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IN VITRO BINDING OF MANGANESE TO SERUM TRANSFERRIN IN CATTLE

By

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The question of transport of manganese by plasma proteins in humans has been elucidated by *Bertinchamps & Cotzias* (1959), who found that the carrier protein of manganese was a β_1-globulin other than transferrin. This protein was named transmanganin. In cattle, preliminary paper and starch gel electrophoretic experiments have tentatively indicated an in vitro binding of radiomanganese to a β-globulin with the same paper and starch gel electrophoretic mobility as serum transferrin (*Panić & Ekman* 1967).

The present experiments were conducted with cattle sera possessing different molecular species of transferrin for the purpose of excluding the possibility of manganese binding in vitro by a β-globulin other than transferrin, but with the same electrophoretic mobilities in paper and starch gel.

MATERIAL AND METHODS

Experimental. In order to obtain the different phenotypes of transferrin, we investigated electrophoretically the serum of 50 cows of Red Danish Milk Cattle from the agriculture combinate "Beograd". Three transferrin phenotypes were selected from these sera for the manganese binding experiments: Tf AE which possesses 6 bands, Tf AA with 3 fast bands and Tf DD with 3 intermediate bands. A fourth phenotype, Tf EE with 3 slow bands, was obtained from Denmark.

To 0.5 ml of serum was added 0.05 ml carrier free $Mn^{54}Cl_2$ which had a total activity of 6 μC/ml. After the addition of Mn^{54} and incubation at 38°C for 1 hr. the samples were subjected to starch gel electrophoresis for the separation of transferrin. The electrophoresis was performed with Poulik's method, modified by *Hesselholt* (1966). Immediately after electrophoresis the gel was carefully cut into pieces of a length of 3 mm, which were put into test tubes and counted in a well type scintillation counter. Electrophoregrams obtained under the same experimental conditions and stained with Amido Black 10 B served as a means of relating the radioactivity to a particular protein fraction.

After the addition of Mn^{54} to serum, several attempts were made to remove excess of manganese through various dialysis procedures using the TRIS-citric acid gel buffer. Dialysis against 2×2 l TRIS-citric acid buffer, pH 8.65 in 2×24 hrs. was selected. When starch gel electrophoresis and subsequent counting were performed after this dialysis procedure, it was observed that apparently the total amount of not protein bound radiomanganese had been removed.

In addition, to ascertain the binding of manganese to bovine serum transferrin, autoradiographs of starch gel electrophoregrams of different transferrin phenotypes were performed. The time of exposure was 30 days.

RESULTS

On the diagrams in Figs. 1—4 are shown the results of the activity directly measured on the starch gel after electrophoresis of sera with different transferrin phenotypes. The position of the activity on electrophoregrams follows that of the electrophoretically different transferrin. These results show that Mn^{54}, added in vitro, is readily bound to serum transferrin. When electrophoresis of serum with added Mn^{54} is performed without previous dialysis, the radioactivity of the electrophoregrams is not limited to the transferrin areas, but the activity also appears elsewhere on the electrophoregrams, indicating the presence of excess Mn^{54}. The transferrin zones, however, showed much greater activity than other zones. When electrophoresis was performed after dialysis, all unbound manganese was removed, and the activity on the electrophoregrams was clearly limited to the transferrin zones (Figs. 1—4).

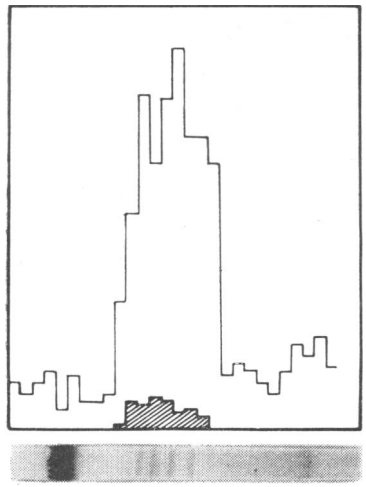

Figure 1. The activity of the starch gel electrophoregram of cattle serum, transferrin type AE, prior to dialysis (white diagram) and after dialysis (shaded diagram).

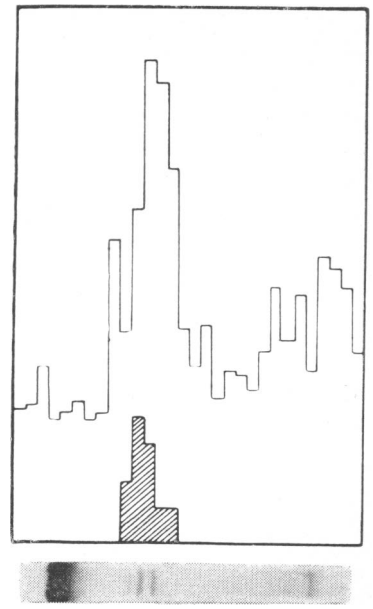

Figure 2. The activity of the starch gel electrophoregram of cattle serum, transferrin type AA, prior to dialysis (white diagram) and after dialysis (shaded diagram).

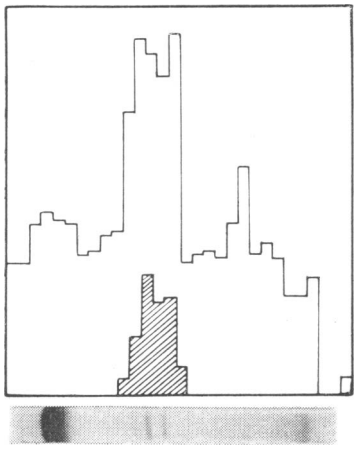

Figure 3. The activity of the starch gel electrophoregram of cattle serum, transferrin type DD, prior to dialysis (white diagram) and after dialysis (shaded diagram).

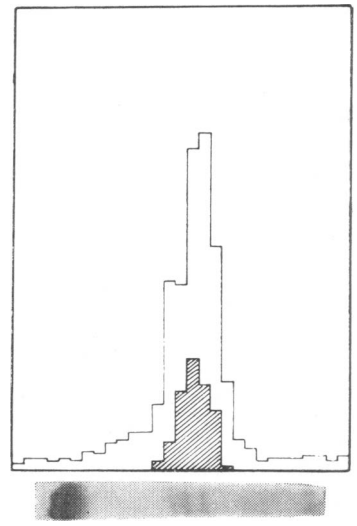

Figure 4. The activity of the starch gel electrophoregram of cattle serum, transferrin type EE, prior to dialysis (white diagram) and after dialysis (shaded diagram).

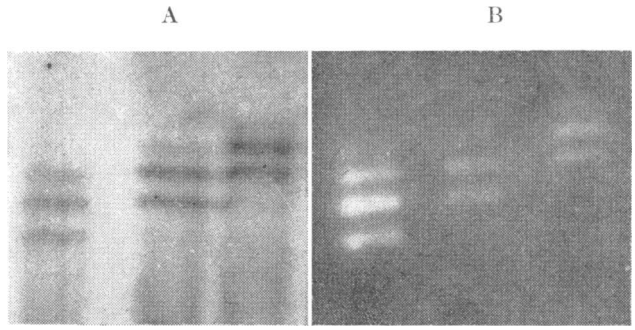

Figure 5. A: Starch gel electrophoregram of three cattle sera with different phenotypes of transferrins: EE, DD and AA.
B: Autoradiogram of the same sera after the addition of Mn^{54} and subsequent dialysis against tris-citric acid buffer.

The autoradiograms of starch gel electrophoregrams of 3 transferrin phenotypes Tf AA, Tf DD, Tf EE showed a clear limitation of the radioactivity to the bands of the different transferrin species. On the autoradiograph (Fig. 5) it is noted that the excess of added manganese was removed entirely by dialysis of serum against tris-citric acid buffer. The albumin fraction did not show any activity. This is in accordance with results of our previous investigations (*Panić & Ekman* 1967).

When the activity attached to the various transferrin phenotypes is considered it is interesting to note the existence of differences in the amount of activity incorporated. In this connection it should be added that a constant amount of radiomanganese was added to the sera investigated.

DISCUSSION AND CONCLUSION

The activity on the electrophoregrams and autoradiograms is clearly limited to the zones and bands of the starch gel electrophoretically different transferrins indicating that manganese added in vitro is attached to serum transferrin. These results exclude the possibility of the existence of a specific carrier protein for manganese, transmanganin, in cattle and the possibility of binding of manganese to other β_1-globulin components.

Simultaneous in vitro experiments in chicken have shown that the addition of radiomanganese to egg white results in a binding of manganese to conalbumin (own observations, to be published elsewhere). Both conalbumin and serum transferrin

are able to bind iron. In addition, the molecular structures of conalbumin and transferrin are nearly identic, the only differences reside in the prosthetic part of the primary structure (*Williams* 1962). The binding of radiomanganese to conalbumin therefore lends additional support to the establishment of a manganese-transferrin relationship.

By comparing the radioactivity of different phenotypes of transferrins, it can be noted that considerable differences exist between them in amounts of Mn^{54} bound. Very likely, these differences are due to individual quantitative variations of serum transferrin. It is well known that the total iron-binding capacity of serum, which reflects the content of transferrin, is subjected to individual, pathological and physiological fluctuations. The apparent variations in amounts of radiomanganese bound to the individual serum samples suggest similar fluctuations in the manganese-binding capacity of serum. The significance of this parameter for resorption, transportation and deposition of manganese will be the object of future investigations.

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SUMMARY

The in vitro binding of manganese to serum proteins in cattle was investigated using sera exhibiting the following transferrin phenotypes: Tf AE, Tf AA, Tf DD and Tf EE. After the addition of Mn^{54} to serum and dialysis, where excess of manganese was removed, it was found by means of starch gel electrophoresis that the radioactivity was confined to the electrophoretically different transferrin fractions. Variations in the amounts of radioactivity bound in the individual sera suggested fluctuations in the manganese-binding capacity of cattle sera.

ZUSAMMENFASSUNG

Bindung von Mangan an Serum Transferrin in vitro bei Rindern.

Die Bindung von Mangan an das Transferrin wurde in vitro untersucht, wobei vier ausgewählte Rindersera verwendet wurden, die die folgenden Phenotypen besassen: Tf AE, Tf AA, Tf DD und Tf EE. Nach Beigabe von 6/μC Mn^{54} auf je ein ml Serum und Dialyse zwecks Entfernung von überschüssigem Mangan wurde Elektrophorese auf Stärkegel durchgeführt. Die Radioaktivität des Elektrophoregramms wurde durch Messung der Aktivität der Gelstücke bei unmittelbarer Verwendung des Scintillationsmessers und auch autoradiographisch festgestellt. Die Aktivität des Elektrophoregramms war klar auf Zonen oder Bänder von elektrophoretisch verschiedenartigen Phenotypen von Transferrin begrenzt. Auf Grund der erhaltenen Resultate wird festgestellt, dass das Mangan an das Transferrin im Serum gebunden wird.

SAMMENDRAG

Binding af mangan til serum-transferrin in vitro hos kvæg.

Bindingen af mangan til serumproteiner hos kvæg blev studeret in vitro under anvendelse af sera med transferrinfænotyperne: Tf AE, Tf AA, Tf DD og Tf EE. Efter tilsætning af Mn^{54} og dialyse, hvor overskud af mangan blev fjernet, blev der ved stivelsegelelektroforese af sera fundet, at radioaktiviteten i gelen var begrænset til de elektroforetisk forskellige transferrinkomponenter. Variationer i mængden af radioaktivitet, der var bundet til de individuelle sera, tydede på fluktuationer i kvægserums manganbindende evne.

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