The Transport of Vitamin D in the Serum of Primates

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(Received 25 March 1976)

'Transcalfiferin' (the serum transport protein for cholecalciferol and related substances) of two New World monkeys, *Cebus apella* and *Cebus albifrons*, was found to be immunologically identical with the transcalfiferin of other monkeys and partially with that of man. In contrast with the α -globulin mobility of the transcalfiferin of other primates, the transcalfiferin of cebus monkey has the electrophoretic mobility of albumin. Most of the serum 25-hydroxycholecalciferol was precipitable with isolated monospecific anti-(human transcalfiferin) γ -globulins but not with anti-(human albumin) γ -globulins. These results indicate that the transport of 25-hydroxycholecalciferol in the cebus monkey is not due to albumin itself but to transcalfiferin with the electrophoretic mobility of albumin. Similar variants of transcalfiferin also exist in man.

Cholecalciferol and 25-hydroxycholecalciferol [9,10-seco-cholesta-5,7,10(19)-triene-3 β ,25-diol] present in human serum are bound to plasma proteins, mainly α -globulins. This was first demonstrated (Thomas *et al.*, 1959) by measuring the antirachitic activity of serum fractions and later confirmed by using labelled cholecalciferol (Avioli *et al.*, 1968) or labelled 25-hydroxycholecalciferol (Haddad & Chyu, 1971). This serum transport protein for ergocalciferol, cholecalciferol and their many hydroxylated derivatives has been isolated from human serum (Bouillon *et al.*, 1976b); we tentatively term it 'transcalfiferin', by analogy with other serum binding proteins (transcortin, transferrin and transcobalamin). During a comparative study of 25-hydroxycholecalciferol-binding protein in 72 mammalian species (Hay & Watson, 1976), labelled 25-hydroxycholecalciferol was bound to a protein with α -globulin mobility on polyacrylamide-gel electrophoresis in all but seven species. In these seven exceptions, which included three species of New World monkeys (*Cebus albifrons*, *Cebus capucinus* and *Cebus apella*), 25-hydroxycholecalciferol was bound to a protein with the electrophoretic mobility of albumin. The same phenomenon was previously described for *Cebus albifrons* (Edelstein *et al.*, 1973). Subsequently albumin itself was reported to be responsible for the transport of 25-hydroxycholecalciferol in these New World cebus monkeys (Hay, 1975).

Because this phenomenon would argue in favour of the superfluous nature of transcalfiferin, we re-investigated the nature of the transport of 25-hydroxycholecalciferol in the cebus monkey.

Experimental

Animals

Serum from different species of monkeys were obtained from the Zoological Institute of Antwerp (Belgium).

Materials

25-Hydroxy[26,27- ^3H]cholecalciferol (specific radioactivity 9.2 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Agarose (Indubiose A 37) was obtained from Industrie Biologique Française, Gennevilliers, France. Monospecific antiserum against human serum albumin was purchased from Dako, Hellerup, Denmark.

Antiserum production

Antiserum to human transcalfiferin was raised in rabbits as described by Bouillon *et al.* (1976b).

Immunodiffusion

Agarose (1%, w/v) prepared in diethylbarbituric acid buffer (0.023 M, pH 8.6) was used for double immunodiffusion (Ouchterlony, 1949) and for the following electrophoresis and immunoelectrophoresis studies. Radial immunodiffusion was performed as previously described (Van Baelen & De Moor, 1974) by using either 0.3 ml of monospecific anti-(human transcalfiferin) or 0.05 ml of monospecific anti-(human albumin) antiserum per 10 ml of agarose.

Electrophoresis

Agarose-gel electrophoresis of serum (5 μ l), previously incubated with 25-hydroxy[3 H]cholecalciferol (10 nCi), was performed at 4°C during 3 h at 15 V/cm. Thereafter half of the slide was stained with Amido Black and the other half cut into 0.25 cm pieces and counted for radioactivity for 10 min in 10 ml of a toluene-based scintillator.

Tandem crossed immunoelectrophoresis

Serum samples from three different species (man, and two species of monkey, *Cebus apella* and *Aotus trivirgatus*) were applied in three consecutive wells. After 1 h, the wells were closed with a drop of agarose and the first electrophoretic run (3 h, 15 V/cm, 4°C) was started. The second immunoelectrophoretic run was done at 4°C at 4 V/cm for 20 h. The immunogel (10 ml) contained 0.4 ml of a monospecific rabbit anti-(pure human transcalfiferin) antiserum (Bouillon *et al.*, 1976b).

Sucrose-gradient ultracentrifugation

Ultracentrifugation of serum from man or *Cebus apella* was performed in a 5–20% (w/v) sucrose gradient (4.2 ml prepared in sodium phosphate buffer, $I = 0.2$, pH 7.4) at 240 000g for 15 h at 4°C in a swing-out bucket of a MSE Superspeed 65 ultracentrifuge. The applied serum sample (0.2 ml) was diluted with the same buffer, either twofold for radial-immunodiffusion studies or 20-fold for radioactivity measurements, after previous incubation of the serum with a trace amount of 25-hydroxy[3 H]cholecalciferol and 14 C-labelled bovine serum albumin, as prepared by Rice & Means (1971).

Immunoprecipitation

Immunoprecipitation of 25-hydroxy[3 H]cholecalciferol-labelled serum was performed with γ -globulins, precipitated from whole rabbit antiserum with $(\text{NH}_4)_2\text{SO}_4$ (20%, w/v) and further purified by Sephadex G-75 gel filtration on a 4 cm $\times$ 20 cm column, eluted with phosphate buffer (0.05 M, pH 7.4). The final incubation mixture of 1 ml consisted of serum (diluted 500-fold) from man or *Cebus apella*, 10 nCi of 25-hydroxy[3 H]cholecalciferol and serial dilution of γ -globulins (from 4 to 0.04 mg/ml). After 20 h incubation at 4°C the tubes were centrifuged (20 000g for 20 min) and 0.5 ml of the supernatant was counted for radioactivity for 10 min.

Measurements of radioactivity

Radioactivity was measured in a Packard model 2450 spectrometer in either a toluene scintillator (Bouillon *et al.*, 1976a) or Insta-Gel (Packard Instruments, Downers Grove, IL, U.S.A.).

Fig. 1. Sucrose-gradient ultracentrifugation of serum from *Cebus apella*

(a) Fraction 1 represents the top and fraction 35 the bottom of the gradient. In each fraction 25-hydroxy[3 H]cholecalciferol (○) and 14 C-labelled bovine serum albumin (●) were measured. For further details, see the text. (b) Fraction 1 represents the top and fraction 46 the bottom of the gradient. In all fractions transcalfiferin (○) and albumin (●) were measured by radial immunodiffusion. The maximum protein concentration present in a single fraction was taken as 100%. For further details, see the text.

EXPLANATION OF PLATE I

Ouchterlony immunodiffusion

(a) Presence of transcalciferin in all primates. The central well contains 5 μ l of a monospecific rabbit anti-(human transcalciferin) antiserum. The outer wells contain 5 μ l of serum from: 1, man; 2, chimpanzee (*Pan*); 3, *Cercopithecus aethiops*; 4, *Ateles*; 5, *Aotus trivirgatus*; 6, *Cebus apella*. (b) Specificity of the antisera against transcalciferin and albumin. (i) The outer wells contain 5 μ l of: 1, serum from man; 2, human serum albumin (30 mg/ml); 3, pure human transcalciferin (3 mg/ml); 4, serum from *Cebus albifrons*. The central well contains 5 μ l of a monospecific rabbit anti-(human albumin) serum. (ii) The central well contains 5 μ l of a monospecific rabbit anti-(human transcalciferin) antiserum. The outer wells contain the same samples as in (i).

EXPLANATION OF PLATE 2

Immunelectrophoresis (a) and tandem crossed immunoelectrophoresis (b) of serum from man, Cebus apella and Aotus trivirgatus

Serum from man ($5\mu\text{l}$), *Cebus apella* ($7\mu\text{l}$) and *Aotus trivirgatus* ($5\mu\text{l}$) was applied in three consecutive wells with a distance between centres of 1 cm. The immunogel in the tandem crossed electrophoresis (a) and the trough in the immunoelectrophoresis (b) contain monospecific anti-(human transcaliferin) antiserum. The distance between the first and the second peak of immunoprecipitation is 1.4 cm, and between the first and the third, 3.05 cm. For details, see the text.

Results

1. Serum from *Cebus apella* contains a protein that is immunologically related to the transcalciferin of other primates

On Ouchterlony immunodiffusion against a monospecific rabbit anti-(human transcalciferin) antiserum a complete immunological identity between transcalciferin from man and chimpanzee (*Pan*) can be demonstrated (Plate 1a). Since spur formation was observed, human transcalciferin must share common antigenic determinants with the corresponding protein of other monkeys, both from the Old World (*Cercopithecus aethiops* and *Macaca mulatta*) and from the New World (*Cebus apella*, *Cebus albifrons*, *Ateles* and *Aotus trivirgatus*).

The monospecificity of our anti-(human transcalciferin) antiserum has previously been demonstrated (Bouillon *et al.*, 1976b) by the presence of a single precipitation line on immunoelectrophoresis of human serum. The absence of cross reaction either between human serum albumin and anti-(human transcalciferin) antiserum or between human transcalciferin and anti-(human albumin) antiserum is further demonstrated in Plate 1(b).

2. The serum transport protein for 25-hydroxycholecalciferol in the *Cebus* monkey is not albumin but transcalciferin with an increased electrophoretic mobility

(a) 25-Hydroxy[³H]cholecalciferol is precipitable by anti-(human transcalciferin) γ -globulins. γ -Globulins isolated from a monospecific anti-(human trans-

calciferin) antiserum were able to precipitate 73% of the 25-hydroxy[³H]cholecalciferol added to diluted serum of *Cebus apella* or *Cebus albifrons*, whereas γ -globulins against human serum albumin only precipitated 24% of the same tracer. Identical incubations with prelabelled diluted human serum indicated that 82% of 25-hydroxy[³H]cholecalciferol was bound to transcalciferin and 16% to human serum albumin. These results demonstrate that 25-hydroxycholecalciferol is largely bound to a specific transport protein, transcalciferin, in both species. The slightly lower binding of 25-hydroxycholecalciferol to the *Cebus* transcalciferin could be due to its lower association constant (Hay, 1975; Hay & Watson, 1975).

(b) Transcalciferin is smaller than albumin. The sedimentation coefficient of transcalciferin from *Cebus apella* was 4.1S (Fig. 1a). This value is identical with that obtained in man (Bouillon *et al.*, 1976b). Accordingly, these proteins are smaller than human or bovine serum albumin (4.6S). It was also shown, by immunochemical localization of transcalciferin and albumin, that both proteins do not coincide after sucrose-gradient ultracentrifugation of serum of *Cebus apella* (Fig. 1b).

(c) Transcalciferin of the *cebus* monkey has an increased electrophoretic mobility. On agarose-gel electrophoresis 25-hydroxy[³H]cholecalciferol added to the serum of *Cebus apella* (or *Cebus albifrons*) is bound to a protein with a greater mobility than the corresponding protein present in human serum (Fig. 2). This confirms previous reports (Edelstein *et al.*, 1973; Hay & Watson, 1976) that in all *Cebus* species (*Cebus apella*, *Cebus albifrons* and *Cebus capucinus*) 25-hydroxycholecalciferol is bound to a transport protein with the mobility of albumin. By using immunochemical detection of the transport protein on tandem crossed immunoelectrophoresis (Plate 2), it is evident that the transcalciferin from man and *Aotus trivirgatus* have the same electrophoretic mobility, since the distance of 3 cm between their respective application wells is identical with the distance between their peaks in the immunogel. The increased mobility of transcalciferin of *Cebus apella* compared with that of man is demonstrated by the distance of 1.4 cm between their immunoprecipitation peaks, whereas the distance between their respective application wells is only 1 cm.

Fig. 2. Agarose gel electrophoresis of serum from man and *Cebus apella*

After electrophoresis of serum from man (○) and *Cebus apella* (●) the gel was sliced in pieces (0.25 cm) and 25-hydroxy[³H]cholecalciferol was measured. The arrow indicates the position of albumin as revealed by staining the gel with Amido Black. For further details, see the text.

Discussion

Hay (1975) reported that albumin itself was responsible for the transport of 25-hydroxycholecalciferol in the *cebus* monkey, because he was unable to dissociate 25-hydroxy[³H]cholecalciferol from the serum albumin peak during electrophoresis or gel filtration. This premature conclusion can now be explained, since we demonstrated that the *cebus*

transcalfiferin (from *Cebus apella* or *Cebus albifrons*) has indeed the electrophoretic mobility of albumin and that both proteins have only a small difference in molecular size. There can, however, be no doubt that these monkeys have a serum protein that is immunologically related to the transcalfiferin of other primates and is able to bind most of the serum 25-hydroxycholecalciferol.

The phenomenon of altered electrophoretic mobility of transcalfiferin not only exists in these New World monkeys but also in the human species. Indeed, several variants of group-specific components of human serum with altered electrophoretic mobility have been described [for a review, see Cleve (1973)]. This group-specific globulin is now known to be identical with transcalfiferin (Daiger *et al.*, 1975; Bouillon *et al.*, 1976b).

The reason for the variation in electrophoretic mobility is, however, still unclear, since the molecular weight and the isoelectric point (H. Van Baelen & R. Bouillon, unpublished work) of transcalfiferin, as studied by means of 25-hydroxy-[³H]cholecalciferol, are identical in man and *Cebus apella*.

Stimulating discussions with Dr. W. Heyns were very much appreciated. The technical assistance of Mrs. K. Vandecaeter-De Ruddere and Mr. Tan Biau Keng is kindly acknowledged. We thank Dr. J. Desmyter and Mr. R. Verlaecht for their supply of the monkey

sera. R. B. is 'Aangesteld Navorser' of the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek.

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