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Healing of Urinary Bladder Wounds. Morphologic and Biochemical Studies.* (31517)

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Connective tissue is ubiquitous and its elements play an important role in almost all physiologic and vital processes. Changes in the connective-tissue cells, ground substance and fibers predominate in wound healing. The cells produce acid mucopolysaccharides, and the fibrous tissue is important in restoration of structural continuity and production of adequate tensile strength. The regeneration process has been studied intensely, and parallelism between tensile strength, microscopic appearance of collagen, and hydroxyproline content has been established(1). Hydroxyproline is an imino acid specific of collagen. All repair processes are characterized primarily by edema, and secondarily by mucinous and fibrous organization of the water(2).

The purpose of this investigation was to study the morphologic changes occurring during healing of linear wounds in the urinary bladder, and to compare these observations with the biochemical findings in the same wounds.

Material and methods. Eighty-nine albino rabbits weighing about 2.5 kg were kept on an adequate laboratory diet for at least one week before operation. Seventeen of the animals served as controls. The animals were anesthetized by intravenous injection of 60 mg Nembutal® supplied with ether inhalation. The abdomen was opened through a low midline incision. The urinary bladder was emptied by puncture and aspiration. An incision through all layers was made in the midline of the anterior wall from the top to the neck. The bladder was closed in 2 layers with continuous sutures of 4-0 non-traumatic silk. In the first layer the suture was passed through the whole bladder near the wound edge. The second suture was seroserous. The abdominal wall was closed in layers with silk sutures. The animals were divided in 12 groups sacrificed 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 21 and 28 days, respectively, after the operation. They were killed by intravenous injection of 300 mg nembutal. The wounds were removed and specimens from the central part taken for histologic examination, while the rest was used for biochemical analysis.

Fixation and staining. The specimens were fixed in a fresh 4% aqueous solution of lead subacetate for 24 hours. This precipitating medium has been found to be the best for metachromatic staining of acid mucopolysaccharides(3,4). After embedding in paraffin, 7 μ thick sections were cut and stained with an 0.5% aqueous solution of toluidine blue, which stains acid mucopolysaccharides metachromatic. Parallel staining with haematoxylin-eosin, van Gieson-Hansen's connective-tissue stain for collagen, and orcein for elastic tissue was performed.

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Biochemical studies. After drying and defatting, the tissue was homogenized in 0.5 N NaOH. Total content of mucopolysaccharides was estimated by determination of the content of hexosamine as described by Kirk and Dyrbye(5) and Dyrbye(6), using the Elson and Morgan procedure(7) modified by Boas(8). The content of hydroxyproline, representing the amount of collagen, was determined by the method of Neuman and Logan(9) modified by Martin and Axelrod (10). The residual tissue homogenate was left for 24 hours for extraction of the acid mucopolysaccharides, which were isolated and precipitated by means of protamine sulfate, as described by Bollet *et al*(11). The uronic acid content in the isolated acid mucopolysaccharides was determined by the carbazole method of Dische(12) and the orcinol method of Brown(13).

Results. Gross appearance. The first 2 or 3 days after the operation the bladders were always somewhat contracted. Later, the capacity seemed to be normal. The urine was never found blood-stained. The first 5 to 7 days there was a marked edema of the wound edges with a maximum on the first and second days. The swelling decreased towards the lateral areas of the bladder but slight swelling was often noticed in the posterior wall. Twenty-eight days after the operation the wounds were visible only by the silk sutures.

In some of the animals calcification of suture loops occurred one or two weeks after wounding. It was found necessary to use silk sutures, because it appeared impossible to remove catgut sutures radically from the scar tissue before biochemical analysis. Catgut sutures have also been shown to produce more pronounced inflammatory reaction than non-absorbable sutures(14).

After one day the wound edges were well agglutinated and remained in contact even after the sutures were removed. Up to the fifth day they could easily be torn apart. Thereafter they could not be separated.

Microscopic appearance. The bladder wall consists mainly of smooth muscle fibers embedded in loose connective tissue, lined by luminar epithelium of the transitional type. Normally, there was just a very faint metachromasia limited to the subepithelial layer. A moderate number of mast cells with metachromatic granulation could be shown, always in relation to blood vessels (Fig. 1).

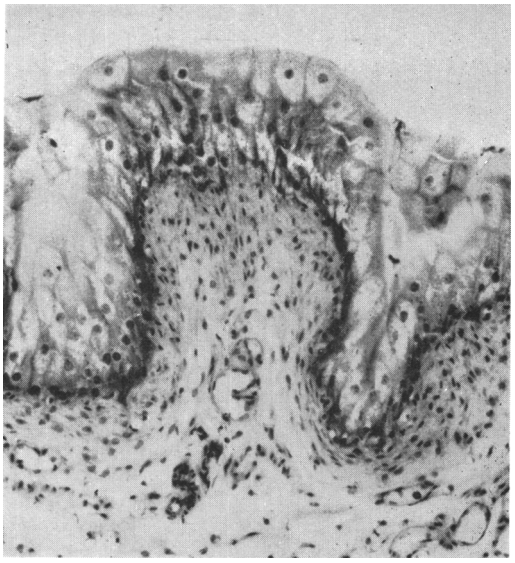

FIG. 1. Photomicrograph of part of normal bladder wall with epithelium of the transitional type, and, in the subepithelial layer, a few mast cells around a small vessel. Staining: Aqueous toluidine blue solution 0.5% (× 125).

Twenty-four hours after wounding the microscopic picture was dominated by inflammatory changes with edema, polymorphonuclear leukocytes and histiocytes. The epithelial defect was covered by fibrin (Fig. 2).

Two days after the operation new fibroblasts and blood vessels could be seen. Up to the fifth to seventh day the number of fibroblasts increased and the first collagen fibers appeared. Both the fibroblasts increased and the first collagen fibers appeared. Both the fibroblasts and the collagen fibers were usually arranged in parallel formations at right angles to the mucosal layer. The earliest proliferation of fibroblasts and formation of collagen fibers appeared around the sutures. From the seventh day, the content of collagen increased steadily during the rest of the examination period. Few elastic fibers were seen in the scar tissue.

At 24 hours the epithelial defect was covered by a fibrinous exudate. Thereafter epithelialization began, and, one week after the operation, flattened epithelial cells covered

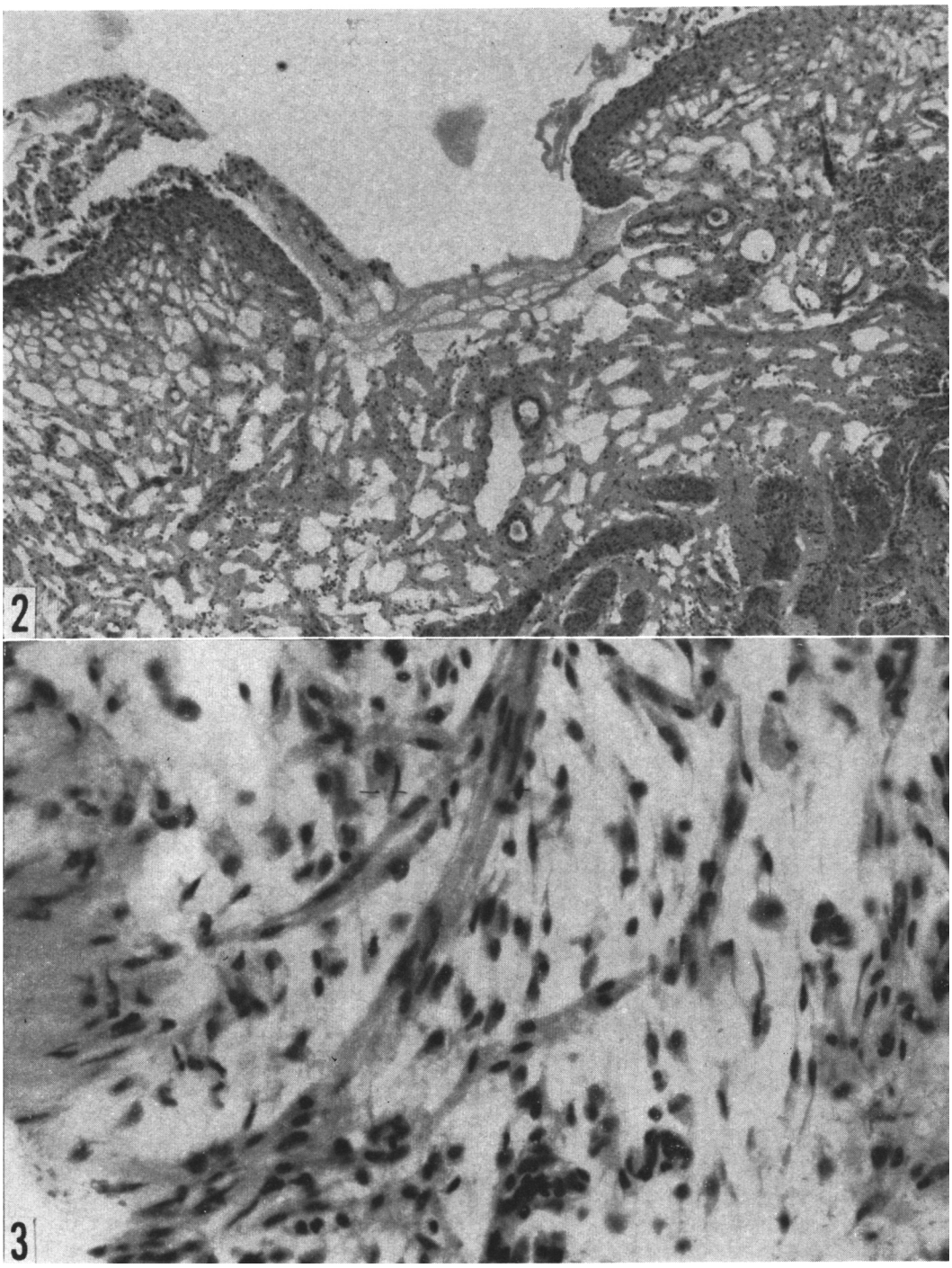

FIG. 2. 24-hour specimen. The epithelial defect is covered by a fibrinous exudate and inflammatory cells are seen in the depth. Staining: Haematoxylin-eosin (× 50).

FIG. 3. Regenerating muscle fibers in a 28-day-old wound. Staining: Haematoxylin-eosin (× 300).

TABLE I. Histologic Graduation of Metachromasia in the Healing Wound.

Days after wounding	Metachromasia	Days after wounding	Metachromasia
0	—	7	++
1	++	9	++
2	++	11	++
3	+++	14	+
4	+++	21	++
5	++++	28	+
6	+++		

entirely the underlying granulation tissue. The origin of the epithelial cells seemed to be the surrounding normal transitional epithelium, where an increased mitotic activity, especially in the basal cell layer, was demonstrated. The new-formed epithelium was reduced in thickness to 2 or 3 cell layers, but was, as a rule, normal 28 days after wounding. The peritoneal surface was covered with mesothelial cells within 24 hours.

Initially, inflammatory changes were seen in the adjacent smooth muscles. After about one week the muscle cells showed proliferation filling the gap from both sides (Fig. 3). The proliferation continued, and, 28 days after wounding, continuity of muscle fibers across the wound was seen.

Toluidine blue stained sections showed increasing metachromasia from the first day until a maximum was reached on the fifth day, declining thereafter. In Table I the changes were graduated by the use of plus signs. The mean values for hexosamine, carbazole and orcinol content in the different groups of metachromasia were calculated. It is evident from Table II that a good correlation exists.

In the normal bladders a moderate number of mast cells were seen. The first day after the operation the amount seemed unchanged, but thereafter mast cells could not be demonstrated until the marked metachromasia had disappeared. From the 14th to the 28th days there was an increased number, compared with control animals. The mast cells were always most numerous in the perivascular areas (Fig. 4).

Biochemical determinations. The results are presented in Table III. The values on different days after wounding were compared statistically with the controls according to students "t"-test.

Discussion. The accumulation of metachromatic material and increase in hexosamine content simultaneous with the fibroblastic proliferation, as well as the decline with the onset of fiber formation, indicate some relationship between concentration of acid mucopolysaccharides and fiber formation. This possible relationship has been studied for several years. It is now generally agreed that the acid mucopolysaccharides do not influence the synthesis of collagen(15). The steady increase in collagen content up to the

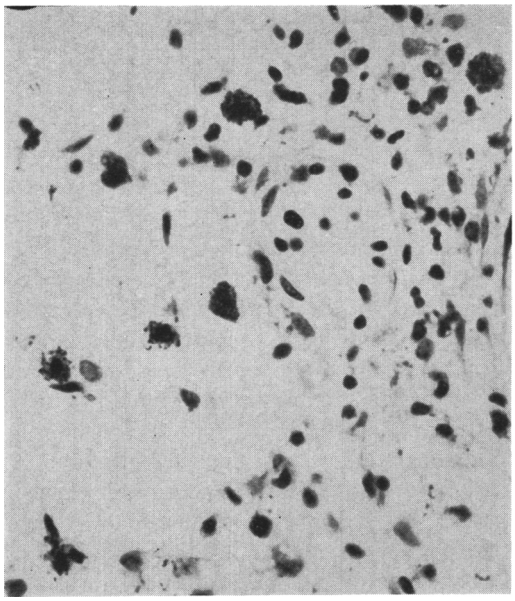

FIG. 4. Several well-granulated mast cells located in perivascular area in a 7-day-old wound. Staining: Aqueous toluidine blue solution 0.5% (× 500).

TABLE II. Comparison Between Degree of Metachromasia and Content of Hexosamine and Uronic Acids in the Healing Wound. Figures represent mean values expressed as μg/mg dried defatted tissue.

Metachromasia	—	+	++	+++	++++
Hexosamine	5.51	5.49	6.10	6.58	7.16
Uronic acid—Carbazole method	1.83	1.96	2.08	2.88	3.09
Orcinol "	1.50	1.80	1.98	2.58	2.59

TABLE III. Content of Hexosamine, Hydroxyproline, Carbazole and Orcinol in Healing Wounds, Given in μg/mg Dried Defatted Tissue. Figures represent mean values and standard error of mean.

Days after wounding	No. of animals	Hexosamine	Hydroxyproline	Carbazole	Orcinol
Controls	17	4.77 ± .09	35.0 ± 1.4	1.26 ± .08	1.21 ± .08
1	3	5.38* ± .26	26.5* ± 1.5	2.14‡ ± .11	1.83† ± .12
2	7	6.22† ± .30	21.9‡ ± 1.1	2.73‡ ± .22	2.44‡ ± .17
3	8	6.16* ± .46	25.2‡ ± 1.0	2.94‡ ± .24	2.16† ± .21
4	8	7.03‡ ± .26	26.1‡ ± 1.4	3.21‡ ± .18	2.66‡ ± .16
5	4	6.49‡ ± .23	30.4 ± 1.5	3.32‡ ± .31	2.96‡ ± .19
6	4	7.17* ± .42	33.1 ± .9	3.37‡ ± .50	2.43† ± .21
7	4	6.32‡ ± .15	35.9 ± 1.7	2.09‡ ± .09	1.93‡ ± .06
9	4	6.12‡ ± .25	38.6* ± .8	1.95† ± .19	2.19‡ ± .12
11	4	6.16‡ ± .18	37.9 ± 1.5	1.99‡ ± .05	1.92‡ ± .12
14	9	5.35† ± .17	41.3* ± 1.7	1.75 ± .21	1.64† ± .14
21	10	5.73† ± .24	39.7* ± 1.4	1.68† ± .06	1.39 ± .10
28	7	5.11 ± .12	41.5* ± 1.7	1.53 ± .16	1.32 ± .07

Significantly different from controls at: * 5% level of probability.
† 1% " " " .
‡ 0.1% " " " .

28th day was in accordance with biochemical increase of the hydroxyproline content.

It appears to the author that no reports of healing of linear wounds in the urinary bladder have been published. However, some investigations of the regeneration process after total cystectomy have been reported. Bohne *et al*(16) removed the entire bladder of dogs and replaced it by an acrylic mould. A new bladder was formed, lined with transitional epithelium and containing smooth muscle fibers in its wall. This extraordinary regenerative capacity is in good agreement with this study, in which, in 4 weeks, the wounds were converted to almost normal bladder wall.

The disappearance of mast cells a few days after the operation might be due to difficulty in identifying the cells, because of the intense metachromatic staining. It might also be due to release of the granules from the cells to the surroundings. Scattered granules were regularly seen in the ground substance. The role of mast cells in wound healing is unknown. According to Glücksmann, they have little to do with fibroplasia(17), while Asboe-Hansen states that these cells are immediately in the center of interest when problems of regeneration and repair, edema, water absorption, etc. arise(18).

Summary. The morphologic and biochemical changes during healing of experimental wounds in the urinary bladder of rabbits were studied. A high regenerative capacity was shown. The muscle layers healed by formation of granulation tissue and collagen, whereafter smooth muscle fibers grew into the scar from both sides. There was a rapid increase in the hexosamine and uronic acid content, while simultaneously the hydroxyproline content decreased. One week after wounding all the values were on the normal level. During the rest of the examination period, which lasted until 28 days after wounding, the hydroxyproline content increased steadily. A correlation between morphologic and biochemical sequences could be established.

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Renal Mechanisms Underlying Actions of Androgen and Hypoxia on Erythropoiesis.* (31518)

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Previous reports have indicated that androgens exert a stimulatory effect on erythropoiesis in endocrine-deficient and intact animals(1,2,3). A resurgence of interest in this field has stemmed from more recent observations that testosterone may induce remissions in some patients with aplastic anemia(4) and with myelofibrosis(5).

Several modes of action of androgen on erythropoiesis have been proposed: 1. a direct effect upon the blood-forming organs (6,7); 2. potentiation of erythropoietin (ESF) action(8); 3. stimulation of production of the ESF(9,10).

Further evidence is presented here to support the concept that androgen stimulates production of ESF in rats and that this action is mediated through the renal erythropoietic factor (REF), a recently demonstrated principle which is not ESF but which when added to normal serum engenders the production of ESF(11,12,13,14). In view of reports (8,15) that animals rendered severely plethoric by hypertransfusion are less responsive to the erythropoiesis-stimulating effects of androgen, both normal and hypertransfused rats were employed in this study.

Materials and methods. Experiments with androgen. In all experiments, adult male rats (250-300 g) of the Long-Evans strain were employed as serum and kidney donors. One group of 5 rats received a single intramuscular injection of 12.5 mg long-acting testosterone cyclopentylpropionate (TCP) (Depo-testosterone, Upjohn Co.) in 0.25 ml cottonseed oil. The other group of 5, constituting the controls, was injected with 0.25 ml of the vehicle, cottonseed oil. Another group of 10 rats was rendered plethoric by intraperitoneal injections, on 2 successive days, of 10 ml of whole heparinized blood obtained from normal male rats. As with the non-plethoric groups, 5 of the plethoric rats were given a single intramuscular injection of 12.5 mg TCP while the other 5 received only the cottonseed oil. All injections were administered 4 days after the 2nd transfusion. Hematocrits at this time ranged from 65% to 70%.

Six days after the injections, all rats comprising the 4 groups were exsanguinated, the bloods allowed to clot at 5°C and the sera of the different groups pooled. The kidneys of the 4 groups were also separately pooled and subjected to the following procedure for extraction of the REF. The organs were weighed and minced. Ten ml of cold 0.25 M sucrose were added for each g of renal tissue. The mixture was homogenized in a Potter-Elvehjem homogenizer. Centrifugation was carried out at 6,300 $\times$ *g* for 15 minutes at 5°C and the sediment discarded. The sucrose supernatant was now recentrifuged at 21,000 $\times$ *g* for 20 minutes at 5°C. The sediment obtained ("light mitochondrial" fraction) was resuspended in 0.02 M phosphate buffer, pH 6.8 and the mixture immediately frozen. After thawing, the material was centrifuged at 37,000 $\times$ *g* for 30 minutes. The super-

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