Effects of bilirubinemia, hemolysis, and lipemia on clinical chemistry analytes in bovine, canine, equine, and feline sera

Robert M. Jacobs, John H. Lumsden, Evert Grift

Abstract

Interferences caused by bilirubin, hemolysis, and lipemia on 25 clinical chemistry analytes in bovine, canine, equine, and feline sera were studied using the Coulter Dacos and commercial reagents. We present the data as "interferograms", which show the anticipated percent change in serum analyte activity or concentration with varying concentrations of bilirubin, hemoglobin, or lipid. Obvious species differences in response to at least one added interfering substance were found for alanine aminotransferase, aspartate aminotransferase, cholesterol, creatine kinase, globulin, total protein, and urea. The remaining analytes were affected in a linear or complex dose-response relationship or were only affected at the highest concentrations of interfering substances. These data will be useful in aiding interpretation of laboratory test results when common interferences are present in the serum.

Résumé

Les effets de la bilirubine, de l'hémolyse et de la lipémie sur les composés chimiques cliniques analysés dans le sérum bovin, canin et félin

L'étude a porté sur les interférences causées par la bilirubine, l'hémolyse et la lipémie sur 25 composés chimiques cliniques analysés dans le sérum bovin, canin et félin, en utilisant le Coulter Dacos et des réactifs commerciaux. Nous présentons les données sous forme de photographies des phénomènes d'Interférence qui démontrent le pourcentage de changement anticipé d'activité dans le sérum analysé ou de concentration avec variations de concentrations dans la bilirubine, l'hémoglobine et la lipide. On a trouvé des différences évidentes reliées à l'espèce dans la réponse à au moins une substance d'interférence ajoutée pour l'aminotransférase alanine, l'aspartate, l'aminotransférase, le cholestérole, la créatine kinase, la globuline, la protéine totale et l'urée. Les autres analyses avaient été affectées de façon linéaire ou selon un rapport dose-réponse, ou avaient été affectées seulement dans les concentrations les plus élevées des substances d'interférence. Ces données seront utiles pour l'interprétation des résultats des tests en laboratoire lorsque les interférences communes sont présentes dans le sérum. *(Traduit par Jean Leroux)*

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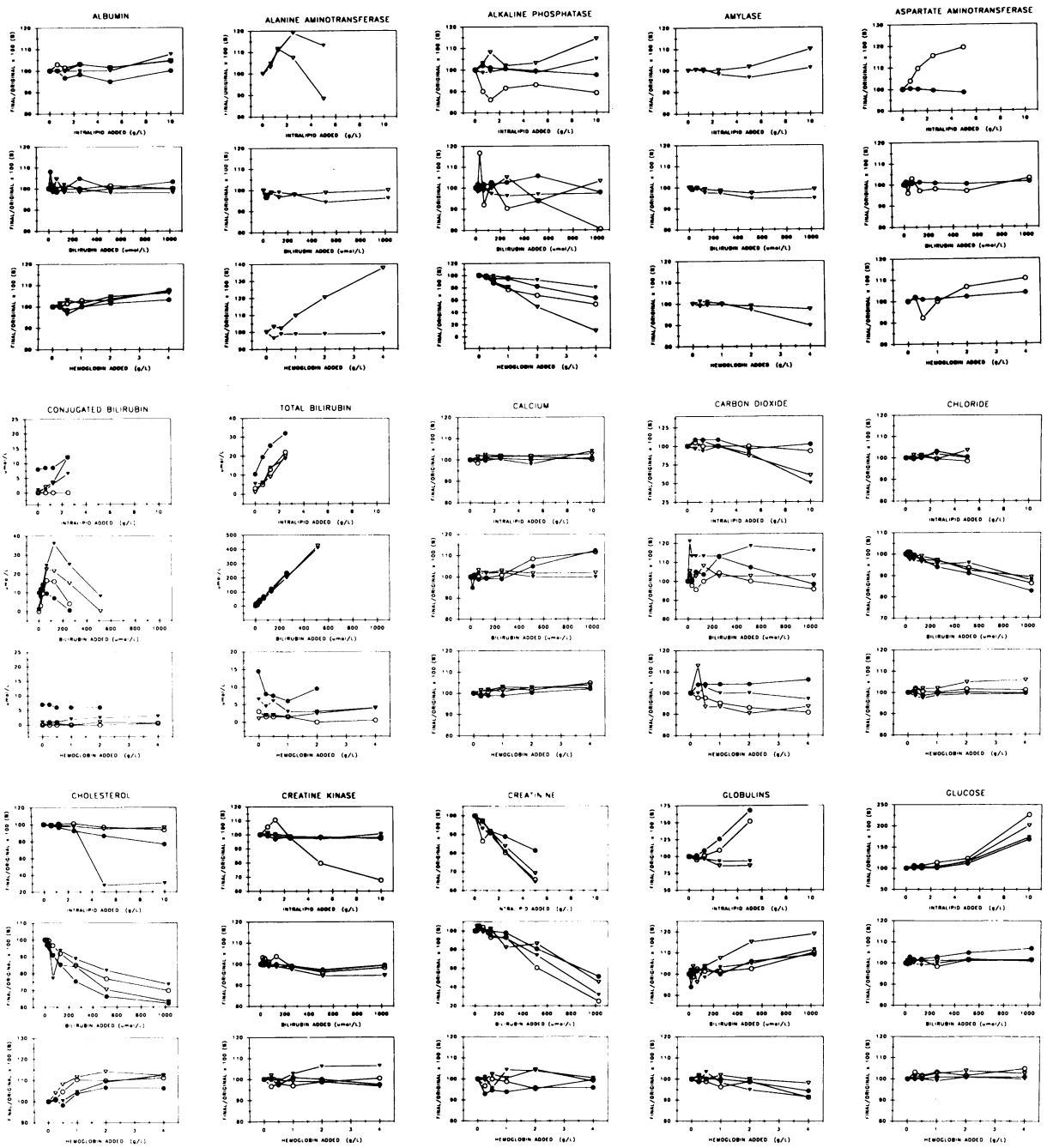
Introduction

The potential effects of common interferents such as bilirubin, hemoglobin, and lipid on serum clinical chemistry test results are often acknowledged, but data concerning their influence on specific analytes according to method, instrument, and species are either unknown or can only be derived from multiple literature sources (1–4). Much of the interference data developed by manufacturers of clinical chemistry instruments have been determined using human sera. Knowledge of the effects of interfering substances on test results is critical for the correct interpretation of laboratory data. Previously, we have reported the effects of interfering substances on two methods for determination of serum creatinine concentration (5). Elsewhere, we have shown the effects of bilirubin, hemoglobin, and lipid on 11 clinical chemistry analytes (6). In the work reported herein, we developed "interferograms" (6,7) showing the effects of bilirubin, hemoglobin, and lipid in bovine, canine, equine, and feline sera for 25 analytes in order to give clinicians assistance in the interpretation of laboratory data complicated by the presence of common interfering substances.

Materials and methods

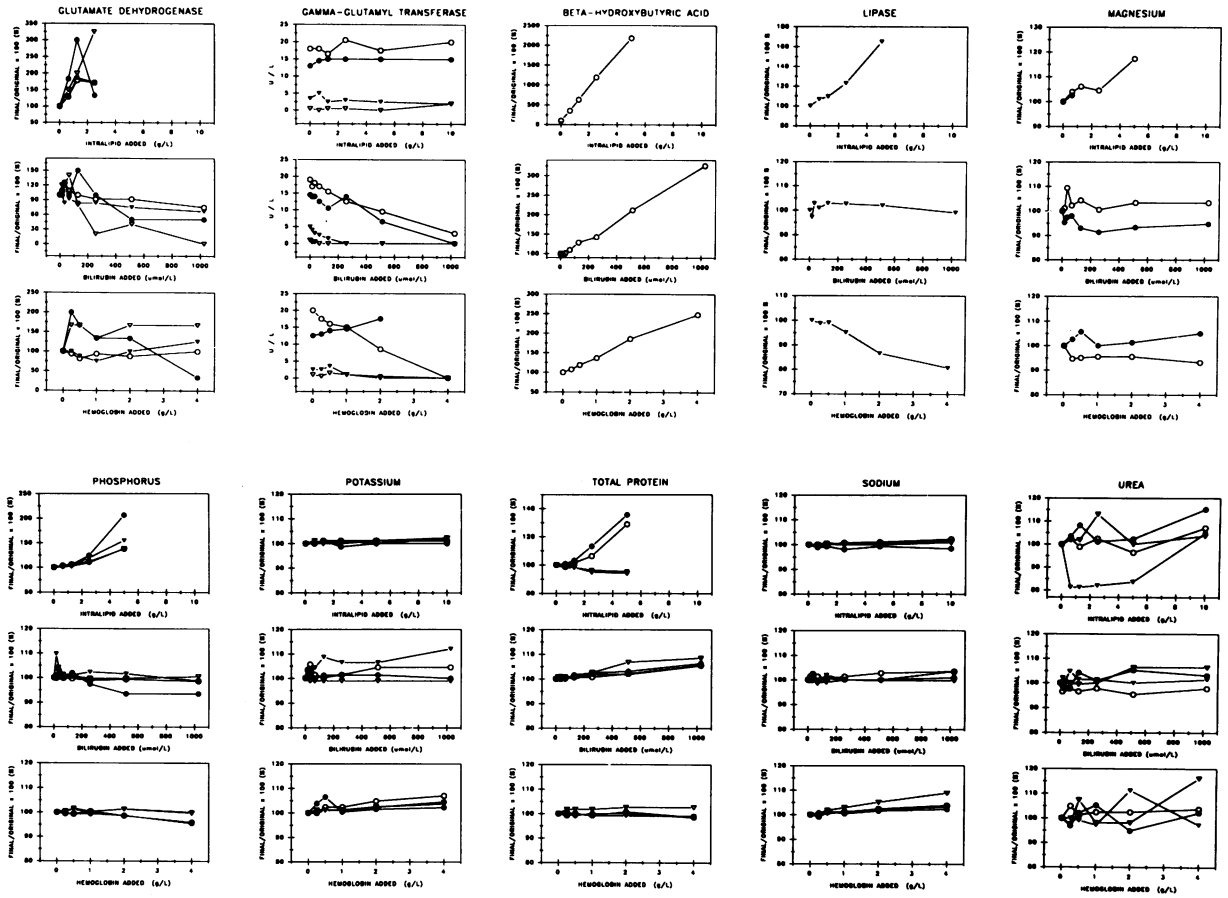
Pools of bovine, canine, equine, and feline sera were made and stored frozen. All clinical chemistry tests were run on an automated analyzer (Dacos, Coulter Electronics, Hialeah, Florida, USA) in a tertiary care veterinary institution. Commercially available reagents were used as directed by the manufacturers. Reagents for all tests were supplied by Coulter Electronics except for the following: glutamate dehydrogenase and lipase reagents, which were supplied by Boehringer Mannheim, Dorval, Quebec; beta-hydroxybutyrate reagents, which supplied by Sigma Diagnostics, St. Louis, Missouri, USA; and magnesium reagents, which were supplied by Synermed, St.-Eustache, Quebec. Bilirubin, hemoglobin, or lipid were prepared and added to serum pools as described previously (5–7). Briefly, bilirubin (Aldrich Chemical Co Inc., Milwaukee, Wisconsin, USA) was initially dissolved in dimethyl sulfoxide and then added to the pooled sera at 0.0, 15, 32, 65, 128, 256, 513 and 1026 μmol/L; fresh hemolysate was prepared from red cells of each species by lysing saline-washed red cells in distilled water and then adding filtered hemolysate to homologous pooled serum at 0.0, 0.25, 0.5, 1.0, 2.0 and 4.0 g/L; lipid (Intralipid 20%, Baxter Travenol, Toronto, Ontario) was added to pooled sera at 0.0, 0.625, 1.25, 2.5, 5.0 and 10.0 g/L. Data points represent the mean of duplicate determinations. "Interferograms" were prepared according to previously reported protocols (6,7).



Briefly, the "interferograms" show, on the X axes, increasing concentrations of bilirubin, hemoglobin, or lipid, while the Y axes show the percentage change (final/original × 100%) in any particular analyte. Presentation of the data in this way allows the observer to anticipate the percent change in analyte activity or concentration at any given concentration of one of the interfering substances. In those instances where the concentration or activity of a particular analyte was numerically small, the absolute values are given on the Y axes. All tests were performed for all species except in instances where particular analytes are not commonly used in one or more species.

Results

Reaction types and test methodologies are summarized in Table 1. Graphical representations of the "interferograms" are shown in Figure 1. Obvious species differences, represented as opposing trends among the automated clinical chemistry tests, were found as follows: alanine aminotransferase, where moderate concentrations of added lipid caused a positive bias in canine serum and a negative bias in feline serum, and where added hemoglobin caused a linear increase in feline serum but essentially had no effect in canine serum; aspartate aminotransferase, where bovine serum was positively biased by added lipid, whereas equine serum was unaffected; cholesterol, where canine serum was negatively biased by the addition of lipid at moderate and high concentrations, whereas sera from other species were essentially unaffected; creatine kinase, where bovine serum was negatively biased by the addition of lipid whereas sera of other species were unaffected; globulin and total protein,



◀▲

Figure 1. Interferograms for pools of bovine (○), canine (▼), equine (●), and feline (▽) sera. The effects of adding bilirubin, hemoglobin, and lipid are shown for 25 analytes using the Coulter Dacos and reagents specified in Table 1. Serum bilirubin concentration and gamma-glutamyl transferase activity are shown in absolute units rather than percent change because of the numerically small values.

where large animal sera were positively biased by the addition of lipid, whereas small animal sera were essentially unaffected; and urea, where canine serum was negatively biased at low to moderate amounts of added lipid. Analytes that were unaffected except at the highest concentrations of added interfering substances were albumin, amylase, calcium, total carbon dioxide, glucose, potassium, and sodium. The remaining analytes (alkaline phosphatase, conjugated bilirubin, total bilirubin, chloride, creatinine, glutamate dehydrogenase, gamma-glutamyl transferase, beta-hydroxybutyrate, lipase, magnesium, and phosphorus) were affected by one or more interfering substances and in two or more species, but generally with similar trends among the different species.

Discussion

Species differences were remarkable, with the most obvious being the differences in protein concentrations. Total protein and globulin concentrations in bovine and equine serum pools were biased positively by the addition of lipid, but there was no effect of lipid on these analytes in canine and feline serum pools. This effect was not due to lipid-induced changes in albumin concentrations. The globulin concentration was calculated from the difference between total protein and albumin concentrations; therefore, the error was in the determination of total protein concentration. Inefficient sample blanking in the presence of lipemia with total protein in cattle and horses likely accounts for this difference. Although species differences in serum lipolytic activity may be involved, we do not know if this same phenomenon occurs under natural circumstances. Hyperlipidemia occurs rarely in cattle and horses, although is not uncommon in ponies. Species differences in red cell constituents likely account for most of the changes seen with the addition of hemoglobin made from red cell lysate. Other phenomena that probably play a role in mediating species differences and differences between analytes are color interferences resulting from excess bilirubin or hemoglobin, additional chemical interactions between interfering substances and serum constituents, or serum constituents acting as substrates in linked enzymatic reactions.

The serum pools were made from "normal" individuals for each species. Thus, "interferograms" are valid for the effects of interfering substances or contaminants on samples with "normal" analyte activities or concentrations. This means that the percent-

Table 1. Test characteristics for analytes determined using the Coulter Dacos[a]

Analyte	Reaction type	Methodology
Alanine aminotransferase	Zero order kinetic	Modified IFCC[b] (alanine and ketoglutarate substrate)
Albumin	First order kinetic	Modified Doumas (bromcresol green)
Alkaline phosphatase	Zero order kinetic	Modified Bowers and McComb (nitrophenyl phosphate substrate)
Amylase	Zero order kinetic	Modified Wallenfels (nitrophenylmaltohexaoside substrate)
Aspartate aminotransferase	Zero order kinetic	Modified IFCC (aspartate and ketoglutarate substrate)
Bilirubin	Equilibrium[c]	Modified Walters and Gerarde (diazo)
Calcium	Equilibrium	Modified Connerty and Briggs (cresolphthalein complexone)
Total carbon dioxide	First order kinetic	Enzymatic phosphoenol-pyruvate carboxylase
Chloride	Equilibrium	Modified Schoenfeld and Lewellen (thiocyanate)
Cholesterol	Equilibrium	Enzymatic (cholesterol esterase/oxidase)
Creatine kinase	Zero order kinetic	Modified Oliver-Rosalki (creatine phosphate substrate)
Creatinine	Initial rate	Kinetic Jaffé
Glucose	Equilibrium	Modified hexokinase/glucose-6-phosphate dehydrogenase
Glutamate dehydrogenase[d]	Zero order kinetic	alpha-oxoglutarate substrate
gamma-Glutamyl transferase	Zero order kinetic	Modified Szasz (glutamylnitroanilide/glycylglycine substrate)
beta-Hydroxybutyrate[e]	Zero order kinetic	beta-hydroxybutyrate dehydrogenase
Lipase[d]	Zero order kinetic	Triolene substrate and colipase excess
Magnesium[f]	Equilibrium	Xylidyl blue
Phosphorus	Equilibrium	Modified Daly and Ertingshausen (molybdate)
Potassium	—	Ion selective electrode
Total serum protein	Equilibrium	Modified biuret (cupric sulfate)
Sodium	—	Ion selective electrode
Urea	First order kinetic	Modified Talke and Schubert (urease)

[a]Located at Clinical Pathology Laboratory, Department of Pathology, University of Guelph. Unless indicated otherwise, all reagents are from Coulter Diagnostics, Hialeah, Florida, USA
[b]International Federation of Clinical Chemistry
[c]Also termed "endpoint" reaction
[d]Boehringer Mannheim, Dorval, Quebec
[e]Sigma Diagnostics, St. Louis, Missouri, USA
[f]Synermed, St.-Eustache, Quebec

age change may not be the same if the initial analyte concentration or activity is outside the reference range, although the trend will likely be similar. With this consideration in mind, the data presented here can be used to estimate the degree to which serum analyte concentrations or activities are influenced by bilirubinemia, hemoglobinemia, and lipemia. Because of the wide variations in different manufactuers' reagents and instruments (6), the data presented here are valid only for the specified reagents on the Coulter Dacos.

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References

1. Alleman AR. The effects of hemolysis and lipemia on serum biochemical constituents. Vet Med 1990; December: 1272–1284.
2. Dorner JL, Hoffman WE, Filipov MM. Effect of *in vitro* hemolysis on values for certain porcine constituents. Vet Clin Pathol 1983; 12: 15–19.
3. Leard BL, Alsaker RD, Porter WP, Sobel LP. The effect of haemolysis on certain canine serum chemistry parameters. Lab Animals 1990; 24: 32–35.
4. O'Neill SL, Feldman BF. Hemolysis as a factor in clinical chemistry and hematology of the dog. Vet Clin Pathol 1989; 18: 58–68.
5. Jacobs RM, Lumsden JH, Taylor JA, Grift E. Effects of interferents on the kinetic Jaffé reaction and an enzymatic colorimetric test for serum creatinine concentration determination in cats, cows, dogs, and horses. Can J Vet Res 1991; 55: 150–154.
6. Glick MR, Ryder KW, Glick SJ. Interferographs: User's Guide to Interferences in Clinical Chemistry Instruments. 2nd ed. Indianapolis: Science Enterprises, 1991: 135–146.
7. Glick MR, Ryder KW, Jackson SA. Graphical comparisons of interferences in clinical chemistry instrumentation. Clin Chem 1986; 32: 470–475.