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IMMUNOCHEMICAL QUANTITATION OF PIG TRANSFERRIN BY SINGLE RADIAL IMMUNODIFFUSION

By

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V. HRISTIĆ and M. MOVSESIJAN: *Immunochemical quantitation of pig transferrin by single radial immunodiffusion.* Acta vet. scand. 1975, *16*, 244—250. — A system has been developed for immunochemical determination of pig transferrin. Direct comparison of immunological values with chemical values showed that monovalent rabbit anti-pig serum transferrin is suitable for the immunological estimation of iron binding protein in swine.

immunodiffusion; transferrin; specific monovalent antiserum; total iron binding capacity; pig.

Analytical methods for the quantitative determination of a protein can be used only for the relatively few serum proteins with defined chemical activities. Thus, chemical methods for the quantitative determination of transferrin are based on the assumption that 1 mg of transferrin can bind 1.25 μg Fe^{3+} (*Laurell & Ingelman* 1947).

A method based upon an immunochemical reaction can theoretically be used to measure any serum protein at almost any concentration. However, immunochemical assays of human transferrin has in some instances shown difficulties since the antisera frequently gave precipitates with human albumin or human gamma-globulin or both. Thus all antisera need to be absorbed with these substances before they are compared with chemical

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methods (*Jager & Gubler* 1952, *Goodman et al.* 1958, *Eckman et al.* 1970).

This report describes the immunochemical quantitation of pig transferrin by single radial immunodiffusion.

MATERIAL AND METHODS

Preparation of swine transferrin. This was prepared according to the method of *Hristić & Movsesijan* (1974). No impurity was detected by the method of double diffusion in agar gel (*Ouchterlony* 1958) and semimacro immunoelectrophoresis in 1 % agar (*Scheidegger* 1955).

Antisera. Anti-pig whole serum and anti-pig serum transferrin were obtained in rabbit according to *Hristić & Movsesijan.*

Single radial immunodiffusion. This was performed using antiserum against pure transferrin according to the method of *Mancini et al.* (1963, 1965) slightly modified as described by *Jensen* (1966). The specific anti-transferrin serum was mixed with 1 % agar solution (Special Agar Noble, Difco Laboratory) at 50°C. The ratio between volumes of antisera and agar gel proved to be appropriate for development of precipitin rings which could be measured without difficulty. Two ring diameters were measured directly at right angles to each other by slight magnification and a scattered light arrangement. The values obtained were used to calculate the areas of the precipitate.

Pig serum. Pig blood was collected at the slaughter-house or on a farm. Serum was obtained by the usual procedure, pooled and stored at —20°C until use. The samples of pure transferrin or serum were diluted with phosphate-buffered saline for preparation of standards or to ensure that ring diameters are within the range of the diameters produced by standard preparations.

Total iron binding capacity (TIBC). Total iron binding capacity of pig serum or plasma was measured by the method of *Fischl & Cohen* (1962) modified by *Wegger et al.* (1964).

Protein determination. Protein concentration of the pure antigen was determined by the method of *Lowry et al.* (1951).

RESULTS

A typical record of different dilutions of pure transferrin determined by the method of *Mancini et al.* (1963, 1965) is shown in Fig. 1.

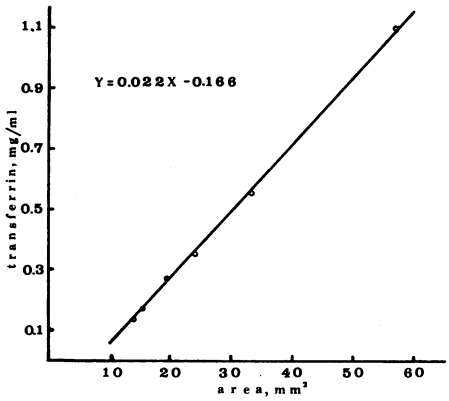

Figure 1. Area of precipitation as a function of transferrin concentration.

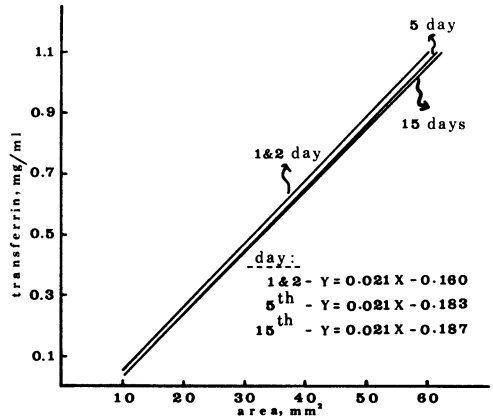

Figure 2. Growth of the precipitate as a function of time. Readings were made after 1, 2, 5 and 15 days of incubation at 37°C.

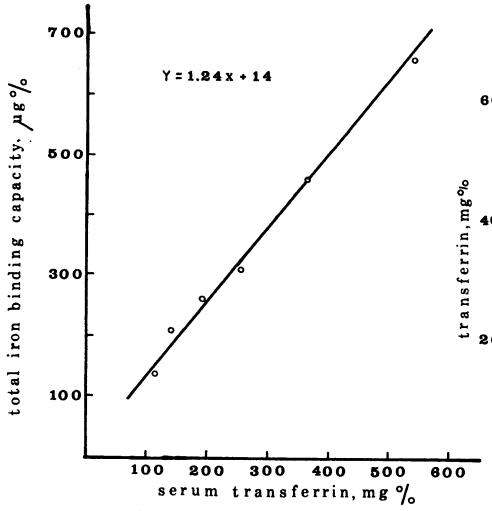

Figure 3. Total iron binding capacity of different dilutions of serum plotted as a function of the concentration of transferrin in the same samples determined by the method of *Mancini et al.* (1963, 1965).

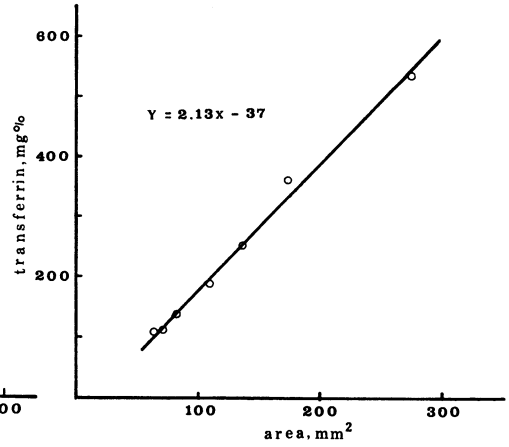

Figure 4. Area of precipitation as a function of transferrin concentration in "standard serum" dilutions calibrated for the protein under determination.

The values for the standards were linear between the concentrations of 0.1375 mg/ml to 1.100 mg/ml with standard deviations from 0.010 mg/ml (7.3 %) and 0.034 mg/ml (3.1 %) for the lowest and highest concentrations, respectively.

The time for the determination of the diffusion of the antigen, i.e. when precipitates have stopped growing, was relatively short because we used the range of 0.1—1.1 mg/ml for a standard curve. The results showed that 24 hrs. of diffusion is a suitable period to obtain termination of precipitate growth (Fig. 2).

The results for transferrin concentration obtained after 15 days were 5 % lower than those found after 24 hrs. with the same slope for all the estimated curves.

In serial dilutions of pooled pig serum we determined the concentration of transferrin comparing the chemical versus the immunochemical technique. The results are presented in Fig. 3.

It is also possible to use pooled pig plasma calibrated for the protein under determination as the routine standard (Fig. 4).

To validate this immunochemical method of assay by independent chemical estimation, the concentration of serum transferrin was determined by measuring its iron binding capacity in 42 sera. Each was also assayed for its transferrin content by the method of *Mancini et al.* (1963, 1965), and the resulting values of TIBC were plotted against the immunochemically determined transferrin concentration (Fig. 5).

The accuracy of repeated determinations of a control serum which contained 360 mg% of transferrin was estimated, and the standard deviation of 35 determinations performed over several weeks was 18 mg% or 5 %.

DISCUSSION

Since there is good evidence concerning the physiological importance of transferrin it is customary to estimate the concentration of the transferrin in plasma in addition to the iron level in clinical studies (*Bothwell & Finch* 1962, *Hristić* 1971). Direct comparison of immunological values with chemical values gave a slope of 1.24 (Fig. 4) and 1.23 (Fig. 5) for serial dilution of a serum and for 42 different serum samples, respectively. These values are very close to that of the theoretical slope of 1.25 which is based on the assumption that 1 mg of transferrin can bind 1.25 μg Fe^{3+}. In similar investigations *Goodman et al.*

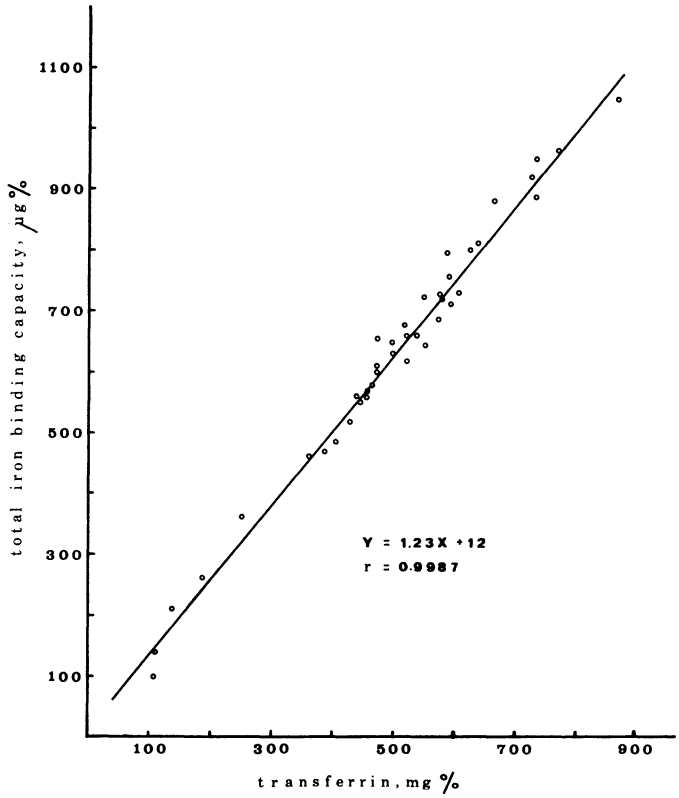

Figure 5. Total iron binding capacity of 42 serum samples plotted as a function of the concentration of transferrin in the same samples determined by the immunochemical method.

(1958) found a slope of 1.25 and *Eckman et al.* (1970) found a slope of 1.26 using an automated method based on the measurement of turbidity. According to the results obtained in this investigation it seems that the immunochemical method could be used with a high degree of accuracy. This is specially true when the transferrin concentration needs to be estimated independently of the plasma or serum iron concentration. This is a method of choice in cases where the concentration of iron in the circulation exceeds the total iron binding capacity.

Also, immunochemical methods could be used when one wishes to distinguish between the iron bound to transferrin and that bound to other iron-carrying proteins as is the case, for example, in colostrum or milk.

Using the immunochemical method for the determination of transferrin concentration many of the difficulties associated with chemical determination, such as the volume of plasma or serum samples needed, anticoagulant used, hemolysis, turbidity, storage and others, could be overcome.

The use of small amounts of pure antigen or "standard serum" with the antisera suggest that it can be used as a routine laboratory technique.

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SAMMENDRAG

Immunokemisk bestemmelse af transferrin hos svin ved enkelt radial immunodiffusion.

Der er udviklet et system til immunokemisk bestemmelse af transferrin. Ved sammenligning af resultater fra immunologiske og kemiske bestemmelser fandtes, at monovalent kanin anti-svine-serum-transferrin er egnet til immunologisk bestemmelse af jernbindende protein i svineserum.

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