PART II

CHAPTER III

MATERIALS AND METHODS
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EXPERIMENTAL VASECTOMY WITH CONTROLS:

A series of twenty-two albino rats including four control animals were utilised for the purposes of the present experimental study. Out of them fourteen animals were brought fresh from the supplier and maintained for a week in the laboratory confinement to make them habituated to the new environments before operations. The rest of the animals were littered in the department. When a litter was born each animal was given a litter number ear mark. The date of birth and colour of the newborn animal were also recorded. Care was exercised to secure animals for control from the blood-brothers of the same litter and killed at the same age as the experimental animals.

During the period of experimentation the animals were confined in separate compartments in animal cases that were kept clean and maintained properly.

FOOD AND NOURISHMENT OF EXPERIMENTAL ANIMALS:

Diet of the animals consisted of gram soaked in water and a mass of paste composed of (1) Ata, 640 gms., (2) Powdered dry fish, 220 gms., (3) Powdered yeast tablets, 40 gms., (4) Sodium chloride, 10 gms., (5) Calcium carbonate, 10 gms. and (6) Celin (Vit.C), 5000 mg. The above food was served
twice a day. Plenty of water was supplied in a separate receptacle inside the case.

That the diet was suitable could be judged from the health of the colony and from the large size of the litters raised, and also from the steady increase in the weight of the animals. Daily personal supervision and observations were done until the conclusion of the experiments. The testes were palpated to see whether they remained inside the scrotum or not. Weights of the animals were recorded regularly twice a month. The animals were kept under conditions as far favourable as the circumstances permitted.

The following different types of experimental operations were performed:

1. Unilateral vasectomy without any interference on the contra-lateral side .. 4 specimens
2. Unilateral vasectomy with 'Mock' operation on the contra-lateral side .. 10 specimens
3. Bilateral vasectomy ... .. 4 specimens

* Against above 4 bilaterally vasectomised cases, 'Control' animals selected from the blood-brothers of the same litter .. 4 specimens

The 'Mock' operation in the contra-lateral side was
performed, obviously to find out the actual changes in the testis and epididymis caused exclusively by vasectomy, apart from those caused by operation trauma, stress and strain which are common factors to both the sides. The more similar the conditions in both operated and control sides, the more scientific shall be the assessment of the effect of the main operation (vasectomy) by comparing it with 'Mock' side (control side). An accurate standardisation is possible by having recourse to this method.

At an interval varying from 7 to 298 days the experimental animals were sacrificed. Anaesthesia, opening of the abdomen, proper exposure and removal of testes with their appendages, measurements of the body weight, weight and dimensions of the testis, and specific gravity of the testicular tissue, histological preparations, staining and micrometric measurements - all were done step by step more or less in the same way as mentioned under MATERIALS AND METHODS in PART I. In addition, the weight of the epididymis and its relative weight in proportion to the weight of the testis were determined and recorded in respect of both the vasectomised and control sides. Gross abnormalities, if any, in the testis and epididymis, condition of the vasa deferentia and the ligatures in them, were carefully observed by proper exposure at the time of sacrifice of the animals and noted accordingly.
During histological study on both the vasectomised and control testes, particular emphasis is given on the following: (i) Whether atrophic changes are evident in the spermatogenic cells with reduction in thickness of germinal epithelium and whether spermatogenesis is at abeyance or active with production of spermatozoa. (ii) Whether there is any evidence of fibrosis with associated narrowing of the tubules. (iii) Whether the intertubular space is dilated or reduced and whether there is apparent increase or decrease in the cellular elements of the interstitial tissue.

With a view to avoiding repetition of the similar phenomenon, illustrations are kept restricted to only representative photographs and microphotographs in this PART II of the thesis.

**TECHNIC OF VASECTOMY AND MOCK OPERATIONS PERFORMED IN THIS STUDY**

All operations were performed with strict aseptic precautions and under ether anaesthesia.

**PRE-OPERATIVE MEASURES:**

Before operation, the hairs on the lower abdomen, scrotum and inner side of the thigh and proximal part of the ventral aspect of the tail were removed with depilatory agent (Depil lotion) and the depilated area of the skin was washed for 2 - 3 minutes under running tap water to remove the debris and depilated hairs. The clean part was then treated with spirit rectified.
ANAESTHESIA:

Both induction and maintenance were done by administering ether by open method. After the pre-operative measures, the animal was put into a round glass jar with a glass cover and into which a little cotton soaked in ether was placed just beforehand. The jar was prevented from being absolutely airtight with the help of a small piece of cotton placed beneath the cover (between the margin of the jar and the glass cover). Thus induced and when the animal was under, as judged by lack of its movements and long breathing inside the jar, the animal was taken out. Its long head with the neck was now pushed into a small empty tin-made container previously perforated like a seive at the bottom with a large single hole on the top. Some cotton was placed at the perforated bottom of the container and ether was dropped from outside as and when required for the maintenance of the anaesthesia. The single hole on the top of the tin container being nicely adopted to the neck of the rat there was no chance of biting the anaesthetist. There was no loss of animal due to anaesthesia during the whole course of experimental operations.

POSITION OF THE ANIMAL:

The animal was put in supine position on the operation table with the limbs tied to the edge of the table. The operator used sterile mask, caps, gown and gloves, and the anaesthetist used only the mask and the cap. Small sized
towel and linen sheets, gauze, cotton, linen threads, instruments and silk threads all were sterilized in a pressure steriliser (Autoclave of Army pattern) for 30 minutes at 20 lbs. pressure.

**OPERATIVE PROCEDURE:**

The skin of the field of operation was swabbed with Tr. of iodine and was protected with sterile linen. The testis of the side to be operated was brought down to the scrotum, if not already there, by light pressure exerted upon the lower abdomen and kept within the scrotum by slight pressure just proximal to the testis between the thumb and the index finger of the operator so as to obliterate slightly and temporarily the wide abdomino-scrotal patent canal. A small longitudinal incision about one centimetre in length was made with a fresh safety razor blade on the ventro-lateral aspect of the scrotum proximal to the point of pressure between the fingers of the left hand as stated above. The skin with the subcutaneous tissue was incised when the fatty body attached to the proximal part of the testis pushing from inside made the peritoneum (processus vaginalis) appear as a tense shiny white bulging. This tense layer of peritoneum was cut through, to make a small slit in it through which underlying portion of the fatty body bulged out immediately. The left hand of the operator was now made free thereby releasing the pressure between the fingers. A pair of fine dis-
secting forceps was picked up with the left hand and was used as a retractor; another pair of fine dissecting forceps could then be introduced by the right hand from the lateral side deep to the fatty body in search of the vas deferens which came in view easily as a well-defined beaded structure along with blood vessels under cover of the fatty body. The vas deferens was caught and gently pulled out with the same right hand forceps and ligated in two places with fine silk (000 No.) A small segment of it about 2 mm. in length was excised from between the two ligatures as stated above. The wound was closed with one or two interrupted sutures of linen, touched with Tr. of Iodine, then sealed with a thin layer of cotton soaked with Tr. Benzoin co. and finally covered with leucoplast. At the end it was verified that the testis lay inside the scrotum.

In case of bilateral vasectomy, the whole process was repeated on the opposite side, and in case of 'Mock' operation on the contra-lateral side the same process was carried upto a point just short of ligature and resection of the vas deferens and vas was released back to its own position and the wound was closed as stated above.

**POST-OPERATIVE:**

Crystalline penicillin (booster dose 1 lac units) was injected I.M. stat, and 50,000 units was repeated once daily for 2 consecutive days. Injections were given in the thigh or calf.

On the 7th day skin stitches were removed and Tr. of Iodine touched. Healing was rapid and uneventful.