Determination of the Protein Concentration in Canine Urine

By

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Introduction

There are many laboratory methods which can assist the clinician in the diagnosis of renal disease. One of the most important is the determination of proteins in urine, for which many methods are available (Martinek, 1970). In this country in veterinary medicine one of the most widely used tests is heat coagulation at acid pH (Henry, Cannon and Winkelman, 1974). The results are expressed as (negative), (trace), (+), (++) etc. They are based on the degree of turbidity and flocculation of the treated sample in comparison to non-treated urine. The main disadvantage of this method is the subjectivity of the reading of the results.

Other widely used tests are the paper-strip tests (Albustix: Ames Company, Div. of Miles Labs., Elkhart, Ind., U.S.A.) in which the reagents (indicator and buffer) are incorporated in a dried form on a strip of filter paper. The test is based on the protein error of indicators. At the pH of the buffer of the strip most of the bromphenol blue indicator is in the yellow, non-ionized acid form (HI), with only a small portion in the blue ionized form (I⁻). If protein is present, it will bind with the I⁻-form. This anion has a greater affinity for the protein than for the hydrogen ion. The removal of I⁻ will cause more of the indicator acid to ionize and the concentration of HI will decrease, resulting in a change of the ratio I⁻/HI. The color presented by the paper strip will depend on the relative proportions of the two forms. The quantity of the indicator is fixed and, if sufficient protein is present, the larger fraction of the indicator will be in the colored form. The greater the quantity of protein, the deeper blue will be the hue of the indicator strip.

As the quantity of buffer is relatively small, applying the strip to strongly buffered urine samples or to samples with a high pH will give false positive readings. This test compares favourably with the other usual qualitative tests (Bradley and Benson, 1969) but the method was developed for human urine. Its sensitivity is greater for albumin than for other urinary proteins (hemoglobin, Bence-Jones proteins and mucoproteins). Bradley and
Benson (1969) reported that the effective range of the Albustix method is from 0.20 to 0.30 g. of plasma albumin per 1,000 ml. of urine. According to the manufacturer the lowest concentration of urinary proteins that can be demonstrated is influenced by the relation between the different types of proteins, the concentration of the chromogenic substances, urinary buffers and salts, light, and the ability of the user to differentiate colors.

The aim of our investigations was to find a simple and reliable method that could be used by the general practitioner with a minimum of laboratory equipment, as with the Albustix or heat coagulation methods.

First it was necessary to find a method which would determine quantitatively the protein concentration in normal canine urine. From our previous experience it was expected that the normal range in dogs would be greater than in men. Such a method would be suitable only where more sophisticated laboratory equipment existed (photometer or spectrophotometer).

A large number of quantitative methods is available. In some methods non-proteins cause interference, such as in the method developed by Saifer et al. (1964), based on the sensitive method of Lowry et al. (1951). Other methods are too complicated. Doetsch et al. (1973, 1975) developed a method of gel filtration combined with a modified biuret method. It combines the sensitivity of the Lowry method and biuret specificity. An improved method of Rice (1975) is less complicated than the above mentioned method, but more so than the method we decided to use (Pesce and Strande, 1973).

Material and Methods

The heat coagulation test (test according to Bang) was carried out as follows. Place in two test tubes about 5 ml. of urine, clarified by centrifugation. Add to both tubes 1 ml. of buffer and bring one to boiling in a flame. Compare the contents of both tubes in ordinary room light. If no difference is observed the test is reported as negative (—). If a barely visible turbidity was present the test is reported as “trace”. Greater turbidities are reported as (1 +), (2 ++), etc.

The buffer consists of a mixture of 56.5 ml. of glacial acetec acid and 118.9 g. of sodium acetate dissolved in 1,000 ml. of water; buffer pH = 4.6 (Noyons, 1951). This method was slightly modified by Henry, Cannon and Winkelman (1974).

The Albustix-method was used according to the manufacturer’s instructions.

The Pesce and Strande method (1973) is a micro-method based on the coagulation of proteins in urine by a trichloroacetic acid Ponceau S mixture.

The test was carried out as follows: Urine was centrifuged for 10 minutes at 3,000 rpm. Two hundred microliters of supernatant or the same amount of a bovine albumin solution (50 or 100 mg. per 100 ml.) were transferred to centrifuge tubes. Two ml. of the TCA-dye-solution was then added and mixed with a Vortex-type mixer. The solution was centrifuged for 10 minutes and the supernatant was then removed by suction. Four ml. of 0.8 % NaOH was added and the sediment dissolved by gently agitating and the absorbance read at 546 nm. (Gilford spectrophotometer-300 N). Trichloroacetic acid, NaOH and all other reagents were of analytical grade.

The TCA-dye-solution was made up as follows: Four grams of Ponceau S (Fluka A. G. Switzerland) was dissolved in 100 ml. of distilled water and diluted with 24.9 g. of TCA and 5 g. of NaCl to 1 liter with distilled water.

The protein stock solution was made by dissolving 0.05 g. of bovine albumin (Baker no 318005, fraction V) in 50 ml. of a solution of 5 % NaCl
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(1 g. per 1,000 ml.). It is advisable to incorporate in each test a protein solution containing 0.5 g. per 1,000 ml.

Results and Discussion

Preliminary investigations showed that the calibration line of the Pesce and Strande method crossed the origin and was at least linear to a concentration of 1 g. per 1,000 ml., when using a Gilford 300-N spectrophotometer. The precision of the method was fairly good. The coefficient of variation was 1.3 %, 1.5 % and 12 % at levels of 2.91, 1.26 and 0.109 g. per 1,000 ml. respectively (n = 20 in all cases). The recovery was 108 %, 93 %, 92 %, 96 % and 100 % at levels of 0.76, 0.87, 1.15 and 1.74 per 1,000 ml. respectively.

Statistical treatment of the results obtained after analyzing 40 samples or urine from normal dogs gave values that ranged from 0 to 0.56 g. of protein per 1,000 ml. of urine.

For the determination of the normal range we used the method of Rümke and Bezem er (1972). This method is a distribution-free one. We used Table 1

<table>
<thead>
<tr>
<th>Albustix</th>
<th>(-)</th>
<th>(trace)</th>
<th>(+)</th>
<th>(2+)</th>
<th>(3+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>0.27</td>
<td>0.02 - 0.37</td>
<td>0.03, 0.04</td>
<td>0.18, 0.43</td>
<td>-</td>
</tr>
<tr>
<td>(trace)</td>
<td>n = 61</td>
<td>n = 14</td>
<td>0.30</td>
<td>0.43, 0.71</td>
<td>-</td>
</tr>
<tr>
<td>(+)</td>
<td>0.55</td>
<td>0.09 - 0.55</td>
<td>0.21 - 0.51</td>
<td>0.43 - 1.49</td>
<td>-</td>
</tr>
<tr>
<td>n = 15</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 13</td>
<td>-</td>
</tr>
<tr>
<td>(2+)</td>
<td>0.36, 0.37, 0.74</td>
<td>0.40, 0.50, 0.70</td>
<td>0.63 - 3.33</td>
<td>1.60, 1.95, 2.27</td>
<td></td>
</tr>
<tr>
<td>n = 15</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 15</td>
<td>n = 10</td>
<td>-</td>
</tr>
</tbody>
</table>

and chose for the percentiles P 2.5 and P 97.5 with a reliability of 90 %. This means that one may state with a reliability of 90 % in at least 2.5 % of the normal dogs that the protein concentration is higher than 0.56 g. per liter. This method resembles the method of Herrera (1958) and of Reed et al. (1971) as described by Henry, Cannon and Winkelman (1974).

The distribution itself was a logarithmic one. The Pesce and Strande method was simple and rapid. In our opinion a definite advantage is that Ponceau S yields equivalent chromogenicity with both albumin and globulin fractions. The normal range of protein concentration in the urine of dogs is much wider than in human urine. Sunderman et al. (1970) found the range for human urine was 0.03—0.12 g. per 1,000 ml. Since albumin in a concentration of 0.05—0.20 g. per 1,000 ml. of urine can be detected as a trace by the Albustix-test, normal urines may show such a reaction.

According to the interpretation of the heat coagulation test (Bradley and Benson, 1969) a barely visible turbidity (++)-reaction is equivalent to 0.4—1.0 g. per 1,000 ml. If this interpretation is suitable for canine urine, all results below (++) will denote normal urine in respect to the protein concentrations.

In a second experiment the protein concentrations were determined by the Albustix, heat coagulation and Pesce and Strande tests. The results are given in Table 1, which shows that a (--) or (trace) Albustix reading means...
that the sample contains normal quantities of protein. When the Albustix reading is \((3 +)\) of \((4 +)\) the sample contains very high concentrations of proteins. A \((+)\) or \((2 +)\) Albustix reading, on the other hand is not decisive. In such cases a heat coagulation test must be performed or a quantitative determination with the Pesce and Strande method. A combination of Albustix \((+)\) and heat coagulation \((-)\), (trace) or \((+)\), leads to the conclusion that the protein concentration is normal, but a combination with heat coagulation \((++)\) cannot be interpreted. A heat coagulation \((++)\) means that the treated urine shows a moderate turbidity without flocculation. The protein concentration can be high/normal or low/abnormal.

If the Albustix reading is \((++)\) and the heat coagulation is \((+)\), the conclusion is the same.

Heat coagulation \((++)\) means a distinct turbidity but print is still visible through it.

A concentration of Albustix \((++)\) with heat coagulation \((++)\) of \((++++)\) means that the sample contains varying abnormal amounts of proteins. Heat coagulation \((++++)\) is a heavy turbidity without flocculation and \((+++)\) is a moderate turbidity.

All determinations were performed on samples of naturally voided urine and so proteins of other nature than plasma proteins were determined also (seminal fluid, prostatic fluid, vaginal discharge etc.).

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Summary

A method for the determination of proteins in canine urine is described, based on a combination of the Albustix and heat coagulation tests. As a reference system the Pesce and Strande method was used. The range of proteins in normal canine urine was \(0-0.56\) g per 1,000 ml. The method is suitable for use by the general practitioner.

The Pesce and Strande method gave excellent results and is recommended for use in suitably equipped laboratories.

Zusammenfassung

Bestimmung der Eiweißkonzentration im Urin von Hunden


Im normalen Hundeurin schwankt die Proteinkonzentration zwischen \(0-0.56\) g pro Liter. Die Methodik kann auch in der klinischen Praxis angewendet werden. Die Methode von Pesce und Strande gibt ausgezeichnete Ergebnisse, sofern ein entsprechend ausgerüstetes Laboratorium zur Verfügung steht.

Résumé

Détermination de la concentration des protéines dans l'urine des chiens

Une méthode de détermination des protéines dans l'urine de chiens est décrite; elle est basée sur une combinaison d'Albustix et du test de coagulation
à chaud. La méthode de Pesce et Strande fut utilisée comme système de référence.

Le taux normal de protéines dans l'urine canine varia de 0—0.56 g/litre. La méthode est applicable dans la pratique clinique. La méthode de Pesce et Strande donne d'excellents résultats et peut être recommandée pour les laboratoires convenablement équipés.

**Resumen**

Determinación de la concentración proteica en orina de perros

Basada en una combinación de Albustix y coagulación térmica, se elaboró una técnica para determinar las proteínas en la orina de perros. Como análisis de referencia se usó el método de Pesce y Strande.

La concentración proteica oscila en la orina normal de perro entre 0—0.56 g por 1000 ml. El método se puede emplear en la práctica clínica. El procedimiento según Pesce y Strande ofrece resultados excelentes, recomendándose su uso en los laboratorios provistos de equipo adecuado.

**References**


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