ROLE OF DEXTRAN IN INTRACELLULAR METABOLIC REACTIONS IN THE PROXIMAL CONVOLUTED TUBULES OF THE KIDNEY

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Experiments using cytochemical and electron-microscopic methods have shown that after administration of dextran (polyglucin, strain SF-4), only glucose formed by the splitting of dextran in the body is utilized in the proximal convoluted tubules of the kidney for structural and energy-producing purposes of intracellular metabolism. Most of the reabsorbed dextran is deposited in the cell as large droplets and takes no part in metabolic reactions.

The components of a plasma substitute must be readily assimilated by the body tissues for plastic purposes [7]. This is one of the chief requirements of a plasma substitute, but unfortunately it has been inadequately studied.

According to reports in the literature, the various dextran preparations, structurally allied to polyglucin, are incompletely eliminated from the body. In the first 24 h, about one-third of the administered dextran is excreted in the urine [12, 14, 18]; it is not known what happens to the rest of the dextran. Dextran has been found in various tissues of the body 1-3 months after its administration [11, 14, 15].

Data have been obtained indicating that dextran is partially broken down in the body with the formation of glucose molecules [3-6, 16].

After administration of C^{14}-labeled dextran, it was found in the expired CO_2 and in glucose excreted in the urine [13, 14, 17], additional evidence that dextran participates in metabolic reactions.

Since plastic and energy metabolism, assimilation, and dissimilation are interconnected [1], and since the mitochondria play a leading role in metabolism and in the production of cell energy [8], the state of cell nutrition can be judged from the number, size, and localization in the cell of the mitochondria [10]. To investigate the state of intracellular metabolism, histochemical tests were carried out to determine polysaccharides, lipids, phospholipids, proteins, RNA (connected with protein metabolism), and alkaline phosphatase (connected with carbohydrate metabolism), and to study the changes in these components during various metabolic reactions of the cell in relation to changes in the mitochondria.

In this respect the proximal convoluted tubules of the kidneys provide an excellent model, because they reabsorb not only plasma proteins of low and high molecular weight, glucose, and electrolytes, but also substances foreign to the organism, from the primary urine.

The Soviet preparation polyglucin, obtained from strain SF-4 of dextran, was used in these investigations.

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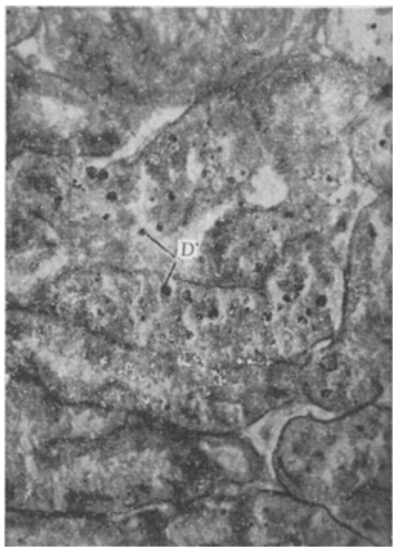


Fig. 1. Proximal convoluted tubule of the kidney 48 h after injection of polyglucin. Large conglomerates of dextran (D) can be seen in the cells. PAS reaction, 400×.

EXPERIMENTAL METHOD

Experiments were carried out on 27 rats weighing 200 g. To synchronize their intracellular metabolism, the animals were fasted for 24 h, after which they received 5 ml polyglucin parenterally. The animals were sacrificed by decapitation 30 min and 1, 2, 3, 24, 48, and 72 h after injection of the plasma substitute (3 animals at each time). Six rats were kept under the same conditions as the experimental animals and acted as controls. The kidneys were investigated after histological and histochemical treatment. To detect mitochondria, the material was fixed in Champy's and Regaud's fluids, and stained by the methods of Cowdry and Kuhla, while polysaccharides were detected by the PAS reaction, alkaline phosphatase by Gomori's reaction, lipids were stained with Sudan black and Nile blue, phospholipids were detected by Menshik's method, and proteins were stained with a solution of bromphenol blue in mercuric chloride. Pieces of tissue for electron-microscopic investigation were fixed for 1.5-2 h in the cold in a 2% solution of osmic acid in veronal-acetate buffer, pH 7.4. The material was embedded in a 4:1 mixture of butylmethylmethacrylate and catalyst. Sections were cut on the Sjöstrand LKB Ultratome and examined in the UÉMB-100 electron microscope.

EXPERIMENTAL RESULTS

Dextran was found in the cells of the proximal convoluted tubules of the kidney 30 min after its injection, as a result of its filtration through the glomerular filter of the kidney and its active reabsorption by cells of the proximal convoluted tubules.

Staining with hematoxylin-eosin showed that in the first hours after injection (30 min, 1,and 2 h) dextran was visible in the cells of the proximal tubule in the form of yellow droplets. Yellow dextran droplets could also be seen in the lumen of the tubules and in the blood vessels between the tubules. Not only separate droplets, but whole conglomerates of dextran were found in the cells of the proximal tubules 24, 48, and 72 h after its administration.

Staining for RNA revealed a picture almost identical with that observed by the use of hematoxylin-eosin: all the large drops were yellow in color, and the fuchsin dye was not taken up. A very small quantity of RNA could be found only in small granules.

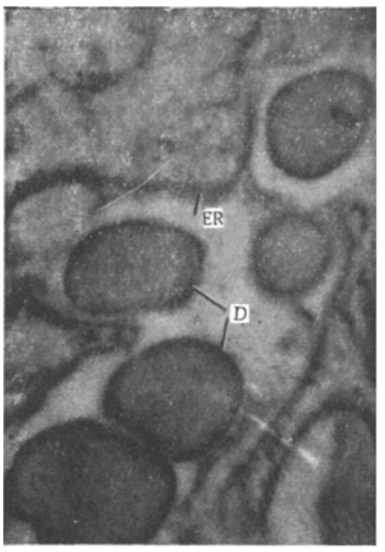
By the PAS reaction, small and medium-sized granules stained as glycoprotein granules, while the larger granules and conglomerates did not take up the stain and appeared in the sections as yellow granules and droplets (Fig. 1).

No reaction for neutral lipids likewise was obtained by staining with Sudan black and Nile blue. Only during staining for phospholipids did the small granules appear bluish-green in color, i.e., they took up some stain for phospholipids. The larger drops remained yellow. The dextran droplets inside the tubules and in the spaces between them also remained yellow in color.

In sections stained for protein with a solution of bromphenol blue in mercuric chloride, the small granules stained blue for protein while the large droplets remained yellow.

Staining for alkaline phosphatase showed that the small granules possessed alkaline phosphatase activity.

The small granules of reabsorbed polyglucin which, as described above, reacted with polysaccharides, proteins, phospholipids, RNA, and alkaline phosphatase, when stained for mitochondria appeared under the optical microscope like granular mitochondria. The large drops, on the other hand, remained yellow and did not stain for mitochondria.



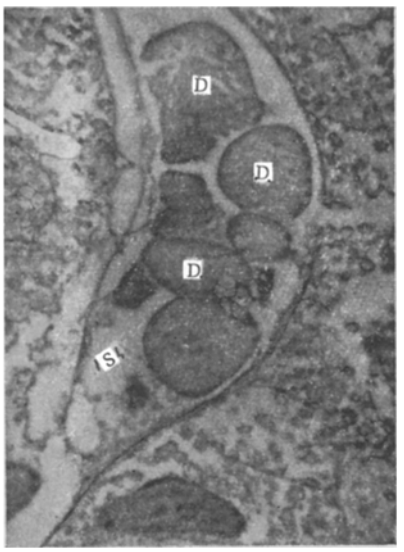


Fig. 2 Fig. 3

Fig. 2. Part of cell from proximal convoluted tubule of rat kidney 24 h after administration of polyglucin. Reabsorbed dextran (D) visible in endoplasmic reticulum (ER), 40,000 ×.

Fig. 3. Space between tubules 24 h after injection of polyglucin. Space (S) filled with dextran drops (D), 30,000 ×.

The preliminary results of electron-microscopic investigations confirmed the results of optical microscopy. Dextran penetrated into the cells of the proximal convoluted tubules of the kidney and could be detected in the endoplasmic reticulum of the cell (Fig. 2). The large drops of dextran also filled the spaces between the tubules (Fig. 3).

The authors' investigations, in which animals were loaded with protein in order to determine the morphological picture of intracellular protein metabolism, showed that during protein reabsorption complexes are formed in the proximal convoluted tubules of the kidney between the reabsorbed protein and polysaccharides, phospholipids, alkaline phosphatase, and the RNA of the cell. These complexes are directly linked with the cell mitochondria. The dimensions and localization in the cell of the mitochondria were similar to the dimensions and localization of the granules. Changes were reversible, and 2-3 days later the cell components once again had the characteristic appearance of intact cells.

Similar changes were observed after glucose loading. This suggests that organic substances (protein, glucose), when they enter the cell, are used by it for plastic and energy-producing purposes. This is manifested morphologically by the formation of complexes which are intimately linked with the cell mitochondria.

Dextran loading showed that the reabsorption of this substance takes place mainly in the proximal convoluted tubules of the kidney. This evidently explained the smaller percentage of breakdown of dextran by extracts prepared from the whole kidney [3].

The morphological investigations not only confirm the results of biochemical tests, but also demonstrate the topographic distribution of the injected material and shed light on the dynamics of assimilation of dextran by the cells.

Histochemical staining, even with Schiff's reagent alone [2], enables the degree of splitting of dextran and its partial participation in metabolic reactions of the cell to be estimated.

Polyglucin was observed in the cells of the proximal convoluted tubules of the kidney 30 min after its injection, as small mitochondrial granules containing complex aggregates of substances in which were similar in type whether the animals were loaded with protein [9], glucose, or polyglucin. Only a small proportion of the polyglucin reacts with the intracellular components, in all probability the 5% glucose

solutions which are formed by the hydrolysis of this substance [6], and it must be supposed that only this glucose participates in cell metabolism, because the granules formed were identical with the granules produced after glucose loading. It thus follows that the cell uses only the organic substances for its plastic and energy-producing purposes, while the greater part of the reabsorbed dextran, possibly the dextran dextrins formed by the detachment of glucose [6], are deposited in the cell in the endoplasmic reticulum as large drops. Further investigations will be carried out to determine the action of these substances on intracellular metabolism.

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