischen Arbeitsmethoden, 1912, pt. v, 836; Ebeling, A. H., *Jour. Exper. Med.*, 1913, xvii, 273.

The first animal was etherized, the hair on the anterior part of the neck was removed with sodium sulphide, and the skin was washed with alcohol and sterilized with iodin. The neck was opened by longitudinal incision and one of the carotid arteries was dissected and opened after the circulation had been interrupted by a *serre fine.* The blood was washed out from the lumen of the vessel with Ringer solution, and the end of a glass cannula about thirty centimeters long was introduced and fixed by means of a circular ligature into the lumen of the artery. The cannula was not paraffined and no oil was used, on account of the danger of fat embolism of the coronary arteries of the second animal. Then the animal was kept etherized until it was time to transfuse the blood to the second animal.

The second animal was etherized, and the skin on the anterior part of the neck, thorax, and abdomen was shaved and sterilized by means of the technique previously described. The operation comprised four stages.

1. A long incision was made in the middle line from the upper part of the neck to the lower part of the abdomen, and the skin was dissected on each side of the line of incision. The trachea was dissected, and the esophagus was isolated, ligated, and cut under aseptic precautions. In some cases a catheter was introduced into the esophagus as far as the stomach, and fixed in such a way as to prevent any infection. Then the trachea was cut transversely and a curved glass cannula was inserted into its lumen and fixed by two circular ligatures. A gum catheter was introduced into the glass cannula and the trachea, as far as the bifurcation, according to the Meltzer-Auer method. The end of the catheter was connected with a foot bellows or a small electric blower.

2. The abdominal wall was opened on the middle line from the sternum to the pubis. The abdominal viscera were wrapped in a Japanese silk towel sterilized in vaselin, and were pushed up into the upper part of the abdomen. Branches of the mesenteric artery were ligated and cut, and the small intestine was prepared for section at a short distance from the large intestine. Then it was severed after having been ligated, and was cauterized with carbolic acid. The aorta and vena cava were exposed near the point of their bifur-

cation, ligated, and cut. The ureteral vessels were ligated and the ureters were severed. When the bladder had to be kept the ureters and their vessels were dissected as far as the posterior part of the bladder. The bladder itself was isolated from the surrounding tissues, the urethra was cut, after having been ligated, and the bladder was raised to the level of the kidneys. Then the aorta and vena cava were isolated from the abdominal wall and their posterior branches were tied. The peritoneum surrounding the kidneys was dissected and cut. All the small vessels uniting the aorta and the vena cava to the posterior part of the abdomen were also ligated and cut. The splanchnic nerves were cut and also the pillars of the diaphragm. Then the abdominal viscera, being completely separated from the abdominal wall, were wrapped in a Japanese silk towel. They remained united to the animal by the aorta and vena cava and their circulation was still active.

3. A transverse incision was made in the third or fourth intercostal space. The mammary arteries were clamped. Then the costal cartilages were cut on the right side of the sternum, and the thorax was widely opened. The diaphragm was severed at a short distance from the thoracic wall, and artificial respiration was established. It consisted of a current of air introduced into the trachea by means of a catheter and interrupted about sixteen times every minute. The lungs and the heart were protected by a Japanese silk towel, impregnated with vaselin. Then the innominate arteries or the carotid arteries were ligated and cut. The superior vena cava and the azygos vein were also tied and cut. After a few minutes the animal died, but the heart continued to pulsate. The vagus, sympathetic, and phrenic nerves were severed. Then the pleurae were cut on each side of the thoracic aorta. All the posterior branches of the thoracic aorta were clamped by means of a small Gentile hemostatic forceps and cut close to the thoracic wall. Generally the heart pulsated weakly and the blood pressure was low. The lungs and the abdominal organs were pale and no pulsations could be seen in the small arteries. In some experiments the heart stopped pulsating entirely.

4. Then the thorax and abdominal viscera, united by their blood vessels, were removed from the cadaver of the animal and placed

in a tray containing Ringer solution at a temperature of about 38° C., in such a manner that the lungs floated on the surface of the fluid and the heart was suspended underneath in the liquid. When this position was reversed and the heart was placed above the lungs the circulation was somewhat impaired. The temperature of the Ringer solution was maintained constant by means of an electric pad placed under the tray, or simply by the addition from time to time of Ringer solution at the right temperature. Ordinarily the heart still pulsated slowly and regularly, but the blood pressure was low and the appearance of the organs anemic. After a few minutes the blood pressure began to rise, and in a few cases became almost normal. Generally it remained low and sometimes the heart entirely ceased beating. Then a transfusion was made from the carotid artery of the first animal to the inferior vena cava or abdominal aorta of the visceral organism. When the pressure was very low or when the pulsations of the heart had completely stopped, the transfusion was made directly through the aorta, in order to reëstablish immediately a normal circulation through the coronary arteries. The heart started almost immediately to pulsate normally. As the condition of the heart improved the transfusion was made through the inferior vena cava. It was possible in that way to inject quickly a large quantity of blood into the visceral organism. Immediately after the transfusion the lungs became pink, the heart beat strongly from 120 to 150 times a minute, and the blood pressure often rose above normal. The abdominal aorta pulsated violently and strong pulsations could be seen in the arteries of the stomach, liver, kidney, intestine, and even of the ovaries. Peristaltic contractions of the stomach and of the intestines were observed. The spleen, which was bluish, assumed its normal appearance. After a few minutes all the viscera were apparently normal. Then a careful hemostasis of the posterior branches of the thoracic aorta was made and all the forceps were removed. It was important to ascertain that no hemorrhage, even one from a very small vessel, was taking place. If a few minutes after the transfusion the pressure was still above normal, a certain quantity of blood was allowed to flow from the lower part of the abdominal aorta. Then the appearance of the viscera exactly resembled that of a normal animal.

5. The visceral organism was placed in a tin box filled with Ringer solution, and covered with Japanese silk and protected by a glass cover. The tracheal and esophageal tubes were fastened to proper openings in the anterior part of the box. The intestine was pulled through a glass and rubber tube fixed in the posterior wall of the box. The end of the intestine was fixed by circular suture to the edge of the rubber tube, an artificial anus being made. The box was put into an incubator at a constant temperature of about 38° C. Artificial respiration was carried on by a current of air interrupted about twelve times a minute. The compressed air was furnished by means of an automatic electric apparatus pumping air into a tank, from which it was given to the animal under the proper pressure. Dr. Meltzer kindly examined the respiratory conditions in these experiments and showed me how a proper ventilation of the lungs could be obtained. His advice contributed in a large measure to the rapid success of the work. Water or food could be injected into the stomach through the esophageal tube. The urine could be collected from the bladder through a tube, but it was generally aspirated from the bladder with a needle, when samples of urine were taken for study. The feces and intestinal secretions were received outside of the box from the artificial anus.

It was then observed that during the hours following the operation the viscera had the same appearance as those of a living animal. The contractions of the heart and the circulation of the organs were apparently normal. Pulsations could be seen in the smaller branches of the mesenteric artery. The intestine emptied itself through the artificial anus by means of regular peristaltic contractions. When the intestine was empty, bile and intestinal juices were evacuated. In an experiment in which the stomach was full of meat at the time of death, digestion took place. Dr. Van Slyke found that at least 90 per cent. of the amino acids injected into the intestine were rapidly absorbed. There was also an abundant secretion of urine, which was collected into the bladder. After five or six hours hyperemia of the peritoneum of the intestine appeared. It seemed as though a peritonitis developed progressively, and in some cases the intestines became paralyzed after eight or nine hours, although their

circulation was still very active. Abundant hemorrhage could still be produced by section of a small branch of the mesenteric artery. Some of the visceral organisms died almost suddenly after three or four hours, but most of them were in a normal condition ten and even twelve hours after the death of the animal to which the organs belonged. The death of the organism was announced by some irregularities in the pulsation of the heart, which was also weaker. Then the heart stopped suddenly. In one experiment the death of the visceral organism occurred thirteen and one quarter hours after the death of the cat from which it was taken.

The technique of the operation can be modified in many different ways. The number and the nature of the viscera composing the organism were varied. The intestines, spleen, or kidney can easily be removed. It is also possible to exclude the liver from the circulation by ligation of the portal vein after a lateral anastomosis between the portal vein and the vena cava. The thyroid gland can be kept with its circulation or removed completely. The composition of the organism should be adapted to the problems which are to be studied.

The technique of preserving the organism is far from being definitely settled. The organs were generally kept in Ringer solution. Other kinds of fluid, such as serum or ascitic fluid, can also be used. I attempted to keep the viscera without any fluid at all in fine Japanese silk towels impregnated with vaselin. In this case the organism was put into a bag of very fine rubber, open at each end. Through one end was fixed the tracheal tube and to the other end the anal tube. The sac was then suspended in water. There was less danger of infection, but so far the results have not been as satisfactory as when the organs were placed directly in Ringer solution. In another experiment the entire thoracic cavity was kept. The operation consisted merely of a double amputation, through the abdomen and through the neck. A circular incision was made around the abdomen and the skin was dissected. The abdominal cavity was opened, and the intestine, aorta, and vena cava were ligated and cut. Then the anterior wall of the abdomen was sutured to the posterior wall and the abdominal cavity completely closed. The spinal column was cut

and the skin sutured. Afterwards the head was amputated and the stump was covered with the skin, leaving only an opening for the tracheal tube. A transfusion was made through the lower part of the aorta and the heart went on pulsating almost normally. Then the reduced organism was put into an incubator. It died of emphysema, because the air pressure became too high and produced rupture of the lung.

The technique will probably be progressively modified and adapted to the various problems of pathology, physiology, and biological chemistry, for the study of which the visceral organisms can be used.