

Importance of urinalysis in veterinary practice – A review

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Abstract

Urinalysis is a remarkable tool that can reveal many of the diseases that could go unnoticed and undiagnosed because they generally do not produce striking signs or symptoms. Examples include diabetes mellitus, various forms of glomerulonephritis, and chronic urinary tract infections. Observing the colour, transparency, microscopic and chemical characteristics of urine and urinary sediments coupled with microbial culture and sensitivity test is likely to identify majority of the lower urinary tract disorders in domestic animals. Urinalysis though being a readily available and an inexpensive tool for the diagnosis and management of numerous urinary tract abnormalities, it is still a much neglected facet in veterinary medicine. This review is aimed at highlighting the various beneficial aspects of urinalysis which is an indispensable diagnostic tool in veterinary practice.

Keywords: bilirubin, glucose, ketone, kidney, protein, urine, urinalysis

Introduction

Urinalysis, despite being an immensely useful tool, is perhaps the most underused test in veterinary practice. When performed properly a urinalysis, specifically the measurement of urine specific gravity (SG), can be a measure of tubular function. Finding casts, WBCs, and bacteria in urine is the best way to detect renal diseases before the onset of renal failure (Table-3). Urinalysis can also help detect metabolic diseases such as diabetes mellitus through measurement of glucose and ketone concentrations, liver diseases based on bilirubin measurement, and intravascular haemolysis as indicated by increased haemoglobin values [1]. A complete urinalysis, therefore, includes determining SG, verifying the urine's chemical properties and microscopically examining the urine sediments. Unfortunately it is a much neglected aspect in the current veterinary medicine; hence this attempt was contemplated to review the various aspects and advantages of urinalysis in veterinary medicine and to encourage veterinarians to utilize this valuable and easy-to-use tool during their practice for easily diagnosing certain diseases, which otherwise would require complicated protocols.

Urine Sampling

The method of collecting the urine sample may affect the results of analysis. For consistency, a standardised volume of urine should be obtained each time, to allow comparison of results of urine sediment examination with subsequent samples. Urine samples

can be obtained as free catch (mid stream voided) or through other collection techniques like cystocentesis and catheterisation. Of the three techniques, cystocentesis is the most practical and accurate one [2]. Collection by cystocentesis is accomplished swiftly and safely by inserting a needle through the abdominal wall directly into the bladder [3]. This method simplifies interpretation of the results by eliminating the possibility of contamination from the urethra and genital system [4]. Urine samples should be collected into a clean container with a minimal contact of the voided urine with the animal body. Ideally, new clean containers with tight-filling lids should be used. Asepsis should be strictly maintained while collecting the urine samples. Urine samples should be analysed as rapidly as possible after collection, ideally within 30 minutes. If this is not possible, samples should be refrigerated immediately and stored preferably for no more than 6 – 12 hours after collection. Refrigerated urine should be brought to room temperature and thoroughly mixed prior to the analysis. However, freezing of urine should be avoided, if possible, because it may lead to disintegration of casts and other cells like RBCs, besides inducing changes in the urine pH [5].

Physical Examination of Urine

Colour and transparency: The colour and transparency of urine is recorded while observing it in a test tube or in a urinometer cylinder. The colour is always considered in association with SG and volume. The normal colour of urine is yellow to light amber in cattle and it depends primarily on the concentration of urochromes, whose output is relatively constant (Tables-1)[6]. Urine may be light to dark yellow and pale pink in colour in

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Table-1. Reference values for various urinary parameters in different species [6,39]

Sr. No	Parameters	Cattle	Sheep	Goat	Horse	Dog	Cat	Rabbit	Humans
1.	Urine volume	16-50ml/kg	10-40ml/kg	10-40ml/kg	8-30ml/kg	14-50 ml/kg	18-25 ml/kg	20-350ml/kg	1-2L/day
2.	Colour	Pale yellow – dark brown yellow	Pale yellow-dark brown yellow	Pale yellow-dark brown yellow	ochre	Pale yellow-brown yellow	Yellow –strong dark yellow	Pale yellow – red brown	Colorless –umber
3.	Transparency	clear	Clear	clear	turbid	clear	clear	clear	Clear
4.	Odour	Aromatic	Indifferent aromatic	Indifferent aromatic	Aromatic	Garlicy	Sharp	n.s	Coffee, Safron, Onion
5.	Specific gravity	1.020-1.040	1.020-1.040	1.020-1.040	1.020-1.040	1.001-1.065	1.001-1.080	1.003-1.036	1.003-1.030
6.	pH value	7.0-8.4	7.5-8.5	7.5-8.5	7.6-9.0	5.5-7.0	5.0-7.0	8.2	4.6-8 {7}
7.	Protein	negative	negative	negative	negative	negative	negative	0-20mg/dl	
8.	Glucose	negative	Negative	negative	negative	negative	negative	negative	Negative
9.	Ketones	negative	Negative	negative	negative	negative	negative	negative	Negative
10.	Bilirubin	negative	Negative	negative	negative	Negative-weak positive	negative	negative	Negative
11.	Urobilinogen	Negative-weak positive	Negative-weak positive	Negative-weak positive	Negative-weak positive	Negative-weak positive	Negative-weak positive	Negative-weak positive	0.2-1mg/dl
12.	Blood	negative	Negative	Negative	negative	negative	negative	negative	Negative
13.	Leukocytes	negative	Negative	Negative	negative	negative	negative	negative	0-2/high power field(Hpf)

bovines suffering from urolithiasis [7]. Freshly voided urine from the healthy animals is usually clear, except in horses where it is usually thick and cloudy due to the presence of calcium carbonate crystals and mucus [8]. Cloudy urine may not necessarily indicate pathology, as many samples become cloudy upon standing [9]. Interestingly, in bovine obstructive urolithiasis urine may still be transparent and clear [3, 7]. In a study conducted on calves suffering from obstructive urolithiasis, urine was found to be of different colours and of different appearances than normal. The variation in the colour of urine of the affected animals on day zero probably could be due to the variation in the concentration of urine, accumulation of sediments and haemorrhage. Dirty yellow coloured urine might be due to presence of sedulous materials in the urinary bladder. Brownish urine is indicative of mixing of blood in the urine, which could be due to haematuria or nephritis. Reddish colouration of urine is indicative of haematuria, which could be due to injury by calculi or inadvertent haemorrhage while performing surgery (Table-1) [10].

Specific gravity: Specific gravity (SG) which is directly proportional to urine osmolality, measures solute concentration and urine density, or the ability of the kidney to concentrate or dilute the urine over that of plasma. It is thus a valuable test, as the loss of concentrating ability of the kidneys is among the first signs of renal tubular disease. Urine with a SG outside the range i.e. > (1.020-1.040) suggests alteration by the renal tubules. SG of urine can be recorded either using urinometer method or by a refractometer in cases when the urine quantity is very small (Table 2). Most of the refractometers are calibrated for use with human samples, although instruments for veterinary use are also available. The latter have one calibration scale for dogs and large animal urine samples, which is similar to the scale for human urine samples and a separate scale for cat urine samples [11]. In a specially calibrated refractometer, SG can be readily obtained by

measuring the refractive index (RI). The instrument measures the degree by which light is bent (refracted) when it passes through a liquid. The amount of refraction/RI is a function of the amount and type of solute (particles) present in that liquid. RI and SG are correlated, as SG is the ratio of the density of a substance compared to the density (mass of the same unit volume) of a reference substance and RI is expressed as a ratio of the speed of light in vacuum relative to that in the considered medium. The SG of a solution thus depends on the number and the molecular weight (size) of particles in the solution. Urine always has a SG greater than that of distilled water, which has a SG of 1.00 (Table-1).

SG is a valuable test for evaluating kidneys. The low urine specific gravity, could be caused by osmotic diuresis, loss of medullary tonicity (medullary washout), resistance, and deficiency in the antidiuretic hormone. Osmotic diuresis occurs in diabetes mellitus, Fanconi syndrome and primary renal glucosuria, where an excess amount of glucose in the glomerular filtrate prevents water being reabsorbed in the distal tubules. Loss of hypertonicity in the renal medulla may result from hypoadrenocorticism (loss of sodium), liver disease, prolonged or vigorous fluid therapy. Resistance to Anti diuretic hormone (ADH) termed nephrogenic diabetes insipidus, occurs commonly secondary to many conditions including hypercalcaemia, chronic liver diseases, pyometra, hyperadrenocorticism and hypokalaemia. Specific gravity in health varies with the state of hydration and fluid intake. The range of specific gravity of urine in normal cattle is 1.025-1.045 with an average of 1.035 [10] and in obstructive urolithiasis it ranges from 1.008 to 1.025 [3]. Under normal conditions, urine SG ranges between 1.015 and 1.040 in healthy dogs and between 1.036 and 1.060 in healthy cats (Table-1) [12].

Chemical analysis of urine

Chemical analysis of urine can be used to identify

Table-2. Clinical conditions that can be identified using urinalysis [40]

Parameters	Clinical conditions
High specific gravity (>1.035)	Nephrotic syndrome, dehydration, acute glomerulonephritis, heart failure, liver failure, or shock
Low specific gravity (<1.005)	Diabetes insipidus, nephrogenic diabetes insipidus, acute tubular necrosis, or pyelonephritis.
Protein in urine	Renal disease, fever, congestive heart failure (CHF), hypertension, tumors, and others.
Glucose in urine	Sugar, levels are obviously important in diagnosing diabetes.
Ketone bodies in urine	Ketonuria occurs in diabetes mellitus and starvation.
Bilirubin	Liver damage or disease.
Blood	Hemoglobin or myoglobin, Kidney damage, infection, kidney or bladder stones, kidney or bladder
cancer	or blood disorders, among other conditions.
Red blood cells (RBC's) in the urine	Haematuria
White blood cells (WBC's) in the urine	Infection, urinary tract infections (UTI)
Crystals	Hypercalcemia

Table-3. Characteristics of urinary casts and conditions in which casts are typically observed [41]

Type of casts	Component	Common clinical conditions
Hyaline	Mucoprotein	Normal health, fever, exercise, diuretics, renal disease
Granular	Degenerating cellular casts or aggregated proteins	Glomerular disease, tubular disease, pyelonephritis, viral infections
Waxy	Final stages of granular cast degeneration	Advanced renal failure or other conditions with dilated tubules with diminished flow
Fatty	Lipid-containing renal tubular cells	Nephrotic syndrome
RBC's	Red blood cell	Glomerulonephritis, tubulointerstitial nephritis, acute tubular injury/necrosis
WBC's	White blood cells	Pyelonephritis, glomerulonephritis, tubulointerstitial nephritis
Epithelial cell	Renal tubular cells	Epithelial cell Renal tubular cells

the composition of the calculi [13].

Urinary pH: Urine pH is a measurement of the kidneys ability to conserve hydrogen ions, thus it provides a rough but useful estimate of the body's acid-base status. However, urine pH does not necessarily reflect the body's pH, as it is highly influenced by diet, recent feeding, bacterial infection, storage time, metabolic and respiratory alkalosis, and urinary retention [14]. Diet can have both immediate and short term effects on urine pH. High protein diets, such as those consumed by carnivores produce neutral to acidic urine. Herbivores tend to produce alkaline urine. Any animal may produce alkaline urine immediately after eating due to buffering that occurs in response to gastric acids. Alkaline nature of the urine is frequently linked to urinary tract infections. The bacteria break down urea and forms ammonia contributing towards the alkalinity of urine. Obstruction and renal tubular disease may also create alkaline urine. Acidic urine is commonly observed in animals with diabetes mellitus, especially if the animal is ketoacidotic. Excess or deficient dietary protein may lead to acidosis, as can Fanconi syndrome and metabolic acidosis (Table-2) [15]. The urinary pH in normal cattle is usually on the alkaline side and may range from 7.4 to 8.4 [14]. An accurate measurement of urine pH is critical for clinical decision making especially in urolithiasis cases [16]. Measurement of urine pH can be made using urinalysis strips, narrow range pH meter and portable pH meter. Of these, portable pH meter is highly accurate for the measurement of the urinary pH [17]. In bovine obstructive urolithiasis, urine is usually alkaline [7], however acidic urine is also not an uncommon finding [12]. The release of ammonia due to the breakdown of urea in the retained urine renders it alkaline [18]. Urine pH plays an important role in the formation of uroliths. Struvite and calcium apatite uroliths are mostly found

in urine with alkaline pH [8, 19], while cystine stones are formed at the acidic pH. However, pH is variable in the formation of urate, silicate and calcium oxalate stones [20].

Urinary proteins: Chemical analysis of urine for assessing its protein as an index of disease dates back to 1881 when Cotungo first demonstrated that the urine of some patients precipitated upon heating [21]. Protein in urine is most frequently evaluated using a dipstick, which primarily assesses the albumin content. Dipsticks detect protein by production of colour with an indicator dye. Bromophenol blue, is most sensitive to albumin, but detects globulins and Bence – Jones proteins poorly. Precipitation by heat is a semi-quantitative method, but overall it is not a highly sensitive test. The sulfosalicylic acid test is a more sensitive precipitation test as it can detect albumin, globulins and Bence – Jones protein even at low concentrations. When sulfosalicylic acid (SSA) is added to urine, proteins are denatured and form a precipitate which makes the sample turbid [21, 22]. The turbidity can be assessed visually or more accurately using spectrophotometry by comparing the sample turbidity with that of a set of standards. The SSA method is not widely used in commercial laboratories. The most accurate determination of proteinuria is the protein:creatinine ratio. Tubular concentration of urine increases the urinary protein and urinary creatinine concentrations equally so that the ratio remains constant whether the urine is concentrated or diluted. This ratio is normally less than one [18].

Non pathological causes of proteinuria include a high protein meal, exercise and stress. Pathological causes of proteinuria include renal disease, where glomerular leakage of proteins occur, cardiac insufficiency (filter is not working effectively), and

urinary tract infections and haematuria, where protein is associated with the cells present. Many diseases can contribute to proteinuria because the inflammatory response can cause a glomerulonephritis (Table 2 and 3) [15].

Normally urine does not contain proteins [12, 21]. The presence of protein in urine is called proteinuria. The reference range is negative to trace in most animals. Horses frequently have a higher normal level due to the presence of mucus in their urine. The presence of urinary protein could be an indication of predisposing factors for urolithiasis as about two thirds of the matrix of all urinary stones is composed of proteins [21]. A defective mucoprotein has been implicated in urinary stone formation (Table-3). Immunoreactive profiles of urinary proteins (inter α -inhibitor) have been found to be diagnostic tools to identify active stone formation [16]. A small amount of protein can be considered normal. Proteinuria may result from glomerulonephropathy, tubular transport defects, inflammation or infection within the urinary tract. Increased protein level in the urine might be due to acute nephritis or inflammatory exudation resulting from pyelitis, urethritis, cystitis and urolithiasis (Table-2) [23]. Haemorrhage must be of considerable size (macroscopic rather than microscopic) before it causes significant proteinuria [18].

Persistent microalbuminuria (MA) is an indicator of glomerular damage associated with early progressive renal disease in humans. Such low level of albumin loss cannot be not detected using routine dipstick analysis. Recently, new and highly sensitive dipsticks for urine microalbumin have become available for use in dogs and cats. These are immunological tests use a monoclonal antibody specific for canine or feline albumin. Studies have shown that 20 – 25% of healthy dogs and 30% of healthy cats exhibit MA, whilst approximately 40% of dogs and cats with known medical conditions display MA [24, 25]. The prevalence of MA increases with age [12]. Diseases that are associated with MA include cardiovascular disease, urogenital disease, dental disease, airway disease, pyoderma, inflammatory disease, hyperthyroidism, hyperadrenocorticism, diabetes mellitus, infectious diseases and neoplasia [26, 27]. Prednisolone therapy may also lead to MA. [26]. Thus it appears that a significant proportion of healthy animals and animals with diseases that are unrelated to the renal system may also have MA.

Urinary glucose: The presence of urine glucose is called glucosuria. A healthy animal excretes little to no glucose in its urine. Glucose is freely filtered and then reabsorbed in the proximal tubules, preserving it to be utilized as an energy source. If the blood glucose level is too high (hyperglycaemia), it exceeds the ability of the kidney tubules to reabsorb it (i.e., it exceeds the renal threshold), and the glucose is excreted in the urine. The renal threshold in the dogs is 10mmol/L and is slightly higher in cats (14-17mmol/L). Glucosuria in

combination with hyperglycaemia reflects a tubular resorption defect in which the renal tubules fail to reabsorb glucose from the glomerular filtrate (Table-2) [28, 29].

Nonpathologic glucosuria is associated with eating (postprandial), excitement and stress (especially in cats and horses). Pathologic glucosuria is associated with diabetes mellitus, acute renal failure, and urinary obstruction in cats and milk fever in cattle. Numerous factors can decrease urine glucose values. These include refrigeration, ascorbic acid (vitamin C), salicylates, penicillin and presence of bacteria [30].

Ketonuria: Ketonuria is the presence of ketones in urine. Their reference range is negative to trace. Ketone bodies that commonly appear in the urine when fats are burned for energy are acetoacetate and beta-hydroxybutyric acid. Acetone is also produced and is expired by the lungs. Normally, the urine should not contain a noticeable concentration of ketones to give a positive reading. As with tests for glucose, acetone can be tested by a dipstick or by a lab. The results are reported as small, moderate, or large amounts of acetone. Dipsticks detect acetoacetate and, to lesser extent, acetone, but do not detect beta-hydroxybutyrate. Most commonly ketonuria is pathologic, especially in small ruminants and is usually associated with diabetic ketoacidosis. Cold and exercise may cause ketonuria. Animals in late pregnancy and early post parturition may develop ketosis (pregnancy toxemia), a severe and sometimes fatal disorder. Vomiting and diarrhoea may also result in ketosis, as can starvation.

Bilirubinuria: Similar to other urine chemicals, bilirubin should be present in only negative to trace amounts. If large amounts are present, the condition is referred to as bilirubinuria. There is a low renal threshold for bilirubin; hence even small increases in plasma bilirubin can lead to bilirubinuria. This bilirubinuria can be detected prior to hyperbilirubinaemia or jaundice. Unlike dogs, bilirubinuria in cats, even when present in small quantities in concentrated urine, is usually indicative of an underlying disorder. In cats bilirubinuria has been associated with several disorders including primary hepatic disease, diabetes mellitus, feline infectious peritonitis and feline leukemia-related disorders. Bilirubinuria is caused by conjugated (water-soluble) bilirubin, because unconjugated bilirubin is bound to albumin, which does not usually pass through the glomerular barrier in significant amounts unless glomerular disease is present (Table-2). Bilirubinuria is not observed in healthy cats but a small amount of bilirubin may be occasionally found in the urine of dogs. This is partly because dogs have very low renal threshold and in part because canine renal tubular cells are able to catabolise haemoglobin to unconjugated bilirubin, and then secrete it into the urine [29]. Pathological causes for bilirubinuria include bile duct obstruction, hepatic necrosis caused by infectious canine hepatitis,

leptospirosis and other infectious diseases, and haemolytic diseases such as immune-mediated haemolytic anaemia. Bilirubin is unstable and is decreased by exposure to light and high levels of vitamin C [31].

Inorganic constituents of urine: Certain physiological relationships do exist among the dietary intakes of specific minerals and their corresponding levels in the urine and the probability of occurrence of urolithiasis [16]. A seasonal relationship of urinary silica, calcium, magnesium, sodium and potassium with their dietary intake and incidence of urolithiasis exists [31]. Animals that excrete high amounts of phosphorus in their urine will be more susceptible to the formation of insoluble phosphates and urinary calculi [16]. Urinary excretion of phosphorus has genetic basis, as some breeds of sheep like Texel and Scottish blackface excrete more phosphorus in urine when compared to other breeds, and are hence more susceptible to phosphate urolithiasis [16]. Increasing dietary magnesium levels effectively reduces urinary phosphorus excretion and thus reduces the likelihood of calculus formation [32]. However, Jawalekar et al [31] found that lambs fed on high phosphorus, low calcium diets had increased concentration of phosphate in urine, which was associated with a significant decrease in urinary excretion of magnesium and potassium and increased the incidence of urolithiasis [16].

Levels of serum phosphorus in patients with chronic kidney disease remain within the normal range or may even be modestly below the normal range until the glomerular filtration rate declines. About 75% of renal function must be lost before this elevation is detectable. Serum calcium concentration, the counterpart of phosphorus, is elevated in some cases of renal failure. However the body's homeostatic mechanisms appear to be able to regulate serum calcium and phosphorus concentrations very efficiently by the increasing parathyroid hormone (PTH) secretion [33]. Potassium, like phosphorus, is excreted mainly by the kidney. In chronic renal failure where reduction in renal function progresses slowly, the animal's physiological mechanisms compensate and serum potassium levels remain normal [32]. When renal failure occurs more rapidly, as it does in acute cases and post renal disorders, the serum potassium concentration will rise [23]. Because serum potassium can be elevated by diseases other than renal failure, it is not a reliable test for detecting renal disease. It can be used, however, to differentiate the type of renal disease present, because hyperkalaemia suggests acute renal failure [29].

Enzymes: Injury caused by oxalate crystals to the renal epithelial cells may cause increase in the levels of various enzymes like lactate dehydrogenase (LDH), α -Gluta-myltranspeptidase, alkaline phosphatase (AP), Inorganic pyrophosphates, β -glucuronidase, λ -acetyl

γ -D glucuronidase and lipid peroxidase [34]. Hence a positive correlation exists between urinary excretion of oxalate crystals and levels of these enzymes.

Microscopical examination of urine

The microscopic examination of urine is of great clinical importance. The important structures to identify include crystals, erythrocytes, leukocytes, casts and bacteria [35]. Supravital staining technique, using 1% crystal violet and 0.5% safranin in normal saline is considered reliable for analyzing urinary sediments under ordinary bright field microscopy.

Crystalluria: Crystalluria is a frequent finding during the routine examination of urine sediments. In most instances the precipitation of crystals of calcium oxalate, uric acid, triple phosphate, calcium phosphate and amorphous phosphates or urates is caused by transient super saturation of urine, ingestion of specific foods, or by changes of urine temperature and/or pH which occur upon standing after micturition. Crystalluria is also associated with pathological conditions such as urolithiasis, acute uric acid nephropathy, ethylene glycol poisoning, and hypereosinophilic syndrome. Additionally, crystalluria can also be due to drugs such as sulphadiazine [34]. Because different crystals have different appearances, microscopic examination of urine sediments has diagnostic importance for the identifying the diseases of urinary system. Struvite crystals have coffin lid appearance; calcium oxalate monohydrate crystals have picket fence appearance, envelope ditetragonal pyramids/bipyramidal shape, while calcium oxalate dihydrate crystals have a maltese cross or square envelope shape [36]. Urate crystals are thorn apple or fine needle shape and cystine crystals are of hexagonal shape. The urine of a goat fed on a diet containing calcium and oxalic acid was found to contain numerous cuboidal bipyramidal; and unique rectangular parallel piped calcium oxalate dihydrate (COD) crystals [34]. The crystal number is of greater significance than crystal shape and size [34]. Planar or X-shaped morphology of struvite crystals indicate rapid growth while misshapen or octahedral shape indicates a slow growth rate [37].

Cellular Components: Evaluation of the cellular components in the urine sediment is complicated by the fact that cells may originate from several areas such as the vascular system, interstitial tissue, urothelium or the genital tract [14]. A certain number of epithelial and transitional cells in urine are normal. The presence of increased number of white blood cells are evident in cystitis and pyelonephritis (Table-3). Likewise few leukocytes may be present in normal urine. Pyuria indicates a purulent process at some point in the urinary tract especially urethritis or cystitis [38].

Conclusion

Urinalysis is a high-volume procedure that normally requires significant amount of labor. However, para-

meters obtained from urinalysis are still extensively used to guide empirical treatment of urinary tract infections. Urinalysis when performed properly is a highly reliable index of renal disease. This is especially true for minor changes in renal pathology which are not usually accompanied by abnormal blood biochemical values, as in cases of early lupus and other glomerular diseases. Thus, urinalysis is of great importance since it enables us to provide patients with early treatment and thereby enhances the chances of recovery. Nevertheless, urinalysis has gained some undesirable disrepute over the past few years as a result of poor methodology. Hence, rather than condemning the procedure as ancient and outdated, we strongly believe that the need of the day is indeed to promote urinalysis as an extension of bedside clinical medicine. We recommend that urinalysis should be performed by the clinician himself, who is well-versed with the technique. This ensures immediate and accurate examination of urine samples and better standardisation.

Urinalysis is a safe, non-invasive method, simple study of urine which requires only urination on the part of the subject; it creates no discomfort, poses no health-related risks, has no direct side effects, and evinces no adverse responses.

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