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BLOOD-VOLUME DETERMINATION WITH EVANS BLUE DYE IN FOALS

By

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Persson, S. G. B. and L.-E. Ullberg: *Blood-volume determination with Evans blue dye in foals*. Acta vet. scand. 1979, 20, 10—15. — The rate of disappearance of Evans blue dye from the blood after an intravenous injection was studied in young foals, between 4 and 105 days of age. This was found to be age dependant, especially during the first month, the initial dye disappearance being much faster than in the adult horse. This would mean an overestimation of plasma volume, using the single sampling technique, of about 5 % during the first day of life, i.e. negligible from a practical point of view considering the standard error of estimation.

The effect of exercise on the venous haematocrit was studied, too, in foals less than 3 months of age. There was a significant difference between mean values before and after exercise, and this difference seemed to increase with age, indicating an increasing erythrocyte-storing capacity of the spleen. This means that even in young foals, the splenic function should be considered when determining the total blood volume from the plasma volume and the venous haematocrit.

foal; plasma volume; dye-disappearance rate;
total blood volume; splenic function.

A simple and reliable method for determination of the total blood volume (TBV) in the horse using Evans blue dye (T-1824) has been reported earlier (Persson 1967). This method is based on the dye concentration in a single blood sample drawn at the end of the mixing phase and on the venous haematocrit determined after mobilization of the splenic erythrocyte reservoir with exercise or intravenous injection of adrenalin. The overestimation of the plasma volume caused by a single sampling, as compared to extrapolation of the theoretical zero-time dye concentration, has been found to vary rather little between horses and to be small enough to be negligible from a practical point of view (Persson 1967). On the other hand, the effect of

the splenic red-cell reservoir on the venous haematocrit (VH), and, thus, on the body/venous haematocrit ratio, is of a decisive importance as it affects the reproducibility of the venous haematocrit and the blood volume (*Persson 1967, 1969*). This difficulty can largely be overcome, however, by mobilizing the splenic reservoir maximally, or almost maximally, with exercise or adrenalin injection. There are, however, no reports in the available literature of the erythrocyte-storing capacity of the spleen in the young foal.

The purpose of the present study was to investigate the relevance of the simplified Evans blue dye method for determination of TBV in newborn and young foals with respect to the dye-disappearance rate and the blood-storing function of the spleen.

MATERIAL AND METHODS

The study on the dye-disappearance rate was performed on 11 foals, 4 of which were also used in the haematocrit-variation study. The age ranged between 4 and 105 days with a mean age of 44 days. The dye was injected intravenously at rest as previously described (*Persson 1967*). A standard dose of 50 mg of dye was used in all foals. Blood samples were drawn from a permanent catheter in the contralateral jugular vein at 15, 45, 75, 105, 135 and 165 min. after the injection. The plasma concentration of the dye in these samples was determined spectrophotometrically and the regression coefficient of the relationship between the logarithmic values for the dye concentrations and time calculated. This was considered to represent the dye-disappearance rate (*Persson 1967*).

In the haematocrit-variation study 11 foals were used, 4 of which were also used in the previous experiment. The mean age was 48 days and the range 11 to 91 days. Blood samples were drawn from the jugular vein before and after exercise. The pre-exercise sampling was done as quietly as possible although a certain amount of excitation was usually unavoidable. The exercise consisted of about 5 min. running with the mare which trotted at the maximum speed tolerated by the foal. The work resulted in heart rates in the foals immediately after stopping ranging between 180 and 216 beats/min. with a mean value of 196 beats/min. Promptly after exercise and in all cases within 1 min. a blood sample was drawn from the contralateral jugular vein.

The haematocrit both in the pre- and the post-work samples was determined with a Kemila micro-haematocrit centrifuge (12,000 r.p.m. for 5 min.). Using this method the standard error of a single determination is about $\pm 0.5\%$ of the mean in horse blood. Duplicate determinations were made and if differing the mean value was used.

Statistical calculations were made by standard methods (Hald 1952).

RESULTS

The mean time/log. concentration regression coefficient was -766×10^{-6} with a range of -519 to -1201×10^{-6} . There was, however, a tendency towards lower values with increasing age. This inverse relationship was curvilinear and could be fitted to the regression: $y = 1391 \times X^{-0.19}$, with a correlation coefficient of -0.744 (Fig. 1).

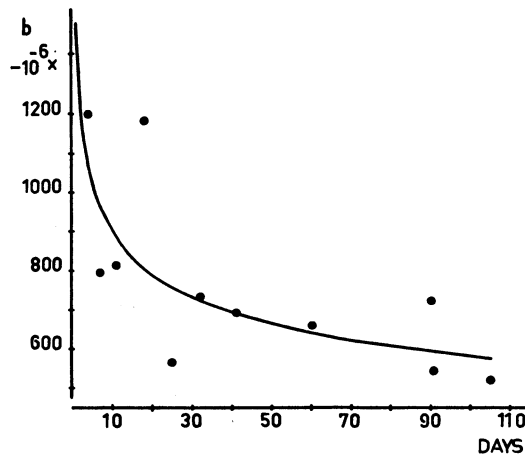


Figure 1. The rate of disappearance of Evans blue dye from the blood after an intravenous injection relative to age in standardbred foals. The dye-disappearance rate is expressed as the regression coefficient for the relationship between the logarithmic values for the dye concentrations and time after the injection ($-10^6 \times b$).

The mean VH was significantly higher ($t = 4.116$; $P < 0.01$) after work than before, 40.2 as compared to 34.3%. The intra-individual difference varied, however, with a tendency toward larger differences at increasing age. The relationship between age and this difference expressed as a percentage of the post-

exercise value was probably significant ($r = 0.62$; $P < 0.05$). The inter-individual variation of VH was greater before exercise, both the standard deviation and the range being about double that found after exercise.

DISCUSSION

The present study reveals 2 phenomena which could be of importance for blood-volume determination in the young foal using a dye-dilution technique. The disappearance rate of Evans blue dye seems to be significantly increased during the first days of life, and disappearance rates comparable with those found in adult horses (*Persson 1967*) do not occur until the age of about 1 month. As Evans blue dye is firmly bound to the plasma albumin (*Gregersen & Rawson 1942*) this should indicate a very rapid turnover of the plasma albumin in the newborn foal, the plasma albumin level being fairly constant, from the first day of life (*Ekman et al. 1975*). The effect of the enhanced dye disappearance in the newborn foal would be an increased overestimation of the plasma volume (PV) calculated from the dye concentration of a single blood sample after the end of the mixing phase, i.e. about 15 min. after the dye injection. However, the total overestimation would be of the order of 5 % during the first day, which is only slightly more than the standard error of determination which is about ± 4 % of the mean value after exercise (*Persson 1967*). Further, this overestimation decreases rapidly during the following days. Thus, from a practical point of view this overestimation is still negligible, and the single-sample dye-dilution method for determination of the plasma volume should be relevant even in the very young foal.

The haemoconcentration brought about by exercise can be caused either by a decrease of the PV by a fluid shift (*Persson 1967*) or by an addition to the circulating red-cell volume of erythrocytes from blood depots (*Persson et al. 1973 b*), or both. The tendency to a larger difference with increasing age seems to favour the assumption that the latter factor predominates. It is also reasonable to postulate that the storing capacity of the spleen in the foal increases by age as it seems to be related to the TBV and the degree of physical activity in the adult horse (*Persson et al. 1973 a*). In the newborn foal the splenic storage capacity seems to be almost nil. The difference between VH before and after exercise at birth extrapolated from the regres-

sion equation amounts to about 4 % of the post-work value which includes the effect of fluid shifts due to exercise. There was no relationship between the post-exercise heart rate and the VH difference which indicates that a complete mobilization of the splenic erythrocytes had occurred in all cases.

It may be concluded that in determining the total blood volume using Evans blue dye and VH the effect of the splenic erythrocyte reservoir should be considered even in the young foal. On the other hand, the overestimation of PV caused by dye determination in a single sample after the mixing phase without extrapolation of the theoretical zero-time concentration seems to be negligible, provided the albumin turnover is normal and no oedematous state prevails.

REFERENCES

- Ekman, L., S. G. B. Persson & L.-E. Ullberg*: The levels of some blood constituents in standardbred horses during their first year of life. Proc. 1st int. Symp. Eq. Hematol. 1975, 289—296.
- Gregersen, M. I. & R. A. Rawson*: The disappearance of T-1824 and structurally related dyes from the blood stream. Amer. J. Physiol. 1942, 138, 698—707.
- Hald, A.*: Statistical Theory with Engineering Applications. Wiley & Sons Inc., New York 1952.
- Persson, S. G. B.*: On blood volume and working capacity in horses. Acta vet. scand. 1967, Suppl. 19, 1—189.
- Persson, S. G. B.*: Value of haemoglobin determination in the horse. Nord. Vet.-Med. 1969, 21, 513—523.
- Persson, S. G. B., L. Ekman, G. Lydin & G. Tufvesson*: Circulatory effects of splenectomy in the horse. I. Effect on red cell distribution and variability of haematocrit in the peripheral blood. Zbl. Vet.-Med. A, 1973 a, 20, 441—455.
- Persson, S. G. B., L. Ekman, G. Lydin & G. Tufvesson*: Circulatory effects of splenectomy in the horse. II. Effect on plasma volume and total and circulating red-cell volume. Zbl. Vet.-Med. A, 1973 b, 20, 456—468.

SAMMANFATTNING

Blodvolymsbestämning med Evans blue på föl.

Försvinnandehastigheten av Evans blue dye från blodet efter en intravenös injektion undersöktes på 4—105 dagar gamla föl. Denna befanns vara beroende av åldern med snabbare färgförsvinnande under de första levnadsdagarna än vid vuxen ålder. Detta innebär en överskattning av plasmavolymer om denna bestäms med en enstaka provtagning efter blandningsfasens slut på ca 5 % under första lev-

nadsdagen. Ur praktisk synpunkt är denna överskattning försumbar, då metodfelet uppgår till ca $\pm 4\%$.

Vidare studerades effekten av arbete på hämatokriten på upp till 3 månader gamla föl. Hämatokriten var i medeltal signifikant högre efter arbete än före och denna skillnad tycktes växa med stigande ålder. Detta antyder en med åldern ökande lagringskapacitet i mjälten av erythrocyter och att mjältfunktionen måste tas i beaktande vid bestämning av totala blodvolymen från plasmavolym och venhämatokrit även på unga föl.

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