THE HISTOGENETIC ACTIVITY OF SUBSTANCES SECRETED BY THE TRANSITIONAL EPITHELIUM

A.Ya. Fridenshtein

Division of Radiation Microbiology and Immunology (Head - Active member of the AMN SSSR V.L.Troitskii of the Gamaleya Institute of Epidemiology and Microbiology, Akad. Med. Nauk SSSR, Moscow

(Presented by Active Member of the AMN SSSR G. V. Vygodchikov)

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The incontrovertible facts dealing with the high osteogenetic activity of transitional epithelium [1-8] naturally pose the question of whether or not there is a secretion from this tissue of an active histogenetic factor, free of the living cells. Discussion may be directed toward the examination of the histogenetic activity in the nonliving mucosa of the organs of urinary excretion, toward the secretion of active extracts from the transitional epithelium, and, finally, toward the testing of the products of secretion which the transitional epithelium develops under experimental conditions [4-6].

In the current work material is presented from the experimental investigations in these three directions.

EXPERIMENTAL METHOD

The urinary bladder mucosa of guinea pigs was cut into small pieces with scissors, and pellets were made from the obtained paste, approximately 3 mm in diameter. The latter were fixed in 96° alcohol or acetone for 12-24 hours. Then the pellets were dried in a vacuum dessicator (those fixed in alcohol were preliminarily washed in acetone), moistened with serum, and implanted in the anterior wall of the stomachs of the guinea pigs. After 8-22 days the implants were fixed. In place of fixation, some of the pellets prepared according to the indicated method were placed in test tube and subjected to five-fold freezing in acetone and thawing. Then they were implanted in the anterior peritoneal wall and fixed afer 5-18 days. For the preparation of the extracts the mucosa of the urinary bladder in the guinea pigs was again cut into small pieces with scissors and pulverized in an ebonite mortar. The homogenate, in which the cells were not observable microscopically, was suspended in an equal volume of physiological saline and,each day for 7 days, injected with a syringe into the same site on the anterior wall of the peritoneum. On the 10th day that portion of the peritoneal wall was fixed. Extraction of the mucigenous portion of the urinary bladder in the guinea pigs was carried out in an aqueous medium – either physiological saline or distilled water containing 1% guinea pig blood serum. The mucosa ground with a pestle was mixed with double its volume of physiological saline or distilled water. The homogenate was centrifuged at 1200 revolutions per minute for 10 minutes. The clear supernatant fluid was injected daily for 10 days into the same site in the anterior peritoneal wall. The control guinea pigs were injected with homogenate or extract of liver, prepared in the same manner. The extracts were prepared in the cold; penicillin and streptomycin were added to the material injected into the animals. The site of injection was fixed after 4-12 days.

For the investigation of the products of secretion of transitional epithelium in adult rabbits,the vessels of the left kidney were ligated. Under such conditions urine is not formed, and the transitional epithelium of the pelvis produces a secretion, directed, in particular, into the cavity of the pelvis [5].

After 10 days the left ureter was ligated at the middle third and cut above the site of ligation. The ends of the ureter were separately sutured to adipose tissue. The contents of the pelvis were drained off via the site of drainage of the central end; the drainage site of the peripheral end served as a control. Both subjects were fixed after 10–30 days. In the project a total of 57 animal-recipients were used. The types of histological fixatives utilized were acetone, alcohol-formol, and Gel' *solution. The sections were stained with hematoxylin-eosin, by the method of Dominichi-Kedrovskii using the PAS-reaction, and by the method of Gomora to alkaline phosphatase.

EXPERIMENTAL RESULTS

Implantation of the pellets prepared from the mucosa of the urinary bladder did not lead to epithelial growth in a single case. A proliferative reaction of the connective tissue was observed in the area surrounding the implant, along with its gradual resorption, the character and rapidity of which depended on the method by which the pellet was prepared. Implants fixed in

alcohol or acetone underwent resorption slowly (3-5 weeks) with the participation of coarse, miltinucleated simplasts. It was characteristic that even after 12-17 days a slight degree of alkaline phosphatase activity was preserved in the fixed implants, while in the surrounding connective tissue the alkaline phosphatase reaction was invariably absent. In association with the implantation of fixed urinary bladder mucosa in the tissues,the usual reaction to a foreign body was observed in all cases. Resorption of the mucosa, implanted after freezing and thawing, occurred more rapidly—within the first 10 days. The process was accompanied by a minimal reaction, with a small number of macrophages, predominantly mononuclear. At the site of the implant, having undergone resorption, there remained fibroblastic tissue with a small amount of collagenous fibers. Osteogenesis was not observed in a single instance.

The absence of osteogenetic activity on the part of the fixed mucosal tissue of the urinary bladder does not contradict the data [7] on the obtaining of chondrogenesis in rabbits at the site of implantation (in the ear or the subrenal capsule) of fixed urinary bladder wall. The positive results in these experiments were dependent, as the authors correctly assumed, upon the induction activity of fixed muscle tissue of any origin, and not upon the osteogenetic properties of transitional epithelium.

With repeated daily administration of the homogenates, as well as aqueous and saline extracts, of the urinary bladder mucosa, into the anterior peritoneal wall of guinea pigs, similar results were observed in all cases. Manifest thickenings formed at the site of injection on the 4-5th day. They became less diffuse, but, in places, denser, by the 8-10th day. Histologically, these thickenings consisted of reactive connective tissue. The main mass of their cellular elements was made up of coarse branching cells with basophilic cytoplasm and a large nucleus containing a prominent nucleolus. Mitoses were frequently observed among these cells. A large number of spherical polyblasts were present here, in the cytoplasm of which there appeared a polysaccharide which did not ferment amylase.

A thin polychromatic (stained by the method of Dominichi-Kedrovskii) granularity appeared in the basophilic cells with the contracted branchings on the 5-8th day, and, simultaneously, there appeared glycogen and a polysaccharide which did not ferment amylase.

Neighboring on these elements were disposed small foci of young myeloid cells and more mature cells of the granular leukocytic series- basophilic promyelocytes, eosinophilic promyelocytes, myelocytes, metamyelocytes, and, finally, eosinophilic and basophilic leukocytes. It was possible to clearly show the stage of conversion of the spherical, polyblastic type cells into the coarse, polychromatophilic, myeloid elements.

The generating foci of extramedullary hematopoiesis were usually distributed in the intermuscular connective tissue and, also, in the site of fatty tissue. With the accumulation of the polychromatic granules in the polyblasts, i.e., with their conversion into myeloid elements, glycogen appeared in them in the form of a compact mass, dispersed in the peripheral portions of the cytoplasm. The young myeloid elements, with the not-yet-segmented nucleus, clearly differed from the agglomerated round-nucleated cells of the lymphocyte type and from the plasma cells in their alkaline phosphatase activity. In such foci of extramedullary hematopoiesis there were not encountered disintegrating leukocytes with degenerating nuclei, and when stained for polysaccharides it was not possible to find extracellular masses of glycogen, although the mature leukocytes present here were very rich in this polysaccharide.

All this distinguished these foci from the accumulation of leukoctyes which can arise subsequent to the insufficient observation of aseptic technique.

Besides the foci of hematopoiesis, actively proliferating connective tissue was also formed in the sites of injection of the extracts, in which the processes of fiber formation were initiated. The cellular elements presented here were mainly coarse fibroblasts with basophilic cytoplasm. Collagenous fibers formed around the cells, creating homogeneous discs, here and there, of the osseoid type. However, the appearance of osteoblasts or the development of typical osseoid structures was not observed in a single case. We also never observed any epithelial cells in the sites of injection which would be able to indicate their contamination in association with the introduction of the material. Extracts of the liver did not give rise to marked thickenings in the site of injection. In this case only a proliferative reaction of the connective tissue was observed, without the formation of foci of hematopoiesis.

The experiments involving the drawing off of the secretion produced by the transitional epithelium in the cavity of the pelvis with ligation of the renal vessels was accompanied, in a portion of the animals, by the suctioning off with a syringe of the contents of the ureter that exited from those kidneys. The rather viscous clear fluid obtained, when tested for polysaccharides (in smears), yielded an intensive PAS reaction, which was not eliminated by amylase. In addition to this, the fluid contained granules of glycogen in large number. Thus, the fluid corresponded to the contents of the pelves in the kidneys with the ligated vessels which was observed in the histological preparations, and also to the contents of the cysts in the transplants of transitional epithelium [4-6]. We named the secretion obtained pelvin [5].

At the site of drainage of the lower end of the ureter (control) no formation of bone or myeloid tissue occurred. In this location there developed only the usual granulation tissue, with a small number of fibers and small foci of round-nucleated lymphoid elements. It should be kept in mind that in rabbits the transplantation of urinary bladder mucosa to connective tissue does not cause the induction of osteogenesis [6].

In contrast to this, at the site of drainage of the upper end of the ureter, via which pelvin drained from the pelvis, the formation of myeloid tissue had already occurred by the 20th day. The development of multinucleated simplasts was noted here, with large quantities of lipoids and a finely granulated polysaccharide, stable to amylase, around which there arose foci of myeloid hematopoiesis similar to those which were formed at the site of injection of the extracts. Even in this case osteogenesis was not observed in a single instance. The fact that the generation of myeloid tissue precedes the formation of the giant cell simplasts is evidence for believing that it actually develops from local elements instead of the fatty tissue to which the transected ureter was sutured.

Thus, non-living transitional epithelium does not possess any kind of specific histogenetic activity.

It should be considered that in association with the death (fixation) of the tissue, substances, contained in the transitional epithelium and responsible for the induction, are destroyed. They, apparently, are also destroyed in conjuction with the slow resorption of the epithelium rendered dead by freezing and implanted in the connective tissue.

At the same time, the homogenates and extracts of the transitional epithelium, prepared in physiological saline and in distilled water, demonstrated, upon repeated injection, the ability to induce myeloid hematopiesis from the local connective tissue cells. This same effect also gave rise to the flow of a secretion from the transitional epithelium (pelvin) into the connective tissue. Thus, in normal transitional epithelium, as well as in the products of its secretion, substances are contained ensuring the myelogenetic activity of that tissue even without contact with living epithelial cells.

However, the injection of extracts and pelvin is insufficient to cause the appearance of osteogenic tissue. They induce only myeloid hematopoiesis, genetically closely related to osteogenesis. This is either due to the fact that all the factors necessary for osteogenesis, are not contained in the extracts of transitional epithelium and pelvin or to the fact that, in addition to chemical factors, osteogenesis also requires the mechanical action of growing epithelial cysts and extensions in the surrounding connective tissue.

SUMMARY

Physiological saline and water extracts of urinary bladder mucosa and secreta of transitional epithelium (pelvin) possess histogenetic activity. They induce myeloid hemopoiesis at the site of injection. There is no histogenetic activity in fixated and frozen tissue of vesicular mucosa.

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