15th day, Purkinje cells are round and irregular in shape. Ribosomes are scattered through the cytoplasm, and 3 to 4 membranous sacs are arranged in parallel and occasionally take the form of clusters. They may be described as a kind of lamellar structure. Mitochondria with irregular profiles are distributed randomly and cristae of them are sometimes destroyed. The membranous sacs are clumped and show swelling, and are not always related to mitochondria. Some of their cavities are filled with electron-dense material. The areas among parallelly arranged slender membranous sacs show a high electron opacity (Figure 2). High magnification of the lamellar structures reveals structural details. On opposed aspects of those clumped sacs to the other sac or mitochondria, ribosomes are not present, but the other free side of the clumped sacs is adorned with ribosomes (Figure 3 and 4). Further, the interstices between slender sac and mitochondria or the other closely associated sacs are occupied with electron-opaque material with patterns of spots or dots (Figure 5). It is interesting that the distance from one saccular structure to the other seems to be twice the diameter of the ribosome.

From these findings, it is clear that, in the initial stage of formation of lamellar structures, mitochondria are closely associated with membranous structures studded with ribosomes, and these structures lose ribosomes from their cisternal surfaces during formation of lamellar structures. The significance of these findings for the etiology of neurolathyrism is under investigation.

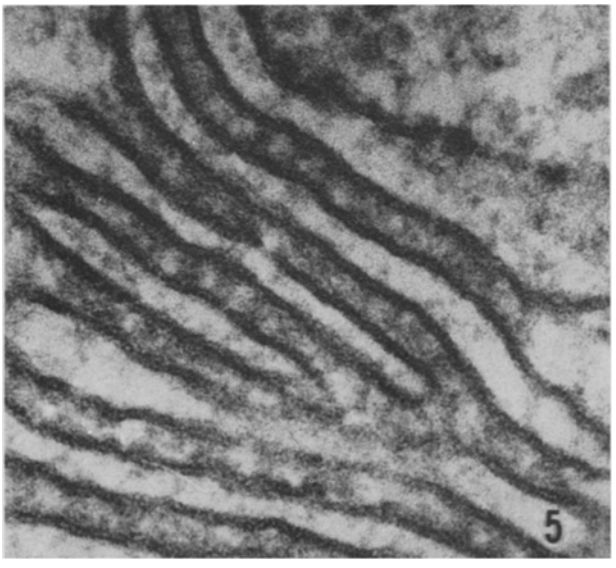

Fig. 5. High magnification of lamellar structure. The spaces speckled with high electron opacity can be seen. ×200,000.

Zusammenfassung. Nachweis, dass durch Gaben von β-Aminopropionitril die Bildung von Membransystemen in den Purkinje-Zellen des Kleinhirns induziert wird.

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The Influence of Insulin and Glucose on the Release of Plasminogen Activator by Isolated Rabbit Kidneys

In subjects with hyperinsulinemia caused by excessive or incorrect nourishment, low fibrinolytic activity and thrombosis occurance are frequent[1]. In experimental hyperinsulinemia induced by infusion of glucose or i.v. injection of the hormone, a fall of blood fibrinolytic activity was observed in rabbits[2,3]. In these experiments, results were obtained suggesting that insulin reduces the fibrinolytic activity by decreasing the level of plasminogen activators in the blood. In the present work the effect of insulin on the secretion of plasminogen activator by the kidneys was investigated.

Materials and methods. Rabbits of mixed strain, males and females, 3.0–3.5 kg weight, were used. The secretion of plasminogen activator by the kidneys was assessed under conditions resembling those already described[4]. The fluid for renal perfusion: citrate rabbit blood obtained by cardiopuncture was centrifuged at 2500 × *g* for 10 min. The erythrocyte sediment was washed 7 times in 20 volumes of 0.9% NaCl. The 40% suspension of washed

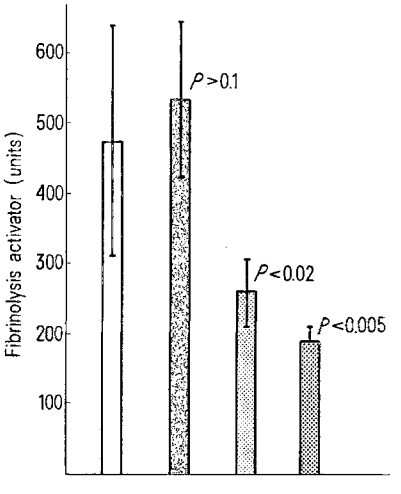

Fig. 1. Results of determination of plasminogen activator secreted into the veins and urinary tract by the kidneys under different experimental conditions. The presence of insulin in the perfusing fluid had no effect on this secretion, while addition of glucose, and particularly glucose with insulin jointly, caused considerable, statistically significant reduction in the renal secretion of plasminogen activator. □, none; ▓, insulin; ▒, glucose; ▒▓, insulin and glucose.

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erythrocytes in 0.9% NaCl was oxygenated 10 min with pure oxygen. This suspension with addition of 1000 μU/ml insulin and 1.5 mg/ml glucose and without any addition to the erythrocyte suspension were used for perfusion.

Renal perfusion: the animals were anaesthetized with pentobarbital (Thiopental, Spofa, Praha) in an i.v. dose of 25 mg/kg body wt., the abdomen was split open, the vascular bed of the kidneys was perfused with 30 ml of 0.9% NaCl through plastic cannules inserted into the renal arteries. After insertion of cannules into the renal veins and ureters the isolated kidneys were immersed in 0.9% NaCl heated to 37 °C. The kidneys prepared in this way were now perfused for 45 min with erythrocyte suspension at a steady rate flow of 1.4 ml/min. Fluids were collected from veins and ureters. After completion of perfusion, sections taken from the renal medulla and cortex were homogenized at 4 °C in Potter homogenizer in 0.9% NaCl (100 mg on 1 ml of 0.9% NaCl) and homogenates obtained were centrifuged for 30 min at 3000 × *g*. The level of fibrinolysis (plasminogen) activator was determined in the fluid from ureters, in the acellular fluid from renal veins and in supernatants of homogenates using method of other autors[5,6]. Each of various erythrocyte suspensions was used in perfusion of 7 different kidneys.

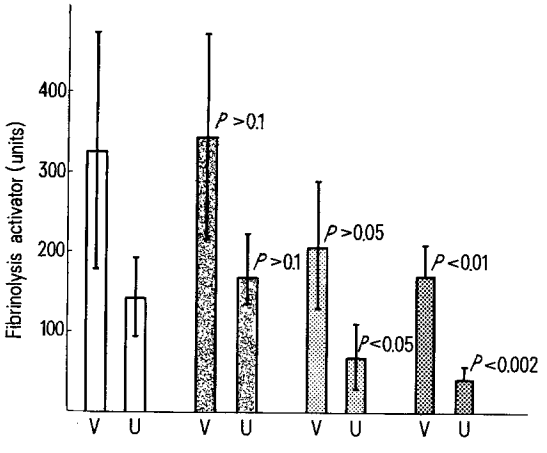

Fig. 2. Results of the measurement of the separate secretion of plasminogen activator into the vascular bed and into urinary tract by perfused kidneys. It is seen that the addition of glucose and insulin to the perfusing fluid decreased the secretion of plasminogen activator into the renal veins as well as into ureters. V, perfusate from veins; U, filtrate from urinary tubes.

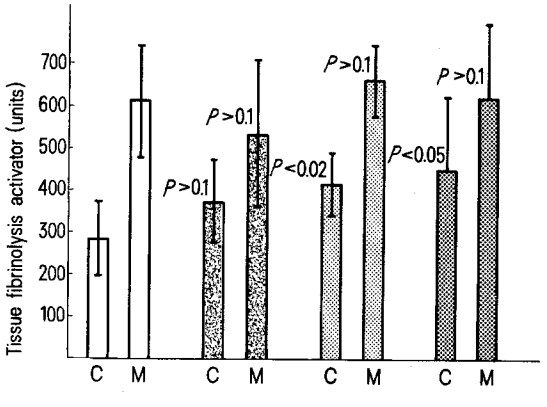

Fig. 3. Level of plasminogen activator in renal tissue after completion of the perfusion. The level of plasminogen activator in the kidneys perfused with fluid containing glucose or glucose with insulin was not only not lower than in the kidneys perfused with suspension of erythrocytes in normal saline, but it was even higher than in these kidneys. C, cortex; M, medulla.

Results and discussion (Figures 1–3). The results of the present investigations demonstrate that insulin fails to reduce the secretion of plasminogen activator by the isolated kidneys when glucose is lacking. Reduction of the secretion of this enzyme occurred, however, during perfusion of the organs with a cell suspension with glucose added without insulin. This effect was greater when the perfusing fluid contained also insulin. For the experiments the kidneys of healthy animals were used. In health insulin is secreted continuously into the blood and a part of it remains in the organs, probably in the form of various connections with appropriate cell receptors. It may be supposed that perfusion of such kidneys with a glucose-containing fluid caused assimilation of glucose by the tissue. The addition of insulin to the perfusing fluid with glucose might increase the assimilation of glucose be the kidneys. This explanation agrees with the observation that inhibition of renal secretion of plasminogen activator was greater when glucose with insulin was added to the perfusing fluid than when this fluid contained only glucose.

The level of plasminogen activator in the kidneys perfused with various fluids was similar. It indicates that the reduction of secretion of this protein caused by addition of glucose and insulin to the perfusing fluid resulted from inhibition of release rather than from decrease of synthesis.

The present results suggest that decreased fibrinolytic activity of the blood observed in experimental hyperinsulinemia[2,3] is due to reduced release of plasminogen activator from the tissue into the blood. This effect may be caused by insulin-dependent rise in cellular assimilation of glucose. In this way the present experiments make it possible to explain the low fibrinolytic activity of blood in subjects nourished excessively or incorrectly.

ВЫВОДЫ. Исследовано воздействие инсулина и глюкозы на секрецию активатора плазминогена в вены и мочевое пути изолированой почки кролика, перфундированой кислородной суспензию эритроцитов. Наблюдается, что глюкоза а особенно глюкоза и инсулин тормозит секрецию активатора плазминогена, но не имеет воздействия на количество его в ткани почек.

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