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HISTOCHEMICAL CHARACTERIZATION OF THE ADRENAL GLANDS AND TESTES IN CRYPTORCHIDISM

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In this study, endocrine disorders in the testes and adrenal glands of experimental animals with true cryptorchidism and after orchidopexy were determined. The identification the succinate dehydrogenase, glucose-6-phosphatase, and adenosine triphosphatase in the tissue of dystopic and orthotopic testes of animals was the aim of this research. The methods of enzyme detection by Nakhlas, Walker, Walker-Zeligmman, and Vakhstein-Maizel were used. The research revealed morphological changes in the testes of experimental and control groups, namely: depletion of germ cells, thickening of the basal membrane of seminiferous tubules, compact arrangement of seminiferous tubules in the testes of animals with cryptorchidism – and less pronounced changes in the testicular tissue of control group animals: germ cells at various stages of development clearly covered the walls of seminiferous tubules. SDH activity in the dystopic testis was lower than in the non-descended testis. Also, SDH activity in Sertoli cells and the vascular wall of tubules was lower than in the testes of control animals. That is, in the orthotopic testis, SDH activity is significantly lower than in the dystopic one. The study also showed that in germ cells, vascular walls, and Leydig cells of the dystopic testis, NAD dehydrogenase activity is higher than in the contralateral testis. Glucose-6-phosphatase activity was observed as clusters of brown color in all structures of the testes in both groups. It was proven that in cryptorchidism, the activity of this enzyme was higher in the dystopic testis than in the non-descended one.

During the study, it was proven that the adrenal glands also underwent morphological changes, namely: the fascicular zone narrowed and the reticular zone expanded, cells were compactly and disorderly arranged, and the capsule was thickened. In cryptorchidism, SDH activity was higher in the reticular zone than in the medullary zone. NAD-oxidized dehydrogenase increased evenly in all zones of the adrenal glands in the norm, but was less pronounced in cryptorchidism. Glucose-6-phosphatase activity increased in cryptorchidism, while SDH and ATPase activities decreased.

The process of moving the testis from the abdominal space to the scrotum was accompanied by a discorrelation of the activity of the zones of the adrenal glands' cortex, which occurred unevenly in the structures of the adrenal glands. As the study showed, the increased activity of enzymes is the result of the reaction of the sex glands and adrenal glands to the stimulating effect of gonadotropins and corticotropins producing by the anterior pituitary. It can be assumed that in this disease, enzymes in tissues are not eliminated by the body, and their accumulation indicates the extinction of the functional activity of organs, which may lead to the malignant transformation of the testes and adrenal glands in the future.

Key words: *testis, adrenal gland, NAD dehydrogenase, SDH activity, glucose-6-phosphatase, cryptorchidism.*

Олена Шарапова. Гістохімічна характеристика наднирників та яєчок у разі крипторхізму

У цій роботі визначені ендокринні порушення в яєчках і наднирниках експериментальних тварин зі справжнім крипторхізмом і після орхідопексії. Метою цього дослідження було виявлення сукцинатдегідрогенази, глюкозо-6-фосфатази, аденозинтрифосфатази в тканині дистопних і ортотопічних яєчок тварин. При цьому використовувалися методи виявлення ферментів за Нахласом, Валькером, Уокером-Зелігманом і Вахштейном-Мейзелем. У результаті проведених досліджень виявлені морфологічні зміни у яєчках тварин експериментальної і контрольної груп, а саме: збідненість на статеві клітини, потовщеність базальної мембрани сім'яних канальців, компактність розташування сім'яних канальців у яєчках тварин з крипторхізмом і менш виражені зміни тканини яєчок у тварин контрольної групи: статеві клітини на різних ступенях розвитку ясно вкривали стінки сім'яних канальців. Активність СДГ у дистопному яєчку визначалася нижче, ніж у неопущеному яєчку. Також СДГ-активність у клітинах Сертолі і судинній стінці канальців була нижчою, ніж у яєчках контрольних тварин. Тобто в ортотопічному яєчку активність СДГ значно нижча, ніж у дистопному. Дослідження також показали, що у статевих клітинах, судинній стінці та клітинах Лейдіга дистопного яєчка НАД-дегідрогеназна активність вища, ніж у контрлатеральному яєчку. Глюкозо-6-фосфатазна активність виявлялася у вигляді грон коричневого кольору в усіх структурах яєчок обох груп. Доведено, що у разі крипторхізму в дистопному яєчку активність цього ферменту була вища, ніж у неопущеному яєчку.

У процесі дослідження доведено, що надниркові залози також мали морфологічні зміни, а саме: звужувалась пучкова і розширювалась сітчаста зони кори наднирників, клітини розміщувалися компактно і безладно, капсула була потовщеною. У разі крипторхізму СДГ-активність більш висока спостерігалась у сітчастій зоні, ніж у клубочковій. НАД-окислена дегідрогеназа підвищувалась рівномірно в усіх зонах наднирників у нормі, але менше

проявлялась у разі крипторхізму. Активність глюкози-6-фосфатази у разі крипторхізму посилювалась, СДГ та АТФ-азна активність знижувались.

Процес переміщення яєчка із заочеревинного простору в калитку супроводжувався дискореляцією активності зон кори надниркових залоз, що відбулося нерівномірно у структурах наднирників. Як показало проведене дослідження, підвищення активності ензимів є результатом реакції статевих залоз і наднирників на стимулюючий вплив гонадотропінів і кортикотропінів, які продукуються передньою часткою гіпофіза. Можна припустити, що при цьому захворюванні ферменти в тканинах не виводяться організмом, а їх накопичення свідчить про згасання функціональної активності органів, що в майбутньому може призвести до злоякісного переродження яєчок та наднирників.

Ключові слова: *яєчко, надниpник, НАД-дегідрогеназа, СДГ-активність, глюкозо-6-фосфатаза, крипторхізм.*

Introduction. In recent literature, reliable data have emerged indicating the significance of endocrine disorders in the pathogenesis of cryptorchidism [1, p. 167–168; 3, p. 57–58; 4, p. 185–187]. During the disruption of testicular migration from the abdominal cavity to the scrotum, the functional and morphological changes are occurred in the adrenal glands as an additional source of androgens and an organ that ensures the body's protective-adaptive reactions. Currently, close functional relationships between the sex glands and adrenal glands have been identified [2, p. 410–412; 5, p. 125–127]. However, studies on adrenal glands have been conducted more frequently under conditions of castration or with the aging-related decline in sexual functions [7, p. 195–198]. Contradictory data on the functioning of the steroid-producing system in pathological conditions of the gonads, the lack of a unified interpretation of the mechanism of interaction between the adrenal glands and gonads, and the compensatory mechanism of the adrenal cortex, as well as disturbances in steroid balance during testicular retention, necessitated the conduct of this study [6, p. 101–102; 8, p. 130–132].

The **aim** of our research is the identification the succinate dehydrogenase, glucose-6-phosphatase, and adenosine triphosphatase in the tissue of testes and adrenal glands of animals with true cryptorchidism and after orchidopexy.

Materials and methods. The study was conducted on the adrenal glands of 15 dogs with true cryptorchidism and 12 dogs after orchidopexy. The age of the animals ranged from 2 to 4 years. The control group consisted of 10 animals of the same age. From experimental and control groups of animals, testes and adrenal glands were removed to create histological specimens stained with hematoxylin and eosin. Additionally, histochemical methods were used to stain enzymes and lipids for the detection succinate dehydrogenase by Nakhlas, Walker, nicotinamide adenine dinucleotide by Nakhlas, Walker-Zeligman, glucose-6-phosphatase, and adenosine triphosphatase by Vakhstein-Maizel.

The research was conducted in accordance with the requirements for handling experimental animals, namely the Law of Ukraine "On the Protection of Animals from Cruelty", and the Order of the Ministry of Education and Science, Youth and Sports of Ukraine № 249 dated 01.03.2012 "On Approval of the Procedure for conducting research, experiments on animals by scientific institutions".

Results and Discussion. During the observation of sections stained with hematoxylin and eosin, it was found that in cryptorchidism, the ducts in the dystopic testis are most often arranged more compactly, creating the impression of their greater quantity. The wall of the tubule is depleted of germ cells. Their membranes are thickened, and only spermatogonia with shrunken compact nuclei are present. Fibrous substance in the form of a dendrite was found in the place of spermatocytes and spermatozoa. There is an increased number of Leydig cells, but their nuclei are mostly shrunken.

In the orthotopic testis, the tubules are arranged less compactly, winding, and irregular in shape. The wall is densely covered with rows of immature germ cells, their order is somewhat disturbed, and they are condensed. Spermatozoa are often found in the center of the tubules. Individual tubules with thin walls containing only a row of spermatogonia with a shrunken nucleus were encountered.

In the testes of the control group of dogs, SDH activity in germ cells appeared as blue formazan grains in large quantities, especially in the center of the tubule, in spermatogonia, and in Sertoli cells. The grains were located in the cytoplasm of cells around the nucleus. In the interstitial spaces, SDH activity was much lower. Leydig cell grains were more than in germ cells. In Sertoli cells, SDH activity is so high that it forms clusters resembling "cherries".

In the dystopic testis, SDH activity is lower than normal, and formazan grains are localized in spermatogonia and Leydig cells. SDH activity in Sertoli cells and the vascular wall of the tubules is reduced.

In the orthotopic testis, SDH activity is significantly higher than in the dystopic and normal testis, especially in Leydig cells and mature germ cells.

NAD dehydrogenase activity in the testes of control animals manifested as blue grains locating in all

layers of germ cells lining the tubules. Larger grains were deposited in spermatogonia and Sertoli cells. The enzyme activity is lower in more mature germ cells and connective tissue.

In the dystopic testis, NAD dehydrogenase activity is higher than normal and in the contralateral testis. It is particularly high in immature second-order germ cells. High activity is observed in the vessels and Leydig cells. In the orthotopic testis, overall activity is much higher than normal but lower than in the dystopic testis. Enhanced activity is observed in Leydig and Sertoli cells. NADH dehydrogenase activity in a normal testis is expressed as large blue grains, mainly in Leydig cells and spermatogonia, as well as in Sertoli cells and the lamellar connective tissue surrounding the seminiferous tubules. In dystopic and orthotopic testes, there is a significant increase in activity in spermatogonia, Sertoli cells, and Leydig cells.

In control testes, glucose-6-phosphatase activity is manifested as clusters composed of small brown-colored granules. Overall enzyme activity is low, mainly localized in mature cells in the center of the seminiferous tubules, Sertoli cells, and Leydig cells.

In cryptorchidism, glucose-6-phosphatase activity is higher than normal in the dystopic testis but lower than in the orthotopic testis. Its distribution changes, primarily in spermatogonia and first-order spermatocytes.

In the orthotopic testis, glucose-6-phosphatase activity is significantly increased compared to dystopic and normal testes, especially in first-order spermatocytes; activity is normal in Leydig cells.

Adenosine triphosphatase activity, manifested as brown and black granules, in normal testes is slightly more abundant in Leydig cells and very high in the lamellar connective tissue around the tubules.

In undescended testes, ATPase activity is lower than normal. Adenosine diphosphatase activity in normal testes is much lower than ATPase activity. Individual brown-colored grains are evenly distributed in the cytoplasm of germ cells. Higher activity is observed in Leydig cells and the vascular wall. In retained and orthotopic testes, ADPase activity is very high, exceeding normal levels, primarily in Leydig cells.

In control testes, lipids are present as small orange-colored droplets located in the cytoplasm of germ cells, including spermatogonia and first- and second-order spermatids. The quantity of lipids in these cells is small. Lipids are particularly evident in the cytoplasm of Leydig cells. In retained and orthotopic testes, the lipid content in Leydig cells is low.

Thus, the activity of NAD, NADH-glucose-6-phosphatase, ADP increases in cryptorchidism in both dystopic and orthotopic testes. SDH, ATP, and ADPase activity is reduced.

When studying the features of the adrenal cortex structure in cryptorchidism compared to normal, it was found that the nuclei of adrenal cortex cells are wrinkled, stained intensely blue, protein-depleted, especially in the fascicular and reticular zones. The cell structure is disrupted. The fascicular zone is somewhat narrowed, and the reticular zone is expanded. The adrenal capsule is thickened. The cytoplasm of the glomerular zone is protein-depleted, and cells adjacent to the glomerular zone are densely and irregularly arranged. Adenomas were often found in the capsule and fascicular zone.

In the normal state, SDH activity was highest in the reticular zone and lowest in the glomerular zone. In cryptorchidism, SDH activity increased in the reticular and glomerular zones, while decreasing in the fascicular zone. Thus, there was a discrepancy in the activity of the zones of the adrenal cortex.

The activity of NAD oxidized dehydrogenase is much lower in normal conditions than SDH. In cryptorchidism, NAD oxidized dehydrogenase activity increased uniformly. The highest activity among the enzymes examined was exhibited by NAD dehydrogenase. In normal conditions, its highest activity was found in the glomerular zone.

In cryptorchidism, NAD dehydrogenase activity decreased in all zones. However, there was an increase in the activity of glucose-6-phosphatase, ADP, and ATP, especially in the fascicular and reticular zones.

Lipids in the form of orange-colored droplets were deposited in all zones in control testes. Highest amount lipids are deposited in glomerular zone, lowest – in reticular zone, and middle amount – in fascicular zone. In cryptorchidism, amount lipids in reticular zone was low.

Conclusion. The movement of the testis from the abdominal space to the scrotum results in partial activation of adrenal gland function. However, it does not lead to significant morphological disturbances observed during organ's development under the influence of hormonal imbalances in cryptorchidism.

In the context of testicular displacement, changes in enzymatic activity are noted not only in the dystopic and orthotopic testes but also in the adrenal glands. The changes in the adrenal glands are uneven and show a discrepancy in the activity of different zones. The increased enzyme activity is likely a response of the target organ to the stimulating influence from the anterior pituitary, which produces gonadotropins and corticotropins.

It is suggested that the elevated enzyme levels are a result of unknown mechanisms, and the enzymes in the tissues are not effectively utilized. The high enzyme content may not necessarily indicate high functional activity of the organ. The accumulation of active enzymes in the tissues of target organs could potentially lead to the development of dysplasia or malignant transformation in the future.

In conclusion, the study suggests that the process of testicular movement and the associated enzymatic changes, not only in the testes but also in the adrenal glands, may have implications for the long-term health of these organs. The accumulation of active enzymes could be a marker of abnormal cellular processes and may contribute to the development of pathological conditions.

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