

in some communities hideous tales of cruelty, and are leading people to believe that these horrors are typical of what is daily occurring in laboratories. Pictures are published tending to harrow the heart of any one who has in him a love for animals. Meanwhile, nothing is being done to counteract the falsity of these impressions. That medical investigators are also men with tender sympathies and are sensitive to the infliction of pain, that they approach their work with reverence for the wonderful mysteries that every living body reveals, that with a keen sense of human suffering they are patiently searching for new truth in the spirit of far-seeing humanity—these facts are not made known.

The time is approaching, I believe, for this education to be started. The movement should not be confined to the medical profession, but should be taken up as a duty by every intelligent person who believes that any restraint on medical research is a danger to human welfare. Already, in England, a Research Defence Society has been started with Lord Cromer as its president. The purpose of the society is to make known the facts, the facts as to the use of animals in experiments, and the immense importance of these experiments to the well-being of mankind. These facts the society intends to diffuse among the people by published articles and leaflets, by lectures, and by answers to inquiries. Although the society has existed only a few months, it already has hundreds of members, from all departments of public life, representing every class of educated Englishmen and Englishwomen, and including many who have been actively engaged in preventing cruelty to animals.

Thus somewhat tardily in England, after research and teaching have been hampered by restrictive legislation for more than thirty years, it has seemed wise to appeal to the good sense of the people. In this country we have thus far refrained from open opposition to those who are hostile to animal experimentation. But a campaign of education will become imperative, if the attitude of the public continues to be shaped entirely by those who are opposed to medical research. The experience of England is before us. "In the mother-country our hands are tied by an act which was defined by one of the highest legal authorities as a 'penal' act; and though with us, as with others, difficulties may have awakened activity, our science suffers from the action of the state." These were the words of Sir Michael Foster at the Toronto meeting of the British Medical Association; and at the end of a review of biologic progress during the preceding thirteen years, he sounded this note of warning: "Some there are who would go still farther than the state has gone, though that is far, who would take from us even that which we have, and bid us make bricks wholly without straw. To go back is always a hard thing, and we, in England, can hardly look to any great betterment for at least many years to come. But unless what I have ventured to put before you to-day be a mocking phantasm, unworthy of this great association and this great occasion, England in this respect at least offers an example to be shunned alike by her offspring and her fellows."

Against Medical Inspection of School Children.—According to the *Lancet*, the Devon (England) County Council has brought a hornet's nest about its head by adopting the medical inspection of schools. Here is one of the stinging rebukes received by the head mistress of one of the schools: "Dear Madam, I objects to my child being overroled by a doctor. I clears his blood vessels reglar with brimstone and treacle, and he don't want no more doctrine."

Original Articles

FURTHER RESULTS IN SUPRARENAL TRANSPLANTATION.*

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In a former communication¹ one of us reported a series of thirty-two cases in which suprarenal transplantation had been attempted. In this first series, a part of the animal's suprarenal was transplanted to its own kidney. At least one positive functional survival of such a graft was obtained. Partial proof of functioning suprarenal grafts was obtained in several other instances. The indication of successful transplantation, consists in survival of the animal, in the absence of all suprarenal tissue, other than that of the graft, and in the histologic demonstration of a living graft containing medullary cells.

In the present series of thirty cases, transplantations have been made into the thyroids, testes and kidneys of dogs and rabbits, not only in the same animal, but also from one animal to another of the same species. The results of implantation into the testes and thyroids were all negative. Positive results were obtained only in those cases where the kidney was used as the receiving tissue.

DETAILS OF TECHNIC.

The complete experiment of transplantation into the kidney, whether the giving and receiving animal are the same, or the receiving animal is another of the same species, consists of three stages, as follows:

First stage.—One suprarenal gland is removed in its entirety. A sagittal third of this, or of a gland from another animal, is introduced into an opening in the lower pole of the kidney cortex, patterned as nearly as possible after the graft to be implanted. The graft is held in place by means of two or more silk sutures passed over it and through the kidney capsule and cortex, on either side. As a rule, the surface of the graft is brought flush with the kidney surface. By cutting off a thin slice from the lower portion of the graft and from each pole, five raw surfaces are brought into apposition with the fresh surfaces of the kidney wound. Care is taken to prevent the intervention of blood-clot between the surfaces of the graft and the kidney. The kidney is then allowed to drop back into its bed, and the external wound is closed.

Second stage.—A varying period after the first operation, the animal's remaining suprarenal is completely removed. In the absence of accessories or remnants, which may have been overlooked during the first operation, there should be no suprarenal tissue remaining, other than that of the graft. If such is the case and the graft has undergone necrosis, the animal should die of suprarenal insufficiency, unless death occurs earlier through anesthesia, hemorrhage, or shock.

Third stage.—If the animal has survived the second operation for a considerable period, the kidney containing the graft is removed. If other causes of death, such as shock and renal insufficiency can be excluded and death occurs within three or four days, preceded by marked muscular weakness and tremor on exertion, it

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¹From the Physiological Laboratory, University of Buffalo, 1. Am. Jour. Physiol., 1906, xv. No. 5.

is probable that the cause of death is suprarenal insufficiency, and that the graft had been functioning. The proof is completed by failing to find suprarenal tissue at autopsy and by the histologic demonstration of an apparently living graft containing remnants of medulla. Additional proof would be the demonstration of blood pressure raising properties of graft extracts.

PROOF OF FUNCTIONING.

Complete proof of functioning graft survival, as outlined above, we have obtained in two cases. In one of these the rabbit's suprarenal was transplanted into its own kidney. Thirty-six days later, the remaining adrenal was removed, and seventy-seven days after the second operation, the kidney containing the graft was removed. The rabbit recovered rapidly from the immediate effects of the operation and seemed quite normal for the first day. The morning of the second day, the animal appeared apathetic; did not move unless disturbed, but showed no pronounced muscular weakness; did not eat. In the afternoon muscular weakness was more pronounced, and there was slight tremor on exertion. When not disturbed there was still slight tremor of the hind quarters and a gentle swaying motion; dullness and apathy were increased. It was found dead, the following morning, stretched out on side, head thrown back. At autopsy the viscera appeared normal. There was no evidence of peritonitis and no suprarenal accessories or stump could be found.

The left kidney, containing the graft, which was removed at the third operation, was adherent to the omentum over the surface of the graft. These omental adhesions were very vascular. After separation of the adhesions, the graft itself was not distinguishable on the surface, but showed on cross section through the kidney as a dark hyperemic mass with a central yellowish core, about two millimeters in diameter and five millimeters in length. Microscopic examination of the graft, which had been fixed in Zenker's fluid, showed this as an oval mass at the bottom of the kidney wound and consisting of cells which stained well with hematoxylin and eosin. Outside of these and extending toward the external surface there was a mass without definite cellular structure, containing many areas of extravasated blood and occasional islands of what appeared to be cells of the suprarenal cortex. The central cells, as well as several small islands separated from the main mass by new formed connective tissue, appeared to be of the suprarenal medulla type. These latter cells also gave a reaction with chrome salts.

The second case (sixty), in which the three stages were completed, consisted in the transplantation of the suprarenal of one rabbit into the kidney of another. The rabbit's remaining suprarenal was removed thirty-six days after the introduction of the graft. The animal made an uneventful recovery. Twenty-nine days later, the kidney, containing the graft, was removed. Death occurred forty-three hours after the third operation, preceded by symptoms similar to those recorded for the first case.

At autopsy no abnormality was found in any of the viscera and there was no evidence of peritonitis. The abdominal vessels were unusually distended, which was likewise the case in the first experiment reported. No accessory adrenals or adrenal stumps were found. The kidney, which contained the graft, was found, at the time of the last operation, to be rather firmly adherent to the left lobe of the liver. The graft was visible at the

kidney surface as a yellowish body, about two millimeters wide and four millimeters long; the portion imbedded was about two millimeters in its greatest diameter.

Microscopic examination showed the graft as a wedged-shaped mass of cells walled off from the kidney by new-formed connective tissue, but with very little, if any, inflammatory reaction in the adjoining kidney tissue. The cells of the wedge were large in size and spherical to polyhedral in shape, with relatively small nuclei and much cytoplasm. The nuclei stained well with hematoxylin, and the cytoplasm faintly with eosin. It is difficult to identify them with the cells of the normal gland, but from the fact of their reaction to chrome salts and their size and arrangement it seems probable that they are of the medullary type.

Four rabbits survived the third operation—the removal of the kidney containing the graft. These were killed later, and found to possess accessory suprarenals. In two of these cases, living grafts, containing medullary cells, were found. In the third, the graft was partly necrotic and no medullary cells could be demonstrated. In the fourth, there was hydronephrosis and a partly necrotic graft.

One rabbit met with an accidental death through anesthesia, at the third operation, ninety-four days after the introduction of the graft and fifty-one days after the removal of the other suprarenal. At autopsy, no remaining adrenal stump or accessory bodies could be found. The graft itself was living and contained cells of medullary type. In this case, the transplanted suprarenal was obtained from another rabbit.

Another rabbit died through accident at the second operation, thirty-four days after the introduction of the graft. The graft, although partly necrotic, contained some islands of medullary cells.

In still another rabbit death occurred eighteen days after the removal of the second suprarenal. At autopsy no accessories were found, and the graft was necrotic. In this case the graft must have been still functioning at the time of the second operation, the process of necrosis having been completed eighteen days later, at which time the animal died from suprarenal insufficiency.

The best histologic picture of a graft is shown in an animal which died from some undetermined cause, eight days after the primary operation. In this case the cross section of the graft looks like a section through normal suprarenal, distinctly showing all the zones.

Of the remaining cases of this series, some animals died through accident at the primary operation, and the others from suprarenal insufficiency after the second operation.

SUMMARY.

Including the cases of our first report with those recorded here, we believe that satisfactory evidence of functioning suprarenal graft survival has been given in, at least, three cases. One of these consisted in a transplantation from one animal to another. In several other instances where complete proof could not be obtained, there is a strong probability that grafts were functioning. So far as we know these are the first successful cases of suprarenal transplantation with preservation of function.² That the original sympathetic

2. Note made on reading proof: Since the reading of this paper, there has come to our notice a report (Haberer: Arch. f. Klin. Chir., lxxviii, No. 2, 1908), of suprarenal transplantation into the kidneys of dogs and rabbits. In these experiments, however, a portion of the original blood supply was left undisturbed, and there was continuity of tissue between the intra and extra-renal portion of the gland. No adequate evidence of functional graft survival was given.

nerve connections of the gland may be severed without apparent disturbance of function has been shown by the successful cases of transplantation.

The kidney seems to be the best structure for the reception of the grafts. The method of removal of the kidney, with the graft, in the third stage of our experiment, has, we believe, in no instance, been a disturbing factor, since those animals which had accessories survived, with apparently no ill effect, the removal of one kidney.

Whether the graft alone, in any of these cases, would have been sufficient to carry on the suprarenal functions indefinitely, we can not say. The longest period of graft survival which we have recorded is 247 days.

With improved technic and more uniform success in animal experiments, it does not seem unreasonable to attempt suprarenal transplantation as a therapeutic measure in Addison's disease.

In conclusion we wish to thank Dr. James A. King and Dr. W. Ward Plummer for valuable assistance in some of the earlier operations.

DISCUSSION.

DR. W. M. L. COPLIN, Philadelphia: An interesting fact in regard to implantation is the suggestion, which has been accentuated by Dr. Busch's admirable communication, that transplantation in the presence of an adequate, normal suprarenal body, or where suprarenal secretion is sufficient, is followed by hyperplasia of the transplanted suprarenal. A very interesting and highly suggestive phase of this work was brought out two years ago by French workers, during attempts to induce hypernephromata by transplantation of the suprarenals. Some times the graft takes and a tumor results; in other instances no neoplasm is produced, although the transplanted tissue survives; of course, in most cases the graft dies, autolyzes and disappears. When the graft remains and its cells proliferate without evidence of suprarenal intoxication, we at once think of Professor Adami's suggestion that when cells are no longer required to maintain their secretory functions they revert to a more highly manifest hyperplastic and productive activity.

DR. H. G. WELLS, Chicago: In 1900 I performed a small series of experiments with the object of transplanting the suprarenal into the kidney in young guinea-pigs, with the object of seeing if from such misplaced adrenal tissue a hypernephroma might develop, but the results were negative. I never got any tumor formation, and only occasionally evidence of survival of adrenal cells. The results were so negative that they were never published.

DR. A. J. CARLSON, Chicago: Is there any explanation for the fact that the graft takes only in the kidney? Why should it not take in other tissues in the vascular supply?

DR. W. B. CANNON, Boston: Have grafts been placed in the spleen and other organs? Why not make use of the spleen also in these cases?

DR. F. C. BUSCH, Buffalo: The point brought up in regard to hyperplasia is interesting, but one on which I am not competent to speak. In these animals, at the first operation, only one adrenal was removed, a part of this or a similar portion of the gland from another animal, being used for transplantation. The remaining adrenal was sufficient to carry on the function until the second operation, even if the graft was inactive. I have not seen any instance of overgrowth or new growth on the part of the transplanted cells.

It is interesting to note that in those animals in which the graft became necrotic, death occurred soon after the removal of the remaining suprarenal, eight to fourteen hours after the second operation. This was due, I believe, to suprarenal insufficiency, and not to the operative procedure alone. In those subjects surviving the removal of all other adrenal tissue, we have nothing but the graft to account for such survival. After the removal of the graft itself, at the third operation, death occurred later, within two to four days, with progressive

symptoms of suprarenal insufficiency. It would seem that there had been some adaptation of the animal to the small amount of functioning suprarenal of the graft.

The questions of Dr. Carlson and Dr. Cannon as to the reason for success of transplantation into the kidney and failure in other organs, I can not answer definitely. We did not attempt transplantation into the spleen, partly because, in the rabbit, the spleen is much too small for the purpose. The kidney was chosen because of its ease of access, its vicinity to the suprarenal, its vascularity, ease of handling and control of hemorrhage, and because of its close developmental relations to the suprarenal. I have no explanation of the failure of transplantation into thyroids and testes. Survival of suprarenal cortex after transplantation into the testis has been recorded.

The difficulties involved in the three stages of the experiment I have not specifically mentioned. One of these has been the close relation between the adrenal and the vena cava or renal vein, or both. In attempting complete removal of the gland there is great danger of fatal hemorrhage from wounding these veins.

THE PRESERVATION OF ANATOMIC DISSECTIONS WITH PERMANENT COLOR OF MUSCLES, VESSELS, NERVES AND ORGANS, BY A NEW METHOD.

A PRELIMINARY NOTE.*

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Since all times, anatomists have tried to preserve dissections with permanent color of muscles, vessels, nerves and organs, but, as far as I know, they have failed in securing permanency of color.

The specimens I have seen in the northern and European museums have all the tissues bleached.

It is not so very important to preserve permanently the actual color of muscles, which varies from one subject to another, but it is essential that there should exist permanently a marked contrast between the fleshy parts of muscles and the tendons, fasciæ, bones and other white tissues, which must remain as white as possible. A dark brown color of the fleshy parts of muscles is satisfactory. The more red the brown is, the better and the prettier.

The specimens should retain their color at least five or six years or else the result would not compensate for the labor and expense. We have specimens with color that are over eight years old. Since the color has kept that long I believe it will keep indefinitely, provided the solutions are changed as soon as they become cloudy.

In my work I have used no newly-discovered chemical. I employ the old arsenic and alcohol, to which I have added carbolic acid, formalin and glycerin. These are the compounds used in colleges all over the world, but it is the combinations and proportions that make all the difference between success and failure.

I started by determining the properties or action of each combination in all the proportions I could think of and letting them go on to do their best or their worst without interfering with them beyond changing the solutions when they became cloudy or discolored. I also experimented to determine the action on color of the lapse of time when the specimens were placed

* Read in the Section on Surgery and Anatomy of the American Medical Association, at the Fifty-ninth Annual Session, held at Chicago, June, 1908.