DISTRIBUTION OF GLYCOGEN IN THE URINIFEROUS SYSTEM OF THE RABBIT'S KIDNEY DURING ONTOGENETIC DEVELOPMENT AND AFTER INJECTION OF PITUITRIN

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 9, pp. 116-119, September, 1960
Original article submitted July 18, 1959

An important histochemical characteristic of the epithelium of the urinary tract in mammals is its high glycogen content. The transitional epithelia of the urinary bladder, the ureters and the renal pelvis show a particularly high content of this polysaccharide [7, 8, 10]. The collecting tubules, situated between the nephron and the transitional epithelium of the pelvis, also contain glycogen, but in a smaller quantity [11]. Glycogen cannot be detected histochemically in the remaining epithelial structures of the kidney.

The role of glycogen in the urine excretory system remains unclear. For its evaluation, information must be obtained on the distribution of glycogen in different functional conditions and at different stages of ontogenetic development. It is known, in particular, that in newborn rabbits, guinea pigs and man the collecting tubules contain far more glycogen than the same structures in adult animals [6].

In this communication findings are presented relating to the distribution of glycogen in the urine excretory system of the rabbit's kidney during embryogenesis and postnatal development, both in normal conditions and after administration of pituitrin.

EXPERIMENTAL METHOD AND RESULTS

The investigation was carried out on 15-to 28-day embryos and on young animals up to 3 months of age. The kidneys were fixed by Shabadash's method and sections were stained by the periodic acid-Schiff method for polysaccharides with or without counterstaining with alum-hematoxylin. Control sections were stained after treatment with amylase.

In the mesonephros (15-day embryo) the collecting system consists of the Wolffian duct, which passes in its posteromedial part. The single layer of cubical epithelium of the Wolffian duct can be distinguished among the tubules of the mesonephros, for, in contrast to the latter, it contains many large clumps of glycogen, filling the apical and basal parts of the cytoplasm of its cells. In the lumen of the Wolffian duct there are many clumps and flakes of glycogen, secreted by its cells. At the points of entry of the mesonephric tubules into the Wolffian duct there is a sharply defined border (Fig. 1, a) of the glycogen content.

In the metanephros of 17-19-day embryos the superior divisions of the collecting tubules begin in the outer parts of the cortical layer, almost directly beneath the renal capsule. In the cortical layer, at the site of the junction of the collecting tubules with the renal tubules, a clear border is formed in the glycogen distribution. In the renal tubules there is no glycogen, whereas in the most proximal parts of the collecting tubules clumps of glycogen can be seen. As we progress down to the pelvis, the glycogen content of the epithelium of the collecting tubules increases sharply (Fig. 1, b). In the lower parts of the large collecting tubules, near the papilla of the pyramid, the cells are distended with masses of glycogen, so that after treatment with amylase the cells appear empty, and the epithelium has the form of an empty honeycomb (Fig. 1, c). On descending from the beginning of the collecting tubules to their lower divisions, just as the cubical epithelium gives way to a high prismatic epithelium, and very large amounts of glycogen are accumulated therein, so the cell nuclei are displaced, ed, first into the central, and later into the most apical part of the cytoplasm, facing the lumen of the collecting tubule. In the superior parts of the collecting tubules, glycogen is present in the lateral and apical portions of the cytoplasm of the epithelial cells, and in the middle divisions of the tubules, in both apical and basal portions of the cytoplasm; while in the inferior divisions of the collecting tubules it is found beneath the nucleus, filling the central and basal parts of the cytoplasm (Fig. 1, d). The same structure is present in the single-layered cylindrical epithelium of the pelvis covering the papillae of the pyramids, where characteristic polypi are formed,

which are filled with glycogen. The opposite wall of the pelvis is lined with a single layer of flat epithelial cells with a small amount of glycogen.

In the kidney of 25-29-day embryos the collecting tubule system as before is distended with glycogen, and in the middle divisions of the collecting tubules it is also present in their lumen. In the collecting tubules of the medullary layer the whole cytoplasm is filled with glycogen, forming solid accumulations, and the cell nuclei are displaced to the apical pole, i.e., are turned to the lumen of the tubules. In individual cells of the collecting tubules in the medullary layer, the lower half of the cytoplasm does not contain glycogen, and it shows empty spaces like those after discharge of secretion. In the neighboring glycogen-containing cells secretion of glycogen can be seen in the form of grains or clumps from the cytoplasm of the epithelial cells beneath the single layer of cells. There is no visible destruction of the cytoplasm of the epithelial cells during this process, which is more of the character of halocrine secretion. This picture cannot be regarded as an artifact, due to penetration of the fixing agent or to cutting on the microtome (Fig. 1, e).

In the middle and superior divisions of the medullary layer, in the mesenchyme surrounding the collecting tubules, a large quantity of glycogen is secreted in the form of small and larger clumps, lying between the cells, within the mesenchymal cells and in the endothelium of the vessels (Fig. 1, f). Meanwhile, glycogen is never found in the epithelium or in the lumen of the loop of Henle, which is situated here. This shows that this picture is not an artifact, due to diffusion. The character of the relationship between the glycogen in the mesenchyme around the collecting tubules and the process of its secretion has not yet been explained. It should be pointed out that there are very few capillaries in this part of the medullary layer. In the lower divisions of the medullary layer, at the base of the pyramids, where the collecting tubules are surrounded by a dense capillary network, the pattern of distribution of glycogen is quite different. Here, too, in the wall of the collecting tubules, the epithelium of which is also distended with glycogen, cells are encountered in which the lower half of the cytoplasm consists of an empty space, and the secretion of clumps of glycogen from the basal portions of the epithelial cells beneath the single layer of cells may be observed. In the surrounding mesenchyme, however, glycogen is present in small amounts or completely absent. It only reappears in the mesenchyme beneath the epithelium of the pelvis, at the apex of the papilla—a zone, moreover, which contains a small number of capillaries. On its hilar aspect, the pelvis is lined with a transitional epithelium of 3-4 layers, with no vertical anisomorphism, and containing a large quantity of glycogen. Beneath the epithelium there is a dense network of capillaries; no glycogen may be seen in the connective tissue beneath the epithelium. In the large collecting tubules, as in the epithelium of the pelvis, secretion of glycogen takes place in the form of clumps through the surface layer of cells into the lumen, where they aggregate into floccular masses. Discharge of glycogen into the lumen takes place by means of secretion from the apical portions of the cell without destruction of the cytoplasm.

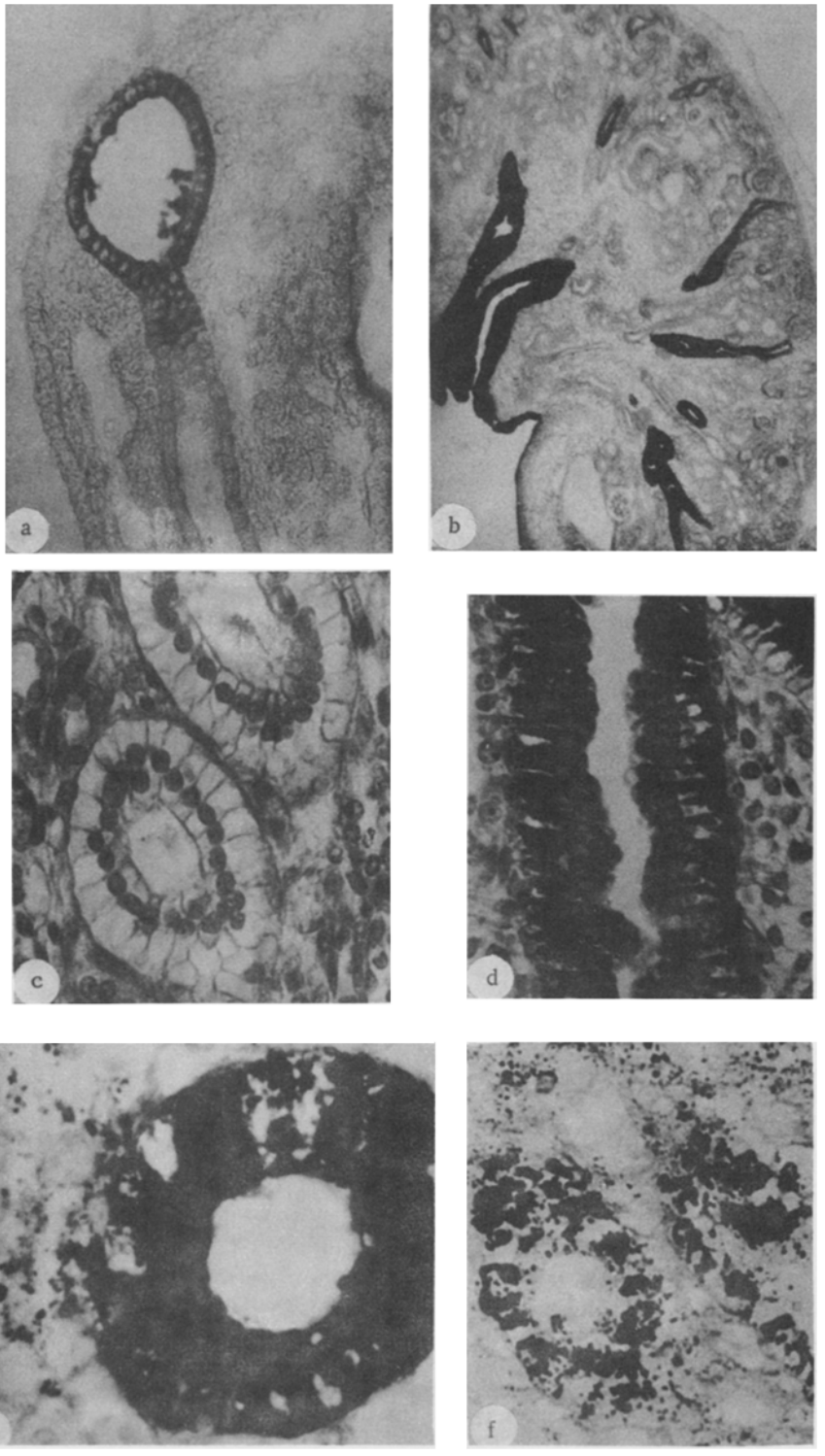
In the newborn rabbit, by comparison with the embryos at the end of intrauterine development, the glycogen content of the upper part of the collecting tubules is diminished where they pass through the cortical substance. In their middle and lower portions, however, lying in the medullary layer, a large amount of glycogen is present as before, filling the cytoplasm of the epithelial cells, especially its basal part. By comparison with the preceding stages, the cell nuclei here have undergone displacement, and instead of apically, are now centrally or even basally situated. In the superior divisions of the medullary layer, as before, the discharge of clumps of glycogen beneath the layer of epithelial cells is observed. In the transitional epithelium of the pelvis appears a layer of smaller, basal cells, not containing glycogen. The overlying layers contain glycogen, but in smaller quantities than in the epithelium of the collecting tubules.

In the young animals, in the course of the first two weeks, a very large quantity of glycogen is present in the collecting tubules. The content of glycogen in their epithelium later diminishes.

In the adult rabbit, the epithelium of the superior divisions of the collecting tubules, lying in the medullary layer, contains a small number of tiny clumps of glycogen. On passing into the subjacent layers, its quantity increases slightly, and the small granules of glycogen are concentrated in the apical and basal portions of the cell. Throughout the whole length of the collecting tubules, the cell nuclei are situated basally. Glycogen is present in the lumen of the large collecting tubules, but there is none in the surrounding mesenchyme. In the large collecting tubules close to the papilla of the pyramid there are intraepithelial spaces filled with the polysaccharides of mucus, resistant to the action of amylase.

The transitional epithelium covering the pad of fat in the region of the pelvis consists of 3-5 layers of cells with characteristic division into zones. The smaller basal cells do not contain glycogen; the overlying layers contain large amounts of it. The single-layered epithelium covering the renal pyramid on its pelvic aspect contains diffuse masses of glycogen in the basal portions of the cells and a small quantity of a polysaccharide not fermented by amylase in the apical parts of the cytoplasm.

Administration of pituitrin, causing a more intensive reabsorption of water in the collecting tubules of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the kidney, is accompanied [1] by significant changes in the epithelium of these tubules. Under these circumstances we observed characteristic changes in the glycogen distribution. In young animals, in which the collecting tubules are normally still rich in glycogen (Fig. 2, a), administration of pituitrin caused after 30-60 minutes a characteristic process of glycogen secretion into the lumen of the collecting tubules (Fig. 2, b) and into the surrounding connective tissue (Fig. 2, c). In the adult animals, in which there is normally little glycogen in the collecting tubules (Fig 2, d), after administration of pituitrin, glycogen accumulates and is secreted through the apical surface of the cells into the lumen of the collecting tubules, and if the dose of pituitrin given is small, this secretion proceeds according to the merocrine pattern, i.e., without destruction of the cytoplasm (Fig. 2, e).

A high content of glycogen is thus a characteristic feature of the urine excretory system of the kidney throughout the whole of embryogenesis. This sign, moreover, sharply distinguishes this system from all other epithelial structures of the kidney, and is common to the Wolffian duct of the mesonephros and the collecting tubules, and to the pelvis of the metanephros. The high glycogen content in the collecting tubules and pelvis of the embryonic kidney cannot be accounted for by their more intensive rate of growth. Both during stages of organogenesis when they proliferate intensively, outstripping in this respect the nephrogenic tissue, and during later stages when their growth is slowed, the epithelium of the collecting tubules is distended with glycogen.

At the period of maximum accumulation of glycogen in the collecting tubules (20-28 days of intrauterine development), the whole cytoplasm of the epithelial cell, facing the subjacent mesenchyme, is filled with massive deposits of glycogen. Under these circum - stances glycogen from the epithelium is secreted into the mesenchyme surrounding the collecting tubule, as a result of a process analogous to secretion in its character. The apical position of the nucleus in the epithelium of the collecting tubules, which is seen only at this moment, is also presumably connected with the process of discharge of polysaccharide from the basal portion of the epithelial cells of the tubules into the underlying tissue. Glycogen may also be secreted into the lumen of the collecting tubules. Morphologically identical secretory processes are observed after the administration of pituitrin.

The importance of the high glycogen content in the urine excretory system, and of its secretion, requires explanation. It can only be postulated that glycogen is of more importance here as a plastic, rather than as an energy-supplying material, being produced in the course of distinctive secretory processes. It is possible that the process of secretion of glycogen by the epithelium of the collecting tubules bears some relation to the normal production of urine. It must be remembered, on the other hand, that when in experimental conditions [2-5] the discharge of substances with the histochemical signs of glycogen from the transitional epithelium into the connective tissue may be induced, and the natural connection of this epithelium with the vessels may be broken, (i.e., the accumulation of this secreted material may be brought about), osteogenesis and hemopoiesis are induced in the connective tissue beneath the epithelium. It may accordingly be suggested that the secretory processes in the epithelium of the urinary tract are related to the histogenetic regulation of hemopoiesis or osteogenesis (and hence, of the calcium metabolism) of the animal body.

SUMMARY

Epithelium of the renal tubules in embryos and young rabbits contains much glycogen and secretes this substance into the lumen of these tubules and the surround-

Fig. 1. Glycogen in the collecting tubules of the embryonic kidney. a) 15-day embryo. Mesonephros. Wolffian duct. Fixation by Shabadash's method. Periodic acid— Schiff reaction. Magnification: objective 10×; b) 17-day embryo. Metanephros. Collecting tubules. Fixation by Shabadash's method. Periodic acid—Schiff reaction. Magnification: objective 10×; c) 19-day embryo. Metanephros. Collecting tubules. Fixation by Shabadash's method. Treated with amylase. Periodic acid— Schiff + hematoxylin. Magnification: objective 24×; d) 19-day embryo. Metanephros. Collecting tubules. Fixation by Shabadash's method. Periodic acid— Schiff + hematoxylin. Magnification: objective 24×; e) 28-day embryo. Metanephros. Collecting tubules. Fixation by Shabadash's method. Periodic acid–Schiff reaction. Magnification: objective 40×; f) 29-day embryo. Metanephros. Collecting tubules. Fixation by Shabadash's method. Periodic acid–Schiff reaction. Magnification: objective 24×.

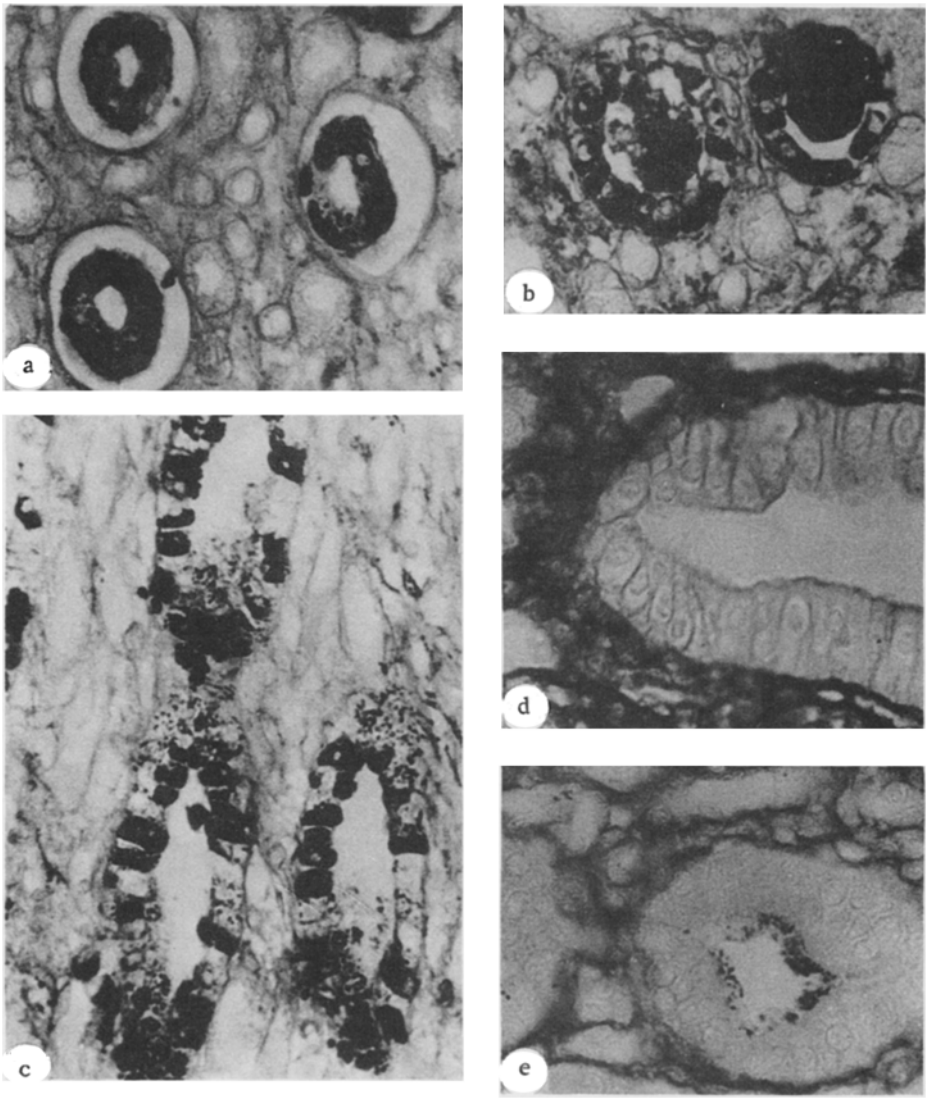

Fig. 2. Effect of pituitrin on the glycogen of the collecting tubules. a) Young rabbit. Normal. Collecting tubules. Fixation by Shabadash's method. Periodic acid—Schiff reaction. Magnification: objective 24×; b) Young rabbit. 30 minutes after injection of pituitrin. Collecting tubules. Fixation by Shabadash's method. Periodic acid—Schiff reaction. Magnification: objective 24×; c) Young rabbit. 60 minutes after administration of pituitrin. Collecting tubules. Fixation by Shabadahs's method. Periodic acid—Schiff reaction. Magnification: objective 24×; d) Adult rabbit. Normal. Collecting tubules. Fixation by Shabadash's method. Periodic acid—Schiff reaction. Magnification: objective 40×; e) Adult rabbit. 30 minutes after injection of pituitrin. Fixation by Shabadash's method. Periodic acid—Schiff reaction. Magnification: objective 40×.

ing mesenchyme. In young and adult animals a similar process of secretion was observed under the influence of pituitrin.

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