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Urinary Pathology - Experimental Modeling

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ABSTRACT

The proposed article is an experience of creating a review on the experimental pathology of the urinary system. Much attention is paid to the nervous mechanisms of disturbance and restoration of the functions of pathologically altered organs. It is also important that most of the proposed methods are easily and simply reproducible. For the analysis of functional disorders caused in the experiment, in addition to physiological ones, clinical and pathoanatomical research methods are included. The article will undoubtedly be of interest to a pathophysiologist, a clinician, and even a pathologist. It will prove to be a useful tool for teaching one of the leading disciplines of medical science - pathological physiology.

Keywords: Methodological Approaches; Urinary System; Experimental Animals

Urinary Pathology

To study diuresis in a chronic experiment, urine is collected in metabolic cells. Small animals (mice, frogs) can be placed in funnels inserted into graduated tubes. To study diuresis in an acute experiment in animals under general anesthesia, the abdominal cavity is opened along the white line, both ureters are dissected and cannulas are inserted into them. Graduated test tubes or measuring cylinders are tied to the cannulas [1]. The study of diuresis in dogs with previously removed ureters allows a more complete study of the dynamic changes in diuresis in a chronic experiment. Technique of the operation of removing the ureters. Small, short-haired female dogs are selected for the operation. An incision is made in the skin and muscles of the lower abdomen along the midline, 7-8cm long. The bladder is removed and placed on a gauze napkin. First, the urethra is cut between two ligatures. The stump below the ligature is stitched with a purse-string suture, its end is invaginated and the suture is tightened. The large blood vessels of the bladder are tied up so that the ligatures fall along the edge of the future flap. Urine is withdrawn with a syringe. The bladder is cut along the midline of the anterior wall to the urethra. The openings of the ureters are found on the mucosa and the posterior wall of the bladder is cut between them. The excess tissue of the bladder walls is removed, leaving only areas around the ureters with a diameter of 2-2.5cm, which are brought to the surface of the abdomen on the right and left along the nipple line. To do this, the skin in these areas is incised by 1.5-2cm, the muscles can be pierced in a blunt way (in order not to damage the artery, feel for its pulsation with your fingers from the inside of the abdominal wall). Closed tweezers are inserted into the gap, which are used to pick up ligatures applied to pieces of the bladder with ureteral openings. Carefully check if the ureters are not twisted. Then the abdominal wound is sutured, after which the removed ureters are strengthened with sutures, achieving the tightest possible fit of the excised area to the edges of the skin incision. The abdominal wound is treated in the usual way, and the skin around the removed ureters is lubricated with vaseline oil or petroleum jelly. The dog should be kept on a bed of sawdust, the cleanliness of the abdominal skin should be monitored (washed with warm water or a weak solution of manganese peroxide, wiped dry and lubricated with petroleum jelly) [2,3].

Urinary Disorders in Disorders of the Nervous System

Demonstration of diuresis disorders in brain lesions. Four frogs are selected for the experiment, the most active, dark in color, better than males, weighing 30-35g. Each frog is injected under the skin of the back with 3ml of distilled water. In two of them, the brain is cut at the level of the visual halls. The frogs are then placed in funnels inserted into graduated test tubes. The funnels are tied with wet gauze. After two or three hours, the amount of urine released is compared (for clarity, urine is tinted with paint). The rest of the urine is squeezed out of the bladder by pressing on the frog's underbelly. The amount of urine excreted in decerebrated frogs during this time is more or less than in frogs with an intact nervous system [4,5]. The different nature of changes in diuresis (increase or decrease in it) with such brain damage apparently depends on the complexity of the function of urination, which is determined not only by water, but also by other types of metabolism. In this regard, the regulation of this process is very complex. A slight difference in the level of the incision of the brain leads to the defeat of the centers, which obviously have a different effect on urination. In the clinical pathology of a person with brain damage, urination can also be increased or decreased. With lesions of the pituitary-hypothalamic region, an increase in urination is more often observed, which reaches 35-40 liters per day - diabetes insipidus [6]. This is due, apparently, to the insufficiency of pituitrin, secreted by the posterior (nervous) lobe of the pituitary gland and inhibiting diuresis.

Demonstration of the Action of Pituitrin on Urination

1) In the experiment, as in the previous demonstration, four frogs weighing 30-35g are selected. Each frog is injected with 3ml of distilled water under the skin of the back. In addition, two of them receive pituitrin (0.1-0.2ml). The frogs are then placed in funnels inserted into graduated test tubes. After 2-3 hours, as in the previous experiment, the amount of urine released is compared. The rest of the urine is squeezed out of the bladder by pressing on the frog's underbelly. In frogs that received pituitrin, urine is not excreted, in control frogs, 1-2ml is excreted after 2-3 hours. Urine is stained and test tubes are shown on a white background. The mechanism of action of pituitrin has not yet been definitively established - it is not clear whether water is retained in the tissues or reabsorption in the kidneys increases. Apparently, the latter factor is of greater importance [7].

2) The experiment can also be carried out on mice. Four mice of equal weight are placed in glass funnels covered with a metal mesh. Graduated test tubes are placed under the funnels to collect urine. All animals are injected with 4 ml of distilled water, and two of them, in addition, 0.1-0.2ml of pituitrin. Experience begins at the beginning of the lecture. After 1/2 hour, 1-2ml of urine is excreted in control animals, and no urine is excreted in experimental animals.

Demonstration of Unconditioned and Conditioned Reflex Changes in Diuresis

1) Experience put on dogs with ureters removed. To collect urine, funnels (rubber tubes or strips) are tied under the openings of the ureters, making sure that they fit snugly. Cylinders with divisions, cones or jars for urine are attached to the funnels (in this form, the dog must stand in the machine every day for several hours, in order to avoid skin irritation with continuously excreted urine). 16-18 hours before the start of the experiment, food and water are taken away. At the lecture for 45 minutes, five-minute portions of urine are collected separately from each ureter. Then the cylinders are removed and cause painful irritation of the skin of the thigh (induction current, voltage 4V, distance between the coils 0, duration of irritation 20-30 seconds). At the end of the defensive reaction, measuring cylinders are put on and urine output is monitored. After 3-5 minutes, the separation of urine almost completely stops for 10-15 minutes, sometimes more. Then diuresis is restored, sometimes exceeding the initial amount of urine. After 2-3 combinations, the dog develops conditioned reflex anuria only when the electrodes are brought to it or when the inductor is knocked [8]. Diuresis disorders also occur with other functional effects on the central nervous system. Fright, fear are usually accompanied by a decrease in diuresis, and small amounts of protein (0.5-1mg%) may appear in the urine. In hysteria, diuresis can be inhibited by self-hypnosis - there are cases when diuresis stopped for several days. During sleep or anesthesia, diuresis is inhibited.

2) The effect of pain stimulation on diuresis can also be studied under conditions of acute experiment without preliminary removal of the dog's ureters. Under general anesthesia, an incision is made along the white line of the abdomen, both ureters are dissected and cannulas are inserted into them to collect urine. Graduated test tubes or graduated cylinders are tied to the cannulas. The sciatic nerve is dissected, it is cut and the electrodes from the induction coil are applied to its central end. Five-minute portions of urine are measured within 15 minutes. Then the nerve is irritated for 30-60 seconds (voltage 2-4V, distance between the coils 5-8cm) and five-minute portions of urine are again measured for 15-20 minutes. Painful anuria develops, as in the previous experiment [9].

Diuresis Disturbances in Kidney and Urinary Tract Impairments

Demonstration of diuresis disorders in focal nephritis. Focal nephritis is caused by a direct action on the tissue of the kidneys of a thermal or chemical irritant. This method allows you to

immediately observe the violations of diuresis arising from this. Damage is caused by introducing into the kidney tissue with a syringe 5-6ml of hot (80°-90°) water or 1ml of a 25% emulsion of turpentine in vegetable oil. The experiment can be performed on a dog with separate ureters taken out or under the usual conditions of an acute experiment (with the abdominal cavity opened and cannulae inserted into the ureters). In an acute experiment, the kidney (more conveniently the left, located below) is damaged through the surgical wound. In a chronic experiment, one of the kidneys is removed under the skin of the back during an operation on the ureters, which makes it possible to damage it by injecting an irritant with a syringe through the skin, without additional surgical intervention. To remove the kidney under the skin of the back, the dog is fixed with its back up, the skin and muscles are cut along the pecking side for 8-10cm parallel to the last rib, 2-3cm away from it. The muscles are cut carefully without damaging the peritoneum. The kidney is removed and attached by the capsule with two or three sutures to the muscles and fascia so that it does not slip away. After that, the incision is sutured. The operation is carried out 2 weeks before the demonstration [10].

16 hours before the experiment, food and water are taken away. Set the initial level of diuresis (measure the amount of urine for every 15 minutes for 30 minutes). 30 minutes after the injury, the diuresis of the affected kidney exceeds the initial one by 2-3 times (polyuria). An increase in diuresis is usually accompanied by a significant drop in the specific gravity of urine (up to 1010 instead of 1020). The specific gravity of urine is measured with a urometer [9]. Urine takes on a reddish tint from the admixture of blood as a result of damage to the vessels of the kidney (hematuria); protein appears in it in an amount of 10-15 mg% (albuminuria). Microscopic examination of the urine sediment reveals many formed elements - fresh erythrocytes and leukocytes. Urination increases not only in a damaged kidney, but also in a healthy one (by 25-30%). Sometimes in the urine excreted by a healthy kidney, protein appears (up to 2mg%). Violations of diuresis in an intact kidney (if the opposite one is damaged) occur reflexively, in response to irritation from the lesion (reno-renal reflex). Reflex mechanisms are of the same importance in violation of the diuresis of the affected kidney. After denervation of the kidney, damaging it with hot saline does not cause an increase in diuresis. The denervated kidney becomes inert to all kinds of irritants (water load, diuretin administration, etc.). To the same extent, this inertness is also manifested under the action of pathogenic stimuli. When setting up the experiment under the conditions of a chronic experiment, diuresis is restored after 2-3 days, although blood cells and small amounts of protein continue to be excreted in the urine. In the clinic, with focal nephritis (for example, with metastatic kidney abscesses), unstable fluctuations in total diuresis and small changes in the composition of urine are also observed. Diffuse glomerulonephritis (unlike focal nephritis) is a local manifestation of damage to the entire vascular system, expressed in spasm of arterioles and increased capillary permeability. With it, there is a persistent decrease in diuresis, a relatively small albuminuria (3-5mg%), hematuria. At the same time, edema develops and blood pressure rises [11].

Urine Protein Tests:

1) Sample by boiling. 2-3ml of filtered urine of slightly acidic reaction is poured into a test tube (acidified with 2% acetic acid). Add 0.5-1ml of saturated sodium chloride solution and heat the top of the tube to boiling. In the presence of protein, a precipitate appears that does not dissolve when 2-3 drops of a 2% solution of acetic acid are added to a hot solution. Soluble precipitate give phosphate and carbonate salts.

2) Sample with concentrated nitric acid. Pour 1-2ml of concentrated nitric acid into a test tube and carefully layer 1-2ml of filtered urine on the acid. In the presence of protein, a cloudy white layer appears at the interface of the liquids. The more protein in the urine, the sooner the ring is formed.

3) Test with sulfosalicylic acid (most sensitive: detects up to 0.0015% protein in urine). Pour 2-3ml of clear acidified urine into a test tube and add 5-6 drops of a 20% solution of sulfosalicylic acid. In the presence of protein, a precipitate or turbidity appears [9].

Demonstration of Impaired Nervous Regulation of Diuresis in Focal Nephritis

Experience put on the same dog as in the previous experience. 30 minutes after kidney damage, against the background of increasing urination, pain irritation is caused. Instead of the usual anuria, there is a small and short-term decrease in diuresis in the damaged kidney. The intact kidney still responds with anuria. The general defensive reaction of the animal is preserved. In the same way, the conditioned reflex reaction (to the presentation of electrodes or the knock of an inductor) also weakens. Such a weakening of the reactions of pathologically altered kidneys to irritation is observed for 1-2 days, then they are restored. However, the composition of the urine, as already mentioned, remains still changed, since the structural disturbances caused by the pathological stimulus still remain by this time. Obviously, violations of urination in nephritis, as well as its restoration, are determined not only by structural changes in the kidneys caused by a pathological agent, but also by changes in the nervous regulation of diuresis associated with them. Nephrons that have fallen out during kidney damage are replaced by others (since normally only 1/3 of the total number of nephrons function at the same time), but this does not happen passively, by itself, but as a result of reflex influences on the central

nervous system from the lesion. The dependence of the restoration of kidney function on the state of the nervous system has been proven by direct experiments. In dogs with experimental neurosis, recovery of function in kidney damage occurs later [12].

Demonstration of Diuresis Disorders in Sublimate Nephrosis

1) To demonstrate violations of diuresis in nephrosis, you can use the poisoning of animals with sublimate. The experiment is performed on a dog with protruded ureters or with a bladder fistula. An hour before the lecture, the dog is injected under the skin of the thigh with a 2% sublimate solution at the rate of 20mg per 1kg of body weight (the introduction of a sublimate solution into the mouth causes severe vomiting). 1-2 hours after the introduction of sublimate, diuresis increases sharply (5-10 times). The specific gravity of urine decreases to 1,007-1,001. After a few hours, diuresis begins to decrease, and on the 2nd day oliguria or anuria develops. The specific gravity of urine rises to 1045-1049. A relatively small amount of protein (4-8mg%) appears in the urine. At the same time, the general picture of sublimate poisoning develops - vomiting, sometimes bloody, bloody diarrhea, increased salivation. The dog becomes lethargic, refuses food, drinks a lot. Death occurs on the 3rd-10th day after poisoning as a result of retention of nitrogenous wastes in the body (uremia) [13]. An autopsy reveals slightly enlarged kidneys, the tissue of which has a reddish-brown color with multiple petechial hemorrhages. Hemorrhages are found along the gastrointestinal tract. Such a clinical picture is also characteristic of sublimate necrotic nephrosis in the human clinic.

2) Changes in diuresis during sublimate poisoning can also be studied on frogs. Two frogs of the same weight are injected into the dorsal lymphatic sac with 3ml of distilled water. One of them, in addition, injected under the skin of the thigh 0.05ml of 1% sublimate solution. Both frogs are placed for 2-3 hours in funnels inserted into graduated test tubes, where excreted urine is collected. At the end of the experiment, the rest of the urine is squeezed out of the bladder by pressing on the lower abdomen of the frogs. It is noted that in a poisoned frog, diuresis drops sharply. It is not possible to establish a preliminary increase in diuresis with this form of experiment [14]. Amyloid-lipoid nephrosis (unlike sublimate nephrosis) is one of the manifestations of profound metabolic disorders throughout the body [15]. Along with a decrease in diuresis, albuminuria and edema are pronounced with it.

Demonstration of Impaired Nervous Regulation of Diuresis in Sublimate Nephrosis

Experience put on a dog poisoned by sublimate. 1-2 hours after sublimate poisoning, against the background of high diuresis, the reaction of the dog to electro cutaneous pain stimulation is examined. It turns out to be weakened - instead of reflex anuria, only short-term oliguria occurs. In the future, against the background of oliguria, the reaction to pain stimulation is almost completely absent, although the defensive (motor) reaction persists until the very last days of the animal's life. Obviously, in the analysis of changes in diuresis in nephrosis, it is necessary to take into account not only structural disorders in the kidneys, but also changes in the nervous regulation of urination [9].

Demonstration of Changes in Diuresis During the Accumulation of Nitrogenous Slags

The retention of nitrogenous wastes in the body causes compensatory polyuria with a low specific gravity of urine. Such changes occur with nephrosclerosis. An increase in diuresis under the influence of nitrogen metabolism products is demonstrated by the following experience. Experience put on 4 mice placed in funnels, covered with nets. Graduated test tubes are placed under the funnels. At the beginning of the lecture, two mice are injected subcutaneously with 3-5ml of a 2% urea solution. An hour later, the amount of urine released is compared. After the introduction of urea, diuresis increases, the specific gravity of urine decreases [16]. The experiment can also be done on a dog with ureters removed or in an acute experiment (a dose of urea is 5 ml of a 2% solution intravenously). When compensatory polyuria is insufficient and the residual nitrogen in the blood rises sharply, a picture of uremia develops [9].

Demonstration of Uremia caused by Lesions of the Urinary Tract

Uremia can develop both with diffuse kidney damage and with damage to the urinary tract, which prevents the excretion of urine from the body. 2-3 days before the lecture, the dog's abdomen is cut along the midline, the ureters are found and ligatures are applied to them. The wound is sutured. Already on the 2nd or 3rd day, the dog becomes depressed, refuses to eat, but greedily drinks water. Immediately after drinking, she vomits and all the water pours out. This picture is shown at the lecture. Nitrogenous products are excreted through the lungs and gastrointestinal tract, so the smell of urine is mixed with exhaled air and vomit [17]. Gradually, the animal falls into a coma, its blood pressure drops, its temperature drops, and death occurs on the 3-5th day after ligation of the ureters. At autopsy, the renal pelvis is distended with accumulated urine. The cavities and tissues are dry (anhydremia), which is explained by a large loss of water during vomiting and diarrhea (according to the observations of clinicians, with the appearance of uremia, the renal edema that was previously in the patient disappears). The abdominal and pleural cavities, and especially the stomach cavity, emit the smell of urine [18].

Demonstration of the Toxicity of Nitrogenous Products Contained in the Urine

The frog is injected under the skin of the back with 2-3 ml of urine. There comes inhibition of acid reflexes, adynamia, sometimes death. The urine of patients with uremia is not toxic, since it contains few products of nitrogen metabolism. In the pathogenesis of uremia, in addition to the retention of nitrogenous slags, one should also take into account the shift in the reaction of the blood to the acid side - acidosis. The reason for it is the delay in the excretion of acidic metabolic products, mainly acidic phosphate salts. Acidosis is compensated by increased release of carbon dioxide through increased respiration (uremic asthma), as well as the formation of ammonia [9].

Diuresis Disturbances for Disorders of Circulation

Demonstration of Diuresis Disorders in Anemia of the Kidney

1) The dog under general anesthesia is fixed with the stomach up. The jugular or femoral vein is dissected. Open the abdominal cavity, find the ureters and cut them at the bladder. Long, curved cannulas are inserted into the central ends of the ureters and lowered into test tubes to collect urine. Summing up the ligature under one of the renal arteries (more convenient under the left). Within 30 minutes, the diuresis of the right and left kidneys is determined (collecting five-minute portions), then the ligature is tightened for 2-3 minutes. This is usually enough to cause anuria that lasts 10 to 20 minutes after the ligation is removed. Diuresis in a healthy kidney increases slightly. Protein appears in the first portions of urine, the specific gravity of urine is increased [9].

2) The dog is injected intramuscularly with 5-10ml of a 1% solution of indigo carmine or intravenously with 50ml of a 1% solution of methylene blue. An anemized kidney begins to secrete dye later than a normal one.

3) Loading with urea (5-10ml of a 2% solution intravenously) also reveals insufficiency of the affected kidney.

4) Bandage the ureter of a healthy kidney. The diuresis of the anemic kidney increases.

Demonstration of Diuresis Disorders in Venous Congestion in the Kidney

The preparation for the experiment is the same as in the previous demonstration, but the ligature is brought under the renal vein. Determine the amount of urine in 30 minutes and tighten the ligature for 5-10 minutes. Anuria occurs, lasting 20-30 minutes after the clamp is removed. Protein appears in the urine. It is also expedient to perform this experiment with a test for the excretion of indigo carmine and a load of urea [18,19]. Demonstration of oliguria with a fall in blood pressure. Establish registration of blood pressure in the carotid artery. The preparation for the experiment is the same. A cannula is inserted into the femoral artery to drain blood. The amount of urine excreted by both kidneys in 30 minutes is determined, then 1/3-1/4 of the total blood volume is released from the artery. Note a sharp drop in blood pressure and at the same time a sharp oliguria. When blood pressure falls below 40 mmHg, anuria occurs. A drop in blood pressure can also be caused by transection of the spinal cord under the oblongata [20,21].

Demonstration of Polyuria with Plethora

The preparation is the same. Set the amount of urine for 30 minutes, then injected into the femoral vein saline (at 38°) in a volume equal to the volume of the entire blood of the dog. Urination increases, the specific gravity of urine decreases [9]. Thus, the data presented in the review on modeling the pathology of the urinary system in the experiment represent a fundamental basis for further study of this system, deepening and detailing the pathogenesis of diseases, allowing you to create a basis for clinical research.

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