Ken Jenning’s wrote *Because I Said So! The Truth Behind the Myths, Tales, and Warnings Every Generation Passes Down to its Kids.* In his book, he considered the evidence that supports or refutes common dogmas within the United States. For example, “Was the nail you stepped on rusty? You’ll get tetanus!” Is the statement true, sometimes true, sometimes false, or false? Or, “Your first answer is usually the right one.” Is the statement true, sometimes true, sometimes false, or false?

A dogma is “a settled or established opinion, belief, or principle.” “Blind belief in authority is the greatest enemy of truth.” (Albert Einstein) “Education has failed in a very serious way to convey the most important lesson science can teach: skepticism.” (David Suzuki) “Most institutions demand unqualified faith; but the institution of science makes skepticism a virtue.” (Robert King Merton)

Are dogmas of clinical pathology valid? The following sections explore a few dogmas that have been or are being passed down from one generation to the next generation of veterinarians.

### Adjusted calcium formula can be used to determine if the hypocalcemia is due to hypoalbuminemia.

This dogma initially arose from a retrospective study that was published in 1982 (JAVMA 180: 63-67, 1982) in which two formulas were derived from measured concentrations of serum $tCa^{2+}$, albumin, and total protein (values expressed in non-SI units).

- Canine-adjusted $[tCa^{2+}] = measured [tCa^{2+}] - [Alb] + 3.5$
- Canine-adjusted $[tCa^{2+}] = measured [tCa^{2+}] - 0.4 \times [TP] + 3.3$

The proposed concept for the calculated adjusted $[tCa^{2+}]$ values was that if the value was within the reference interval for $[tCa^{2+}]$, the hypocalcemia was due to hypoalbuminemia (or hypoproteinemia) and there is not a decrease in the $[fCa^{2+}]$. If the calculated adjusted $[tCa^{2+}]$ was decreased, then there was a decreased $[fCa^{2+}]$. [Note: $fCa^{2+}$ (free calcium ion) is frequently called ionized calcium even though all calcium in the body is ionized; some Ca$^{2+}$ ion exists as free ions; other Ca$^{2+}$ ions are bound to a variety of anions.]

There are three major aspects of the 1982 article that are frequently ignored. The derived formulas represented the regression lines for the raw data that contained considerable individual animal variation. Considering the 95%–confidence intervals for the regression line, the formulas should be as follows. Accordingly, there is considerable variability in the calculated adjusted $[tCa^{2+}]$ values.

- Canine-adjusted $[tCa^{2+}] = measured [tCa^{2+}] - [Alb] + 3.5 \pm 1.3$
- Canine-adjusted $[tCa^{2+}] = measured [tCa^{2+}] - 0.4 \times [TP] + 3.3 \pm 1.6$

Second, the authors stated that about one-third of the variability in the $[tCa^{2+}]$ was due to changes in albumin concentrations. Lastly, the formulas were derived from data obtained in one clinical laboratory many years ago and people use the formulas for data obtained from different analytical methods without establishing analytical agreement.

Some of the same authors of the 1982 article wrote another article that was published in 2005 (Am J Vet Res 66: 1330 – 1336, 2005). They concluded that adjusted total Ca$^{2+}$ concentrations are unacceptable for predicting free Ca$^{2+}$ (ionized calcium) status in dogs.

The adjusted calcium statement is mostly false. However, adjusted calcium formulas do emphasize that the total protein and albumin concentrations do influence a patient’s $[tCa^{2+}]$ and thus should be considered when interpreting laboratory data.

### Pseudohypocalcemia is present when the hypocalcemia is due to hypoalbuminemia (or hypoproteinemia).

This more recent statement should not be used. When the $[tCa^{2+}]$ is decreased, there is a hypocalcemia if there is or is not a hypoalbuminemia or hypoproteinemia present. Those who wish to use the term “pseudohypocalcemia” in this context should consider what they should call the neutrophilia that occurs due to shifting of cells from marginated to circulating pools, or the hyperproteinemia that occurs due to decreased plasma water, or the erythrocytosis that occurs due to splenic contraction. Just because there is not a convenient term for a decreased $[fCa^{2+}]$, let’s not use terms that are incorrect.

### In acute inflammation, the release of endogenous cortisol causes the lymphopenia or Stress of the acute inflammatory disease causes a lymphopenia.

These statements have been in the veterinary literature for decades – but where is the evidence that they are true? The statements reflect the concept that the inflammatory state stresses the animal sufficiently to cause a release of cortisol which induces the movement of lymphocytes from the circulating blood and thus a lymphopenia develops.

Increased cortisol activity (or activity of other glucocorticoids) are known to create a lymphopenia. If the lymphopenia in an acute inflammatory state is due to excess cortisol, should we also see other evidence of excess cortisol such as mature neutrophilia,
monocytosis, hyperglycemia, increased ALP activity (dogs), hypercholesterolemia, or polyuria? Why do we accept stress as the cause of the lymphopenia when we do not find other clinical abnormalities that are attributable to excess cortisol?

In a 1995 article in *Adv. Immunol.*, B.A. Imhof described the effects of inflammatory cytokines on blood leukocytes. There is evidence that the cytokines promote the homing of blood lymphocytes to lymph nodes and the migration of lymphocytes to inflamed tissues; these processes can create the inflammatory lymphopenia.

We have evidence that the acute inflammatory reaction alters the movement of blood lymphocytes to create a lymphopenia. To my knowledge, we do not have evidence that inflammation creates a sufficient increase on plasma cortisol to cause a lymphopenia.

A transudate occurs because of hypoalbuminemia; usually when plasma [albumin] is < 1.5 g/dL. (or < 1.2 g/dL, or < 1.8 g/dL) Or A pure transudate is hypocellular (< 1000/µL) and has a TS concentration < 2.5 g/dL.

First, let’s consider the statement that “a transudate occurs because of hypoalbuminemia.” An inherited disorder is recognized in people in which there is no synthesis of albumin by hepatocytes; i.e., analbuminemia. Their albumin concentrations are < 0.1 g/dL and they typically do not develop pleural or peritoneal transudates. How can we attribute the formation of transudates to hypoalbuminemia when people with analbuminemia do not have transudative effusions? Also, how can we state that certain albumin concentrations lead to transudation when analbuminemia does not lead cavity transudates?

A transudate is an effusion produced by changes in mechanic factors such as oncotic pressure or hydraulic pressure in capillary beds. Basically, the determining factor for the accumulation of cavity transudates is the difference between the hydraulic pressure gradient (hydraulic pressure within vessels – hydraulic pressure in interstitial fluid) and the oncotic pressure gradient (oncotic pressure within vessels – oncotic pressure in interstitial fluid). When this difference leads to more fluid leaving the vascular bed than what can be removed by lymphatic vessels, a transudate forms. If transudation occurs in blood vessels that have minimal protein permeability, then a protein-poor transudate accumulates.

It is important to recognize that the plasma oncotic pressure is due to both albumin and globulins; albumin molecules are the major contributors to oncotic pressure but combined contributions of the globulin molecules are also important.

Two common canine disorders that cause the formation of protein-poor transudates are protein-losing nephropathies and hepatic cirrhosis. In these disorders, hypoproteinemia does reduce the plasma oncotic pressure but there also is an increased hydraulic pressure gradient in the portal blood vessels created by the retention of Na and H2O. The combination results in transudation and the formation of protein-poor transudates; the transudation is not solely caused by hypoalbuminemia.

A less common reason for the formation of a protein-poor transudate is presinusoidal portal hypertension. In this state, there is an increased hydraulic pressure gradient in the portal blood vessels but not a hypoproteinemia. Accordingly, the transudation is not caused by hypoalbuminemia.

For the second statement, *(A pure transudate is hypocellular (< 1000/µL) is typically true as there is no reason for the migration of leukocytes from blood to the cavity fluid. However, the second portion of the statement (TS concentration < 2.5 g/dL) may or may not be true.*

It is important to recognize that a serum or plasma “total solids concentration” is not equal to a “total protein concentration.” The total protein concentration is due to the concentrations of albumin and globulins. The total solids concentration includes the total protein concentration plus the concentrations of all other solids in the serum or plasma; i.e., glucose, urea, electrolytes, and other solutes. This data in the following table was extracted from a complete table in Wolf AV:

**Aqueous solutions and body fluids. their concentrative properties and conversion tables, 1966.**

<table>
<thead>
<tr>
<th>Human plasma [TP] (g/dL)</th>
<th>0.8</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human plasma [TS] (g/dL)</td>
<td>2.5</td>
<td>2.7</td>
<td>3.6</td>
<td>4.7</td>
</tr>
</tbody>
</table>

There are clinical refractometers that have a calibrated total solids scale (e.g., TS Meter Refractometer Model 10400B, Leica Microsystems). Most clinical refractometers have a calibrated total protein scale (even those that are called “TS Meters”) and the lowest unit commonly on the scale is 2.5 g/dL. The [TS] for a protein-poor transudate may be < 2.5 g/dL, but that should not be confused with a [TP] of < 2.5 g/dL.

A modified transudate is a transudate that has been modified by the addition of cells or protein. A modified transudate has a higher TS concentration than a pure transudate and moderate cellularity. A modified transudate has 1,000–7,000 cells/µL and a variable protein concentration (2.5–5.0 g/dL).

Using one or more of these definitions or criteria, a variety of cavitary effusions have been classified as modified transudates including the effusions of heart failure, feline infectious peritonitis, noninfectious exudates, hemorrhagic effusions, chylous effusions, uroperitoneum, neoplastic effusions, and bilious exudates. If we define a transudate as “an effusion produced by changes in mechanic factors such as oncotic pressure or hydraulic pressure in capillary beds,” then only the heart failure effusion qualifies as a transudate. None of the other effusions form via transudation and thus should not be called transudates or modified transudates.

The heart-failure effusions form when there is an increased hydraulic pressure gradient within blood vessels that are permeable to proteins. The classic mechanism occurs when central vein or hepatic vein congestion lead to increased hydraulic pressure with hepatic
sinusoids and the pressure forces out an excess amount of protein-rich fluid. When lymphatic vessels are not able to compensate adequately, then a protein-rich transudate accumulates. Pulmonary vessels are also protein-permeable, but not to the same degree as the hepatic sinusoids.

The acidemia of a lactic acidosis is due to increased production of lactic acid by cells.

This statement sounds logical but it does not reflect the true changes in biochemical pathways that occur in lactic acidosis. The cause of the acidemia was addressed in an article by S.C. Dennis, et al (J. Mol. Cell Cardiol. 23: 1077–1086, 1991).

When tissues have an inadequate supply of oxygen (i.e., when hypoxia is present), the cells attempt to generate ATP via anaerobic respiration (fermentation) (also called anaerobic glycolysis). In the final reaction and for each glucose molecule, this reaction occurs and is catalyzed by lactate dehydrogenase: $2 \text{pyruvate}^- + 2 \text{NADH} + 2 \text{H}^+ \rightarrow 2 \text{L-lactate}^- + 2 \text{NAD}^+$. It should be noted that L-lactate (an anion) is formed and not lactic acid; it should also be noted that H$^+$ is consumed in the reaction and thus makes the medium more alkaline, not more acidic.

Anaerobic respiration is an inefficient method of generating ATP from glucose; only 2 ATP molecules are produce for each glucose molecule. When there is an inadequate formation of ATP, the cells start the rapid hydrolysis of ATP to ADP and finally AMP. For each ATP molecule that is converted to AMP, 2 H$^+$ ions are formed.

One might say – if there is excessive formation of L-lactate and the excessive formation of H$^+$, doesn’t that mean there is excessive formation of lactic acid? Considering the pK$_a$ of lactic acid is 3.86, the ratio of lactate to lactic acid at a physiologic pH is greater than 1000:1.

The acidemia that occurs in animals with a lactic acidosis is due to excessive ATP hydrolysis in hypoxic tissues; not excessive formation of lactic acid.

The acidemia of a ketoacidosis is due to increased production of ketoacids by hepatocytes.

This statement sounds logical but it does not reflect the true changes in biochemical pathways that occur in ketoacidosis. The cause of the acidemia was addressed in an article by K.G. Alberti (Ciba Found. Symp. 87: 1–19, 1982).

The process called ketogenesis involves the conversion of 3-hydroxy-3-methylglutaryl-CoA (3HMGCoA) to acetoacetate, β-hydroxybutyrate, and acetone (the traditional ketone bodies). This process actually consumes H$^+$ and the molecules formed are not acids (i.e., not acetoacetic acid or β-hydroxybutyric acid).

As explained by Alberti, the excess generation of H$^+$ it ketoacidosis occurs before ketogenesis and not during ketogenesis. The greatest amount of H$^+$ is formed from triglyceride molecules when there is β-oxidation of fatty acids to AcCoA in hepatocytes. The processes of triglyceride lipolysis in adipose tissue and the conversion of AcCoA to 3HMGCoA also generate H$^+$.

The acidemia in animals with a ketoacidosis is due to the excessive formation of H$^+$ during the mobilization and catabolism of triglycerides when there is a negative energy status; not due to ketogenesis or the formation of ketoacids.

The increased anion gap seen with renal failure is due the accumulation of uremic acids.

This statement sounds logical. When there is a true increase in the anion gap concentration, there is an increased concentration of anions other than Cl$^-$ or HCO$_3^-$ in the serum/plasma. Are the acids anions?

When an animal is in renal failure, the decreased glomerular filtration rate leads to an accumulation of phosphates, sulfates, and citrate in plasma. At a pH of 7.4, most of the phosphates exist as HPO$_4^{2-}$ and a lesser amount of H$_2$PO$_4^-$ (both anions and both acids). The sulfates exist mostly as SO$_4^{2-}$ and a minute amount of HSO$_4^-$ (both anions, SO$_4^{2-}$ is not an acid). Citrate exists as an anion, there is very little citric acid present at a pH of 7.4; citric acid is not an anion.

As some of the “uremic acids” do exist as anions at a pH of 7.4, the statement is partially true. However to reduce confusion, I attempt to consistently state that increased anion gap concentration is due to anions other than Cl$^-$ and HCO$_3^-$. 

The increased serum osmolality is due to dehydration (i.e., ↓ plasma H$_2$O).

It is important to recognize that serum osmolality represents the total concentration of the solutes in the serum and usually dehydration is not the reason for an increase concentration of solutes. The three major reasons for hyperosmolar serum are azotemia (increase urea concentration), hyperglycemia, and presence of exogenous solutes (e.g., ethylene glycol or mannitol). Dehydration does not cause hyperglycemia or an excess of exogenous solutes. Dehydration can lead to azotemia, but only when dehydration creates sufficient hypovolemia to lead to a prerenal azotemia. Decreased plasma H$_2$O by itself does not create a significant increase in urea concentration.

When dehydration results in hypernatremia and hyperchloremia, then dehydration is the cause of the increased serum osmolality. However, most dehydrated animals do not have hypernatremia and hyperchloremia. Hypernatremic dehydration occurs when there is a loss of “pure water” as it occurs in central and renal diabetes insipidus and when there is an insensible loss of water via respiration. Another cause of hypernatremic dehydration occurs when an animal does not have access to water (e.g., frozen water tank).
A measured or a calculated osmolality should not be used to establish the presence or absence of dehydration in an animal. Dehydration is usually not the cause of hyperosmolar serum.

**An increase in [Pi] will cause the [tCa2+] to decrease because of the calcification of tissues. Or When Ca X P is > 70, soft tissue calcification is likely; mineralization occurs if when > 90.**

The concept of the Ca/P product is based on the mass-law concepts in which higher concentrations of Ca$^{2+}$ or PO$_4$ will shift this reaction ($\text{Ca}^{2+} + \text{PO}_4^{3-} \rightarrow \text{Ca}_3(\text{PO}_4)_2$) to the right and thus more Ca$_3$(PO$_4$)$_2$ forms. On the surface, this concept is flawed because not all of the measured [tCa$^{2+}$] is present as free Ca$^{2+}$ and thus is not available to participate in the reaction. Second, very little of the serum inorganic phosphorus concentration exists as PO$_4^{3-}$. Also, when Ca$^{2+}$ & PO$_4^{3-}$ were added to human plasma, precipitation did not occur until the Ca/P product was > 200 (O’Neill W.C.: *Kidney International* 72: 2007). If the Ca/P product concept is not valid, is it true that “An increase in [Pi] will cause the [tCa$^{2+}$] to decrease”?

If there is a prolonged increase in plasma [PO$_4$] (as it occurs in chronic renal disease), the PO$_4$ inhibits renal 1-hydroxylase and thus there is less conversion of calcidiol to calcitriol. Lower calcitriol concentrations do lead to lower [fCa$^{2+}$] (thus lower [tCa$^{2+}$]) due to less intestinal absorption of Ca$^{2+}$, less mobilization of Ca$^{2+}$ from bone, and more renal excretion of Ca$^{2+}$.

If there is a rapid increase in plasma [PO$_4$], colloidal complexes of Ca$^{2+}$ and PO$_4$ form in plasma and the complexes are engulfed by macrophages and the plasma [tCa$^{2+}$] decreases.