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Frank M. Noice
Moorhead State College

REGENERATION STUDIES BY TRANSPLANTATION OF ADRENAL TISSUE

FRANK M. NOICE
Moorhead State College, Moorhead

INTRODUCTION. The regeneration ability of the adrenal gland has been observed and recorded by several investigators; however, reports of the method of regeneration have been conflicting. This study was conducted to attempt to learn more of the method of adrenal gland regeneration through transplantation studies.

The adrenal gland is defined into three primary tissues, the outer enveloping fibro-elastic connective tissue capsule, the cortex and the medulla. The cortex is further subdivided into three zones, according to the arrangement and type of cells.

The outermost region of the adrenal cortex is the *zona glomerulosa* which is composed of cells having a columnar shape with a pale staining basophilic cytoplasm in which are a few droplets of fat. Their nuclei are small, deeply staining and round. The cells are arranged in round or ovoid masses, but do not enclose a lumen.

The middle *zona fasciculata* consists of cuboidal cells arranged in long, parallel columns. The nuclei are larger than in the glomerular zone, two nuclei occasionally being present in one cell. The cells of the fascicular zone are characterized by large amounts of lipid present in their cytoplasm in the form of small droplets. These cells are known as spongiocytes because of the reagent dissolution of the fat droplets during slide preparation giving the cytoplasm a spongy appearance. The *zona fasciculata* comprises the greater part of the adrenal cortex.

The *zona reticularis* is that portion of the cortex which lies nearest to the medulla. It consists of a network of cellular cords formed by the dividing anastomosing branches of the inner ends of the fascicular columns. The reticular zone cells nearest to the fascicular zone are lighter in color and contain fat droplets. However, many of these cells are filled with pigment, which gives them a dark appearance. Light colored cells containing fat droplets may be seen here and there among the pigmented cells.

The medulla occupies a narrow area between the opposing layers of the cortex. It varies considerably in thickness because of the difference in the amount of cellular material which it may contain. Its cells are arranged in masses and cords which form an irregular

network. The cells are characterized by their large vesicular nuclei and by the presence of fine granules in the cytoplasm which have characteristic reaction to the salts of chromic acid, which gives the cells a yellowish or brownish color. The medullary cells are called "chromaffin cells" because of this reaction.

The enveloping capsule is a rather thick, fibro-elastic connective tissue with its inner layer, adjacent to the *zona glomerulosa*, more loosely arranged and more vascular. Slender trabeculae extend from this inner capsule layer radially through the cortex region separating the cortex cells into their characteristic cords or columns.

Many investigators believe that the capsule could give rise to cortical cells. Martinovitch (1) however, obtained evidence both by in vitro and graft studies to show that cortical cells arise only from pre-existing ones.

It has been the authors experience that following the transplantation of whole adrenal glands to host animals, there is an extensive degeneration of the cortical cells except for those cortex cells adjacent to the capsule. These peripheral cortical cells exhibited a rapid proliferation whereas the mitotic figures observed in cells of the capsule were very slight.

Bachmann (2) reported that in the human adrenal there are always some undifferentiated cortical cells within the capsule. These cells appeared to be precursors to cortex cells and could be induced to undergo rapid division by injection of adrenocortiotrophin hormone (ACTH). The author has noted that upon removal and subsequent microscopic examination of the capsule there are invariably numerous cortical-like cells incorporated in the loose, inner layer of capsule tissue. Such tissue has been grown in vitro and by the addition of 25 units/cc. of ACTH to the culture media, the cortical-like cells rapidly proliferate into cortex typical to that of the outer region of the zona fasciculata. Some of these proliferating cells may differentiate into spongiocytes. The connective tissue cells showed no mitotic response to the ACTH.

Hence, it would appear that the cortex cells arise only from surviving cortical cells found within the capsule and not by a transformation of connective tissue cells common to the capsule.

TRANSPLANTATION STUDIES. All the experimental animals used were Guinea pigs of three weeks of age. At this age the adrenal gland is well defined from the perirenal fat and clearly demarked.

Animal Trial 1: By aseptic technique a unilateral adrenalectomy was performed on 12 animals that had been prepared for surgery by a subcutaneous injection of 50 mg. of sodium pentothal per kilo of body weight. Particular care was employed to remove all the adrenal capsule tissue intact with the gland.

The explanted gland was maintained by watch glass technique according to methods of Martinovitch (3). The culture medium employed was Medium, 199; (Morgan, *et. al.*) (4), and was exchanged at five day intervals. After a culture period of 20 days the glands were

replanted into their respective parent animals. The procedure for replanting the adrenal gland was to form a small pocket in the perirenal fat, insert the gland and draw the fat together over the gland with a single gut suture. At the time of replanting the adrenal glands the perirenal fat was carefully examined to determine if there had been any adrenal gland regeneration from remnants remaining after the adrenalectomy 20 days prior. Two of the animals showed such spontaneous regeneration with a small nodule of adrenal tissue having formed and were discarded from the experiment.

At 5 day intervals following the replantation of the adrenal glands two of the test animals were sacrificed and a necropsy was preformed. In both the animals sacrificed 5 days following the replantation, the test gland appeared to have atrophied as compared to the control gland, and the wet weight of the test gland was about half of that of the control gland. The test and control glands were sectioned and stained with hematoxylin-eosin. The *zona fasciculata* of the test gland was not apparent but the *zona glomerulosa* and *zona reticularis* were evident. In the sections of the control gland all three cortical zones were clearly indicated. In animals sacrificed at 10 days all three zones were evident in both test and control glands, as was the case in animals sacrificed at 15, 20 and 25 day intervals. The wet weight of the test gland was not equal to that of the control gland until the 25 day interval. It would appear that even though the adrenal glands were replanted into the same animals from whence they were originally removed, a period of time was required for re-establishment of the tissue, and that this process was at the expense of the *zona fasciculata.* The temporary atrophy may have occurred until vasculation to the replanted gland was accomplished.

Three animals were prepared as in Trial 1 to determine the in vivo effects of ACTH. These animals were given a subcutaneous injection of .50 mg of ACTH daily from the fifth day following the adrenal gland replant to the 15th day, the time of animal sacrifice. The cellular effects of the cortex of both the test and control glands was one of enhancement of the *zona fasciculata.* The *zona glomerulosa* and *zona reticularis* were not evident. The number of spongiocytes seen in the *zona fasciculata* were more numerous than they were in the previous series of animals of this trial that had not been given ACTH. The wet weight of the test gland was still about half that of the control gland, as was the case in the first series of animals of Trial 1.

In an attempt to determine if the trauma of surgery was responsible for cellular effects attributed to ACTH, three animals having no previous treatment of surgery were given .50 mg of ACTH daily for ten days. Sections of the adrenal glands of these animals also revealed the predominance of the *zona fasciculata* and their spongiocytes and the reduction of glomerulosa and reticularis zones.

Animal Trial 2: Attempts to accomplish a transplant of both adrenal glands from a sacrificed donor animal to a bilaterally adrenalectomized host animal was not successful. The transplanted adrenal

glands atrophied and resulted in death of the host animal within 18 days displaying the classical symptoms of adrenal gland failure.

An animal of this trial was sacrificed at 5, 10 and 15 day intervals following the transplant surgery and sections of the adrenal glands were prepared for microscopic examination. In the 5 day post-surgery slides there was no apparent *zona fasciculata* and at 15 days all the cortical zones were absent except for small islands of cells in the outermost *zona glomerulosa*, a few of which were spongiocytes. There was very active cell proliferation of the *zona glomerulosa* immediately adjacent to the capsule in the 5 day slides, much less so in the 10 day slides and in only a few "island areas" in the 15 day slides.

Animal Trial 3: When both adrenal glands from a sacrificed donor were cultured, as previously described in Medium 199 for a period of 15 days, and then transplanted into a bilateral adrenalectomized host the adrenal gland atrophy was delayed. Such host animals survived from 26 to 49 days before death with symptoms of adrenal failure occurred.

As in Trial 2, an animal was sacrificed at 5, 10, 15 and also at 20, 25 and 30 day post-surgery intervals; the adrenal glands were prepared for microscopic examination. The 5 day interval slides showed *glomerulosa* and *reticularis* zones but no *zona fasciculata.* The 10 day slides displayed an active proliferation of peripheral *zona glomerulosa* cells, but no *zona fasciculata* and no noticeable difference in the *zona reticularis* from the 5 day slide. The 15 day slides showed a reduced reticularis and glomerulosa zones and, a narrow region of *zona fasciculata,* using the criteria of the fasciculata as having spongiocytes and forming the zone medial to the *zona glomerulosa.* The 20 day slides indicated no *zona reticularis,* only limited and interrupted areas of spongiocyte fasciculata zone and a decreased *zona glomerulosa.* The proliferation rate of the cortical cells adjacent to the capsule was active in all slides of the 5, 10, 15 and 20 day intervals. The 25 and 30 day slides were much similar to the slides of the 15 day interval of Animal Trial 2, with the cortex being near entirely atrophied except in limited areas adjacent to the capsule.

One animal of this trial did survive for 49 days. The adrenal gland slides showed a similar pattern of atrophy but being extended over a longer period of time.

Animal Trial 4: Both adrenal glands were removed from the donor animals of this trial and cultured as before. The Medium 199 used in culturing the donor adrenal glands in this trial contained 4% serum from the respective animal that was to be the host. Blood was obtained from the host by cardiac puncture. The adrenal glands were thus maintained in vitro with the serum fortified medium being exchanged at five day intervals. After 15 days in the culture medium the donor adrenal glands were transplanted to the bilateral adrenalectomized host. In each of the eight chimeras thus prepared the host animals were compatible to the donor adrenal glands and have survived for seven months, the present date.

A second series of animals thus prepared were sacrificed at 5, 10, 15, 20 and 25 day post-surgery intervals and adrenal gland slides were prepared as before. The 5 and 10 day interval slides were typical to slides prepared of glands from untreated control animals except for the transplanted glands having a reduced *zona fasciculata.* The 15, 20 and 25 day slides showed all three zones typical to the slides of adrenal glands of untreated control animals. There was noticeable increase of proliferation of cortex cells adjacent to the capsule in the 5 and 10 day slides of transplanted glands but not in interval slides thereafter.

CONCLUSIONS.

1. The trauma of transplantation of adrenal glands is first noted by the disappearance of cells of the *zona fasciculata.* This reaction is rapid and cell restoration, provided the tissue atrophy from transplantation is not progressive, is gradual.

2. The *zona reticularis* withstands the initial transplant trauma successfully but in the event of progressive tissue atrophy, the reticularis cells are the second to degenerate.

3. The cells of the *zona glomerulosa* not only are capable of withstanding the transplant trauma but, even in progressive tissue atrophy, are active in tissue regeneration, up to the time of complete adrenal gland failure. This active proliferation is particularly true of the peripheral *zona glomerulosa* cells that are closely associated with the loose inner region of the adrenal gland capsule.

4. ACTH is highly stimulating to the formation of *zona fasciculata* cells, to the extent of transforming the entire adrenal cortex into that type of tissue.

5. In this experiment a successful heterologous adrenal gland chimera required the culturing of the donor glands in medium containing serum from the prospective host for a period of time prior to the transplantation.

It is of considerable interest to note that though adrenal glands may regenerate to a size typical of normal adrenal glands, recent work by Skelton and Hyde (5) shows that such regenerated glands do not regain the functional ability of normal glands. Their work on the albino rat related that the functional capacity of regenerating adrenals as determined by the elevation in plasma corticosterone following stress progressively increased, during tissue regeneration, but the functional capacity of normal adrenals was never regained.

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