

## PLASMA ALBUMIN MEASUREMENTS IN NEW ZEALAND WHITE RABBITS.

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**SUMMARY** : Following reports in the literature of problems with serum albumin measurements using bromocresol green dye binding methods, the method was re-examined using fifty rabbit plasma samples and solutions of different rabbit fractionated proteins. The value found in this study

(albumin :  $33 \pm 2$  g/l) was generally lower than in most of the other studies (27 to 43 g/l) and the ranges narrower. None of the samples analysed gave values for albumin which were higher than those for total protein ( $53 \pm 3$  g/l).

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**RESUME** : *Mesure du taux d'albumine plasmatique chez des lapins Néo Zélandais Blancs.*

Après avoir passé en revue la littérature consacrée aux problèmes rencontrés dans le dosage de l'albumine sérique avec la méthode au vert de bromocrésol, l'auteur a réexaminé les résultats obtenus quand il l'a appliqué au plasma de 15 lapins et à des solutions contenant différentes

fractions de protéines sériques de lapins. Les valeurs trouvées dans cette étude (albumine :  $33 \pm 2$  g/l) ont été un peu plus faible que celles rencontrées dans la littérature (27 à 43 g/l) mais la variabilité plus faible. Pour aucun des échantillons dosés, la teneur en albumine sérique n'a dépassé celle des protéines totales ( $53 \pm 3$  g/l).

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### INTRODUCTION

Recently, HALL (1992) reported inaccuracies with routine unmodified dye binding methods for serum albumin : using either of the dyes bromocresol green or bromocresol purple yielded falsely elevated values which often exceeded the total serum protein concentrations. He stated that the cause of the spuriously high values was not known and that serum protein electrophoresis results indicated that albumin comprises about 60 to 70 % of total serum protein. FOX (1989) also indicated that analytical problems occurred when using bromocresol green with rabbit sera. Although problems associated with bromocresol purple methods for albumin determination have been reported for several species (EVANS and PARSONS, 1988), bromocresol green is commonly used for determining plasma or serum albumin.

In this study, blood was collected from healthy rabbits, and the heparinised plasma was analysed using bromocresol green reagent with a centrifugal analyzer. This type of analyzer allows rapid absorbance measurements to be made following the addition of bromocresol green to plasma, thus reducing the effect of reactions between the dye and other protein fractions which occur if absorbance measurements are delayed for more 60s. (COWIE and EVANS, 1983).

### MATERIALS AND METHODS

The rabbits, weighing 3 to 4 kg, were singly housed and provided with 140g pelleted diet daily. Blood samples were collected from the marginal ear veins of fifty rabbits, within five to ten minutes following a subcutaneous injection of Hypnorm (Jansen Pharmaceuticals ; WILLS *et al.*, 1993) and the blood was then dispensed into tubes containing lithium heparinate as anticoagulant. These plasma samples were free from haemolysis, lipaemia and icterus.

Following centrifugation and separation, plasma samples were assayed with a Cobas Bio Centrifugal analyzer at 30°C (Roche Diagnostics) using bromocresol green dye for albumin and biuret reagent for total protein respectively (COWIE and EVANS, 1983). An assayed bovine control serum (Survey validated Reference serum, Wellcome Diagnostics) was used to calibrate these assays.

Three rabbit protein fractions were dissolved in sodium chloride 9g/l, to obtain working protein concentrations of 30g/l. The protein fractions were (a) gamma globulin, Cohn fractions II and III, (b)-albumin and (c) Cohn fraction IV-4 containing 50 % alpha-globulin and beta-globulin with the remainder mostly albumin(product numbers : A6059, G0261 and G4640 ; Sigma Chemical Co, St. Louis, USA).

**Table 1: Mean plasma albumin and total protein concentrations, g/l ( $\pm$  1S.D.) and ranges obtained.**

References	Albumin			Total protein		
	Mean	S.D.	Range	Mean	S.D.	Range
<b>THIS STUDY</b>	33	2	29 to 35	53	3	46 to 59
KOZMA <i>et al.</i> (1974)	-	-	-	52	8	-
	-	-	-	63	1	53 to 79
KANEKO (1989)	27	3	-	65	3	-
BORTOLOTTI <i>et al.</i> (1989)	43	15	29 to 75	59	19	44 to 99
FAVARATO and ZATTA (1990)	-	-	-	58	10	-
JENSEN <i>et al.</i> (1992)	42	3	37 to 47	67	3	60 to 73

The albumin method was modified to allow absorbance measurements to be made at 10, 60, 360, 600, and 900s after addition of the protein fractions to the bromocresol reagent.

## RESULTS AND DISCUSSION

The results obtained for fifty samples are shown in Table 1 together with results published by other investigators. There are wide variations between the data sets. None of the samples analysed in this study gave values for albumin which were higher than those for total protein. The values found in this study were generally lower than in most of the other studies and the ranges were narrower.

GUSTAFSSON (1976) reported bromocresol green reacts with human serum proteins in two steps; there is an immediate reaction with albumin within 60s and a second slower reaction with acute-phase proteins, which include alpha- and beta-globulins. WEBSTER (1977) confirmed that bromocresol green reacts with human alpha- and beta-globulins but not human gamma globulins. With the solution of the three rabbit protein fractions and measurement of the bromocresol reaction at several time points in this study, no increased albumin values (or apparent albumin values) were observed with time using any of the rabbit protein solutions. The 30 g/l solution of gamma globulins gave apparent albumin values of less than 0.8 g/l. The 30 g/l solution of Kohn fraction V, containing 50% alpha- and beta-globulins and the remainder as mainly albumin, yielded apparent albumin values of 17 g/l which did not increase with time.

Whilst albumin values may be expected to differ for reasons including blood collection procedures, rabbit strain, nutrition *et cetera*, it remains unclear why some investigators (FOX, 1989; HALL, 1992) have obtained spuriously high values for serum albumin with bromocresol green, and how these values exceeded the total protein concentrations. In this study

and other studies mentioned here, investigators have not experienced such problems with this assay.

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## BIBLIOGRAPHY

- BORTOLOTTI A., CASTELLI D., BONATI M, 1989. Hematology and serum chemistry values of adult, pregnant and newborn New Zealand rabbits (*Oryctolagus cuniculus*). *Lab. Anim. Sci.*, **39**, 437-439.
- COWIE J.R., EVANS G.O., 1983. Plasma albumin determination on the Cobas Bio centrifugal analyzer using bromocresol green. *J. Automat. Chem.*, **5**, 153-154.
- EVANS G.O., PARSONS C.E., 1988. A comparison of two dye binding methods for the determination of dog, rat and human plasma albumins. *J. Comp. Path.*, **98**, 453-460.
- EVANS G.O., PARSONS C.E., 1990. Plasma albumin determination in the Cobas Mira: effect of measurement wavelength. *Clin. Chem.*, **36**, 176-177.
- FAVARATO M., ZATTA P., 1990. Chemico-clinical characteristics of rabbit serum. *J. Appl. Rabbit Res.*, **13**, 14-15.
- FOX R.R, 1989. The rabbit. In: *The clinical chemistry of laboratory animals*. Eds Loeb W.F. and Quimby F.W., Pergamon Press, Inc. New York, 45-46.
- GUSTAFSSON J.E.C., 1976. Improved specificity of serum albumin determination and estimation of

- "acute phase reactants" by use of the bromocresol green reaction. *Clin. Chem.*, **22**, 616-622.
- HALL R.E., 1992. Clinical pathology of laboratory animals. In : *Animal models in toxicology*. Eds Gad S.C. and Chengelis C.P., Marcel Dekker Inc. New York, 765-811.
- JENSEN A.L., WEDO E, BANTZ M., 1992. Critical differences of clinical chemistry components in blood from laboratory rabbits. *Scand. J. Lab. Anim.*, **19**, 179-184.
- KANEKO J.I., 1989. Clinical Biochemistry of Domestic Animals. 4th ed., Academic Press Inc., California, USA, 865.
- KOZMA C, MACKLIN W., CUMMINS L.M., MAUER R., 1974. Anatomy, physiology and biochemistry of the rabbit. In: *The biology of the Laboratory Rabbit*. Eds Weisbroth S.H., Flatt R.E. and Kraus A.L. Academic Press, New York, 60-69.
- WEBSTER D., 1977. The immediate reaction between bromocresol green and serum as a measure of albumin content. *Clin. Chem.*, **23**, 663-665.
- WILLS J.E., ROWLANDS M.A., NORTH D.C., EVANS G.O., 1993. Effect of serial cardiac puncture and blood collection procedures in the rabbit. *Anim. Technol.*, **44**, 39-52.
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