Comparative studies of retinol transport in plasma

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Abstract The comparative immunology and biochemistry of plasma retinol transport were studied using radioimmunoassays previously developed for human and for rat retinol-binding protein (RBP). Serum or plasma from 25 species of verebrates, from the mammalian orders Primates, Artiodactyla, Perissodactyla, Carnivora, and Rodentia and from the classes Aves, Amphibia, and Pisces, were assayed. There was a high degree of immunological specificity within a given mammalian order. Sera from seven subhuman primate species tested reacted in the human RBP immunoassay, and sera from four of five rodents reacted in the rat RBP immunoassay. Primate sera failed to react in the rat RBP immunoassay, and rodent sera failed to react in the human RBP immunoassay. Except for a slight reactivity of canine serum in the human RBP immunoassay, other sera showed no immunoreactivity. Using gel filtration, apparent molecular weights were estimated at 60,000–80,000 for the retinol transport systems in whole serum from cow, swine, chicken, and dog. Canine RBP was isolated and partially characterized. Purified canine RBP was generally similar to human and rat RBP with regard to molecular weight (approximately 20,000) and other properties. In plasma, canine RBP circulates as a protein–protein complex of higher apparent molecular weight. The complex remains to be characterized. These data suggest that mammals in general have a retinol transport system similar to the human and rat transport systems but that immunologically important differences in RBP occur among mammalian orders.

Supplementary key words retinol-binding protein · human · rat · dog · primates · rodents · mammals · vertebrates · vitamin A · comparative immunology · comparative biochemistry · radioimmunoassay

Vitamin A is transported in plasma by specific proteins in both the human (1) and the rat (2). In both species, retinol circulates bound to a low molecular weight retinol-binding protein, which in turn circulates as a protein–protein complex with a larger protein with prealbumin mobility. Despite many similarities between human and rat RBP, the two proteins appear to be immunologically different and distinct (2).

The studies reported here were designed to examine the comparative biochemistry and immunology of retinol transport in a variety of mammalian and other vertebrate species. Using radioimmunoassays previously developed for human and for rat RBP, immunological reactivity was surveyed with plasma or serum from a variety of mammalian species (from the orders Primates, Artiodactyla, Perissodactyla, Carnivora, and Rodentia) and from other vertebrate classes (Aves, Amphibia, and Pisces). In addition, estimates of the apparent molecular weights of the vitamin A transport proteins in whole plasma or serum were made for the cow, swine, dog, and chicken, using gel filtration on Sephadex G-200. In one of these species, the dog, RBP was purified and partly characterized.

EXPERIMENTAL

Animal plasma and serum

Venous blood was drawn from a rhesus monkey (*Macaca mulatta*), sheep (*Ovis* sp.), goat (*Capra* sp.), mouse (*Mus musculus*), hamster (*Mesocricetus auratus*), rat (*Rattus norvegicus*), gerbil (*Meriones unguiculatus*), guinea pig (*Cavia porcellus*), cat (*Felis catus*), two dogs (*Canis familiaris*) 12 wk and 14 months old, toad (*Bufo marinus*), and frog (*Rana pipiens*) into heparinized tubes. The plasma was separated by centrifugation at 2000 *g* for 30 min at 4°C and then kept frozen in the dark at −20°C until used. Fresh pooled and filtered sera from carp (*Cyprinus carpio*), domestic duck (*Anas* sp.), turkey (*Meleagris gallopavo*), pigeon (*Columba livia*), chicken (*Gallus domesticus*), horse (*Equus caballus*), cow (*Bos taurus*), and swine (*Sus scrofa*) were purchased from a commercial source (Colorado Serum Co., Denver, Colo.). Sera from baboon (*Papio cynocephalus*), chimpanzee (*Pan troglodytes*), and cebus (*Cebus albifrons*), cynomolgus (*Macaca fascicularis*), stump-tailed (*Macaca arctoides*), and squirrel monkeys (*Saimiri sciureus*) were the generous gift of Dr.

Abbreviations: RBP, retinol-binding protein; SDS, sodium dodecyl sulfate; BHT, butylated hydroxytoluene.

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Radioimmunoassays

Radioimmunoassays for human and rat RBP were carried out as described previously (3–5). In the immunoassays, animal serum (or plasma) samples were assayed without dilution as well as after serial dilutions with standard assay buffer to a maximum of 1:80,000 (v/v). As used in our laboratory, the radioimmunoassay for rat RBP detects RBP present in about 0.1 μl of normal rat serum, whereas the radioimmunoassay for human RBP detects RBP present in 1 μl of normal human serum.

Protein standards

Purified proteins used for estimating molecular weights by gel filtration and SDS–disc gel electrophoresis were obtained from Schwarz/Mann (Orangeburg, N.Y.) (bovine serum albumin, cytochrome *c*, human hemoglobin and albumin, ovalbumin, sperm myoglobin, and beef trypsin) or from ICN Nutritional Biochemicals (Cleveland, Ohio) (chymotrypsinogen, pepsin, lysozyme, and ribonuclease). Human and rat plasma RBP were isolated as previously described (1, 2).

Gel filtration

Serum and plasma samples were dialyzed for 18–24 hr against 0.02 M potassium phosphate buffer, pH 7.4, with 0.2 M NaCl at 4°C, then centrifuged at 10,000 *g* at 4°C to clarify the solutions prior to gel filtration. Swine serum, containing relatively small amounts of vitamin A, was concentrated approximately twofold by ultrafiltration through a UM-2 membrane (Diaflo apparatus, Amicon Corp., Cambridge, Mass.) prior to dialysis. Serum or plasma samples of volume 25–40 ml (estimated protein content 0.7–2.1 g) were mixed with 1 ml of blue dextran polymer (Pharmacia Fine Chemicals Inc., Piscataway, N.J.), 10 mg/ml, and added to a Sephadex G-200 column (5 × 68 cm) at 4°C. The proteins were eluted with 0.02 M potassium phosphate buffer, pH 7.4, 0.2 M in NaCl, at a flow rate of 30–50 ml/hr (15 ml/fraction) with continuous monitoring of the effluent stream for absorbance at 280 nm with a Uvicord II absorptiometer (LKB Instruments, Inc., Rockville, Md.). Fractions were monitored for vitamin A content by measuring fluorescence at 334 nm excitation and 460 nm emission with an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, Md.). In fractions where fluorescence measurements suggested the presence of vitamin A, specific measurements of vitamin A were made as outlined below.

Vitamin A assay

Vitamin A in serum and plasma samples was measured with the trifluoroacetic acid method of Dugan, Frigerio, and Siebert (6), as modified by Roels and Mahadevan (7), or by the fluorometric method of Thompson et al. (8). All reagents used in the trifluoroacetic acid reaction contained butylated hydroxytoluene (BHT, 1 mg/dl, Eastman Kodak Co., Rochester, N.Y.) as antioxidant. Retinol in the column eluates was measured after saponifying 10-ml aliquots with 10 ml of ethanol and 1 ml of 45% KOH for 45 min under nitrogen at 65°C. The retinol (and other nonsaponifiable lipids) was collected by extraction with 20 ml of hexane; a portion of the extract was then evaporated to dryness under nitrogen and assayed. When retinol was measured with the fluorometric method, 0.6 ml of column eluate was mixed with 0.5 ml of ethanol, then extracted with 2.5 ml of hexane before carrying out the assay in the standard manner.

Verification of fluorescent material as retinol

Total lipid extracts of portions of the column eluates were prepared by extraction with 20 vol of chloroform–methanol 2:1 (v/v) containing BHT, 1 mg/dl (9). The total lipid extract was evaporated to dryness, suspended in hexane, and applied to a column containing 2 g of alumina (Woelm, Alupharm Chemicals, New Orleans, La.), activity grade III (6% H_2O), in hexane. Fractions containing carotenoids, retinyl esters, retinal, and finally retinol, were obtained by successive elutions with hexane (8 ml), 15% benzene in hexane (8 ml), 50% benzene in hexane (8 ml), and finally, benzene (20 ml) (10). With the trifluoroacetic acid reaction, pure retinol showed an absorbance maximum at 620 nm.

Molecular weight estimate by SDS–disc gel electrophoresis

Purified canine RBP, 10 μg in 50 μl of 0.2% sucrose, was incubated at 37°C for 2 hr in a solution of SDS then electrophoresed on polyacrylamide gel (0.4 × 5 cm) as outlined by Weber and Osborn (11). The mobility of canine RBP relative to that of human plasma prealbumin, RBP, and albumin, bovine and rat serum albumin, ovalbumin, carboxypeptidase A, chymotrypsinogen A, myoglobin, ribonuclease A, and cytochrome *c* was measured. The molecular weight was estimated from the regression line of the relative mobility and the log of the known molecular weights, as described previously (2, 11, 12).

RESULTS

Comparative immunoreactivity of plasma RBP in various species

Displacement of ^{125}I-labeled rat or human RBP from rabbit anti-rat RBP antibodies or from rabbit anti-human RBP antibodies by diluted serum or plasma from various species indicated the presence of immunological similarities

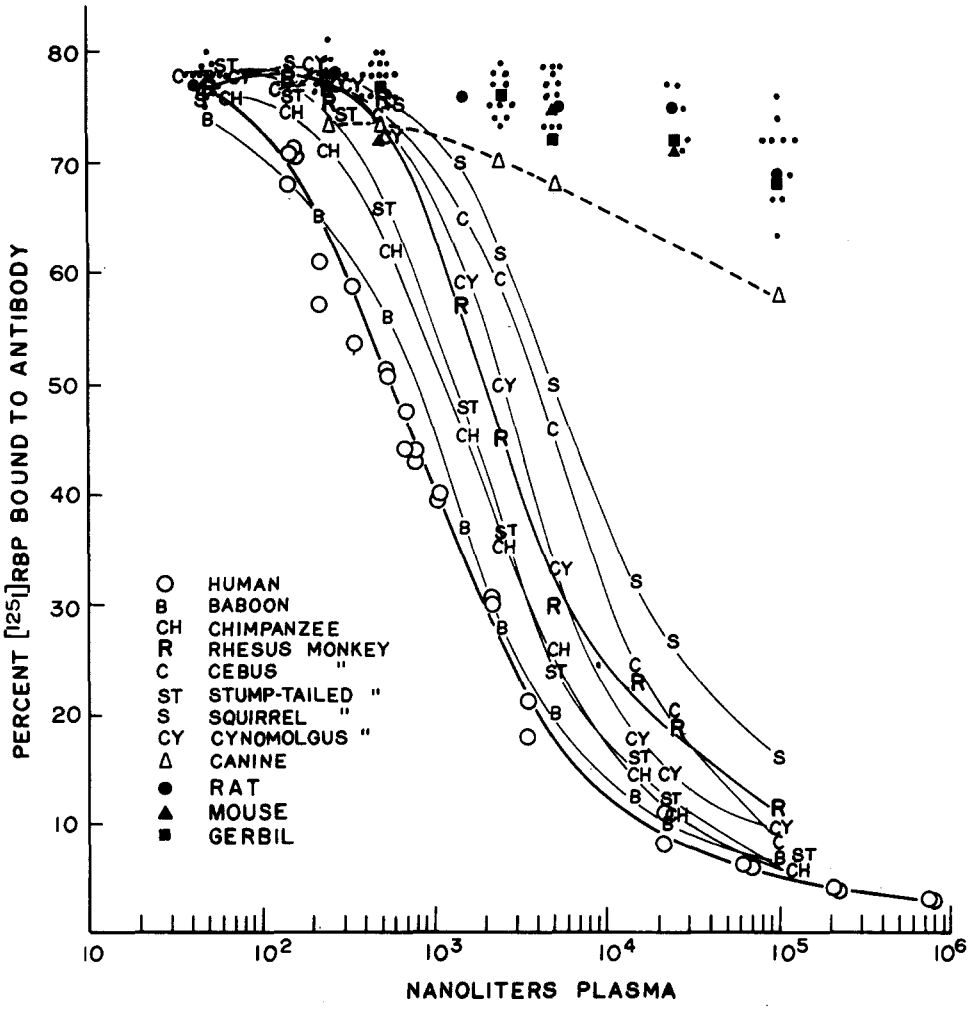

Fig. 1. Comparative immunoreactivity of plasma or serum samples from various species in the human RBP radioimmunoassay. Concentrations of animal sera used in the assay are indicated on the abscissa. Concentrations of vitamin A (μg/dl) in the serum or plasma samples assayed were human 50, baboon 95, chimpanzee 34, rhesus monkey 28, cebus monkey 22, stump-tailed monkey 40, squirrel monkey 19, cynomolgus monkey 21, cow 17, swine 7, sheep 20, horse 11, cat 8, dog 34, rat 21, mouse 22, guinea pig 16, gerbil 18, hamster 34, pigeon 27, turkey 61, chicken 69, duck 78, frog 4, toad 2, and carp 22. Species showing cross-reactivity are identified by specific symbols; nonreactive species are shown as small solid dots without specific identification except for three rodents (rats, mouse, and gerbil).

among certain particular retinol-binding proteins. In general, immunological cross-reactivity was found within a given order of mammals but was not found outside that order. Thus, sera from a number of different primates (including Old and New World monkeys) were able to displace human RBP from the anti-human RBP antibody (Fig. 1), while plasma samples from several rodents (mouse, gerbil, and hamster) displaced rat RBP from the anti-rat RBP antibody (Fig. 2). Rodent plasma failed to show immunoreactivity in the human RBP radioimmunoassay, and primate plasma lacked immunoreactivity in the rat RBP radioimmunoassay (Figs. 1 and 2). No immunological reactivity was observed in either radioimmunoassay with serum or plasma samples from species from a variety of other mammalian orders or from other vertebrate classes (see legend to Fig. 1 for the list of species tested). Immunological cross-reactivity was, however, neither complete within, nor absolutely specific for, a given order of mammals. Thus, within the order Rodentia, plasma from the guinea pig failed to displace significantly rat RBP from anti-rat RBP antibody. Moreover, canine plasma at 100-fold the concentration of primate sera slightly displaced human RBP from anti-human RBP antibody. Immunological similarity between canine and human RBP was also suggested by a line of identity between the purified proteins and anti-human RBP antiserum when diffused through agar.

Although all primate samples tested displaced labeled human RBP from the anti-human RBP antibody, there were quantitative differences in the amount of plasma or serum necessary to produce a given degree of displacement (Fig. 1). The displacement curves were, however, similar

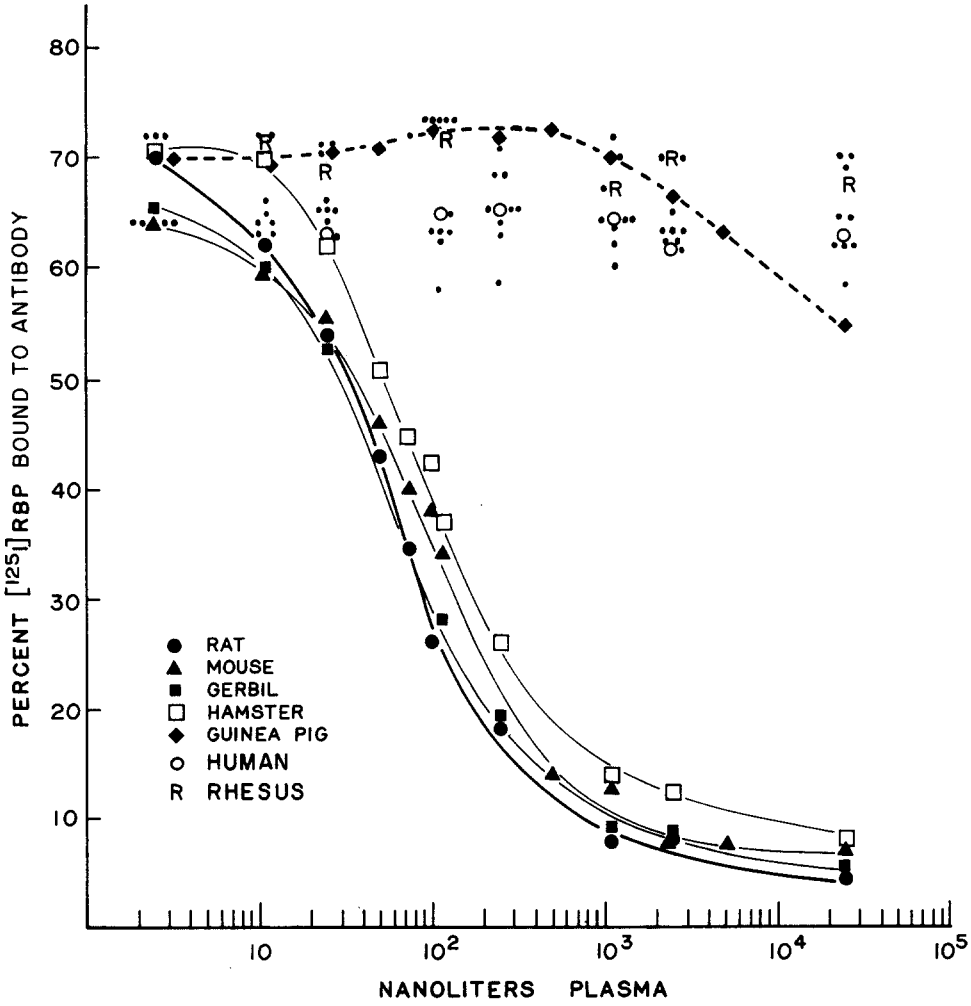

Fig. 2. Comparative immunoreactivity of plasma or serum samples from various species in the rat RBP radioimmunoassay. The samples and their vitamin A concentrations are described in the legend to Fig. 1. Nonreactive species are shown as small solid dots except for human (O) and rhesus monkey (R).

in shape for all samples tested. The differences in the amounts of plasma necessary for displacement could reflect quantitative differences between RBPs of different species with regard to immunological cross-reactivity with human RBP. Alternatively, the differences might reflect differing concentrations of RBP in plasma in various species. Since the amounts of RBP assayed in the various species were not known independently, information is not available to distinguish between these possibilities.

When the minimal displacement of [^{125}I]RBP from antibodies against human or rat RBP by increasing amounts of serum was examined for the species not specifically identified in Figs. 1 and 2, no pattern was apparent. The very small range of displacements by various amounts of serum from the nonreactive species appeared to reflect nonspecific methodological variation.

Sephadex G-200 filtration of vertebrate plasma

When serum or plasma from human, rat, dog, cow, swine, and chicken were eluted from the same Sephadex G-200 column, retinol-containing proteins were eluted in volumes consistent with molecular weights of approximately 60,000–80,000 (Fig. 3). Of the six species studied, the largest apparent size of the retinol transport system was seen in the chicken (V_E/V_O 1.64, molecular weight somewhat larger than serum albumin). In the single adult dog studied and the pooled adult dog plasma used in the purification of canine RBP (see below), almost all of the retinol appeared with proteins in an elution volume consistent with a molecular weight of about 75,000. In the single young dog studied, however, protein-bound retinol was eluted in two peaks, corresponding to proteins of molecular weight of about 75,000 and about 20,000, respectively (see Fig. 3).

Purification and properties of canine plasma RBP

385 ml of pooled plasma from adult dogs was concentrated to 145 ml by ultrafiltration and applied to a DEAE–Sephadex A-50 column (5.0 × 70 cm), and the

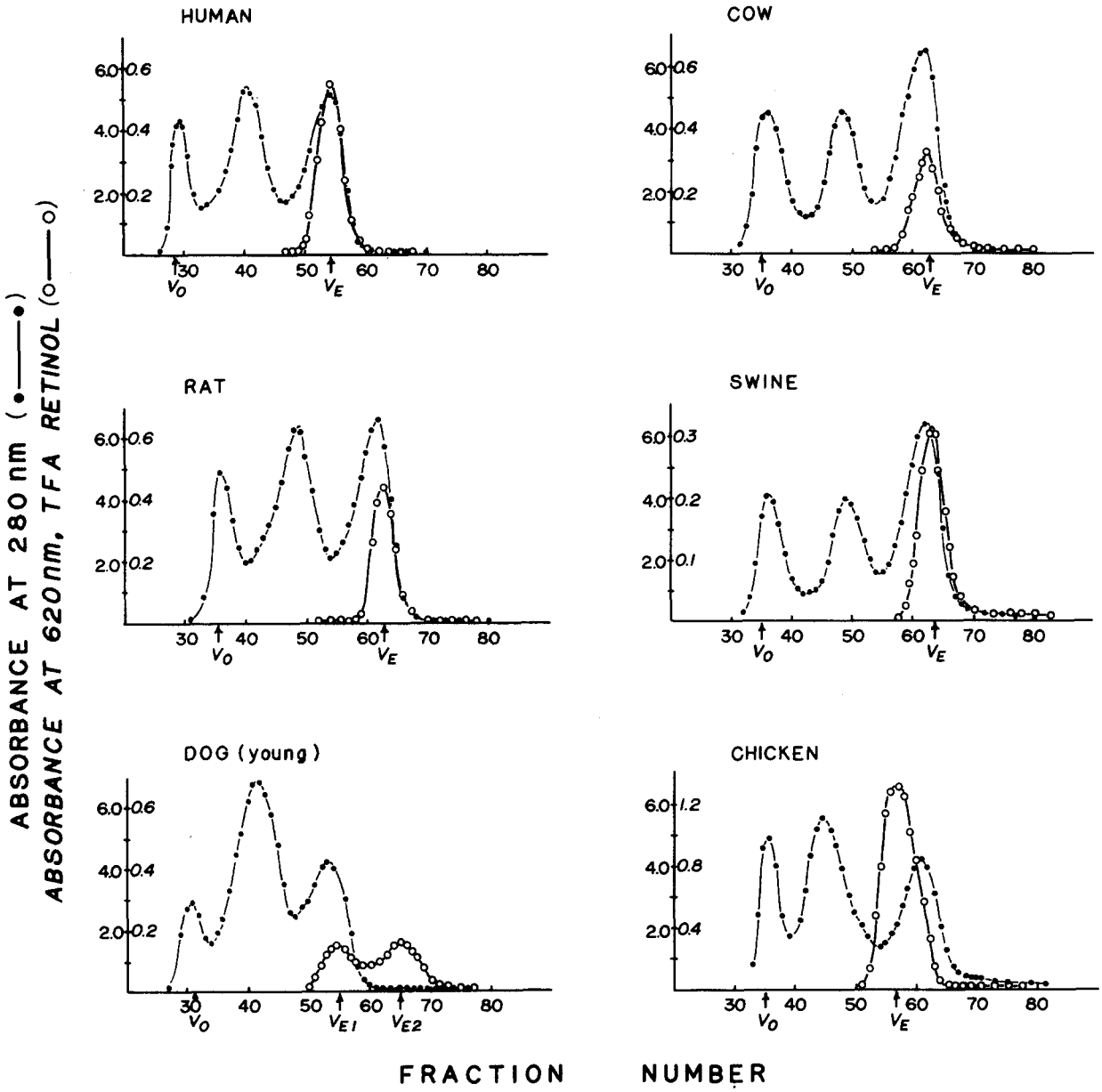

Fig. 3. Molecular weight estimation of the plasma retinol transport system in several species. Gel filtration of whole serum or plasma was carried out on a calibrated Sephadex G-200 column as described under Experimental. Eluted fractions were surveyed for vitamin A by measuring fluorescence at 330 nm excitation and 460 nm emission. Fractions with fluorescence were assayed for retinol with the trifluoroacetic acid reaction. The relative content of retinol is indicated in the figure as absorbance at 620 nm after 30 sec of the trifluoroacetic acid reaction (italic numbers to the right of the *Y* axis). Each sample applied to the column contained a small amount of blue dextran polymer in order to determine the void volume (indicated by the small arrows labeled V_O). The relative elution volumes (V_E/V_O) for the plasma retinol-containing proteins (indicated by arrows labeled V_E) in the sera assayed were human 1.89, rat 1.77, young dog 1.77 and 2.13, cow 1.80, swine 1.84, and chicken 1.64.

proteins were eluted with a linear gradient of NaCl, 0.05–0.3 M, in potassium phosphate buffer, 0.02 M, pH 7.5, at a flow rate of 40 ml/hr. The retinol-containing fractions (assessed by fluorescence) were pooled, concentrated by ultrafiltration, and rechromatographed on DEAE–Sephadex A-50 (2.5 × 65 cm) using a similar gradient. Protein-bound retinol was eluted in a single peak (Fig. 4, top panel). Fractions containing retinol were pooled, concentrated by ultrafiltration, and applied to a Sephadex G-200 column (2.5 × 70 cm) for gel filtration in 0.02 M potassium phosphate buffer, pH 7.4, with 0.2 M NaCl at a flow rate of 10 ml/hr (15 ml/fraction). The fractions containing protein-bound retinol were pooled and subjected to a second gel filtration on a smaller column of Sephadex G-200. As is evident in Fig. 4 (bottom panel), protein-bound retinol was now eluted in two peaks, with V_E/V_O values corresponding to molecular weights of approximately 75,000 and 20,000, respectively. It was assumed, in analogy with the human (1) and rat (2) retinol transport systems, that the second peak represented canine RBP, whereas the first peak represented a complex of RBP with some other protein.

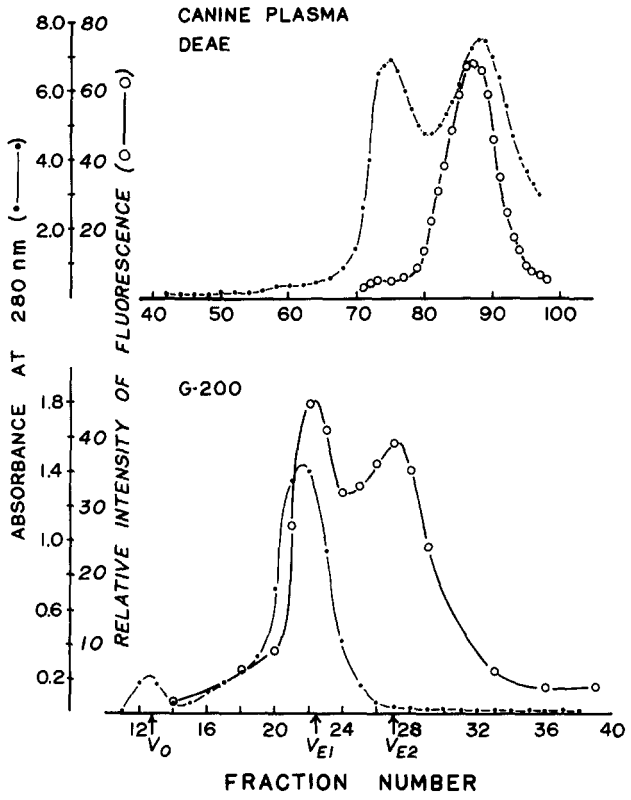

Fig. 4. Purification of canine RBP. Retinol-containing protein was eluted from DEAE–Sephadex as described in the text, concentrated by ultrafiltration, then eluted from a 2.5 × 65 cm DEAE–Sephadex column using a 0.05–0.3 M NaCl gradient in potassium phosphate buffer, 0.02 M, pH 7.5, at a flow rate of 16 ml/hr (15 ml/fraction) (*top*). Fractions 80 through 94, containing the protein-bound retinol, were pooled and subjected to gel filtration on Sephadex G-200 as described in the text. Protein-bound retinol was eluted in a single major peak (V_E/V_O 1.8), the fractions of which were pooled, concentrated, and applied to a second G-200 column, 1.3 × 100 cm, then eluted with potassium phosphate buffer, 0.02 M, pH 7.5, with 0.2 M NaCl at a flow rate of 10 ml/hr (4.1 ml/fraction). During this second gel filtration, protein-bound retinol was eluted in two peaks with relative elution volumes (V_E/V_O) of 1.81 and 2.17 as shown by the arrows (*bottom*).

Column eluates containing the uncomplexed RBP (the second peak in Fig. 4, bottom panel) were pooled and concentrated, and the protein was subjected to preparative polyacrylamide gel electrophoresis as previously described (1, 2). The fractions containing protein-bound retinol (monitored by fluorescence) were pooled, dialyzed against water, concentrated by lyophilization, and subjected to gel filtration on a column of Sephadex G-100 (1.3 × 90 cm), using the buffer indicated above, with a flow rate of 12 ml/hr (4.1 ml/fraction). A single peak of protein was obtained, which coincided with the single peak of fluorescence (protein-bound retinol). Analysis of the protein by analytical disc polyacrylamide gel electrophoresis, as described previously (1), demonstrated the presence of three bands with α_1 mobility, similar to the patterns observed with purified human (13) and rat (2) RBP. Two of the bands were fluorescent under ultraviolet light (before staining for protein) and presumably represented microheterogeneous forms of holo-RBP (containing bound retinol); the third band was not fluorescent and presumably represented apo-RBP.

The purified canine RBP preparation had an ultraviolet absorption spectrum with two peaks, at 280 and 330 nm, similar to the spectra observed with purified human and rat RBP. The absorbance ratio (330 nm/280 nm) of the preparation was 0.58. Fluorescence excitation and emission spectra were also similar to those observed with human (14) and rat (2) RBP. The protein-bound retinol had fluorescence excitation maximum (uncorrected) at 334 nm and emission maximum at 465 nm. On SDS–disc gel electrophoresis, a single band of protein was observed, supporting the conclusion that the preparation contained pure canine RBP. The molecular weight was estimated by SDS–disc gel electrophoresis (see Fig. 11 of Ref. 2 for details of the method as used in this study) to be approximately 19,900.

Studies on the protein that forms a complex with canine RBP

Since purified canine RBP had a molecular weight of approximately 20,000, and since protein-bound retinol in whole dog plasma mainly had an apparent molecular weight of about 75,000, it was clear that canine RBP normally circulates in the form of a protein–protein complex of some sort. Several attempts were made to characterize this complex. In one set of experiments, canine plasma proteins collected during the RBP purification sequence were mixed with a few micrograms of pure RBP, and the mixture was analyzed by gel filtration on a small column of Sephadex G-100 to determine whether complex formation had occurred. The procedure used was similar to that successfully used and described for the studies with rat RBP (2). The proteins tested included canine albumin isolated after Sephadex G-100 chromatography or after preparative polyacrylamide gel electrophoresis, different portions of the protein eluted across the albumin peak on gel filtration, and concentrated fractions representing protein with mobility faster than albumin (collected on polyacrylamide gel electrophoresis). It should be noted that disc gel electrophoresis of whole canine plasma failed to show protein bands with prealbumin mobility. No complex formation was observed in any of the studies even though in some experiments the albumin-to-RBP weight ratio was about 50:1. The possibility was then considered that RBP in whole plasma might be present as a polymer (aggregate) of identical subunits rather than as a complex with a different protein. However, when purified RBP was concentrated (to 2 mg/ml) and subjected to gel filtration, the protein remained a monomer, and no polymerization occurred. The form in which RBP circulates in dog whole plasma was therefore not characterized by these studies and remains to be defined.

DISCUSSION

The system responsible for vitamin A transport in plasma has been characterized in detail in two species, man (1) and rat (2). In both species, retinol is transported bound to a specific protein, RBP. The properties of human and rat RBP are similar in many ways. Both proteins have molecular weights close to 20,000 and α_1 mobility on electrophoresis. The two proteins have nearly identical ultraviolet absorption spectra (peak maxima at 280 and 330 nm) and fluorescence excitation and emission spectra. The amino acid compositions of rat and human RBP are somewhat similar, both with a fairly high content of aromatic amino acids. In plasma, both human and rat RBP circulate in the form of a protein–protein complex together with a protein with prealbumin mobility, with an apparent molecular weight of the complex of the order of 70,000. Human and rat RBP are, however, immunologically distinct from each other (2).

The studies reported here provide some new information about the vitamin A transport systems present in plasma in a variety of mammals and other vertebrate species. Three related studies are reported. In the first study, the comparative immunology of plasma retinol transport was examined by testing serum or plasma samples from 25 different species for immunoreactivity in the radioimmunoassays for human and for rat RBP. In the second study, estimates of the apparent molecular weight of the vitamin A transport system in whole plasma or serum were made for cow, swine, dog, and chicken, as compared with man and the rat. In the third study, RBP from a third species, the dog, was purified and partly characterized.

The comparative immunology study demonstrated a considerable degree of immunological specificity within a given mammalian order. This was particularly evident for the order Primates. Thus, sera from all seven subhuman primate species tested showed immunoreactivity qualitatively similar to that of human plasma when tested in the immunoassay for human RBP. Of the serum and plasma samples tested from nonprimate species, only dog plasma showed slight but definite immunoreactivity. Within the rodent order, less immunological specificity was apparent. While serum or plasma from the rat, mouse, gerbil, and hamster produced comparable displacement of rat RBP from the anti-rat RBP antibody, guinea pig serum failed to displace significantly rat RBP even at 100-fold the volume used with other rodent sera. The guinea pig, although taxonomically a rodent (15), is unusual among rodents in lacking the enzymes for synthesizing ascorbic acid (16). None of the samples tested from species other than rodents showed immunoreactivity in the immunoassay for rat RBP.

In interpreting these results, it should be noted that both the antiserum against human RBP and the one against rat RBP were made in rabbits. The antibodies studied would therefore recognize only antigenic sites on human or rat RBP that are not present in common with those on rabbit RBP. The failure of guinea pig serum to react in the rat RBP radioimmunoassay indicates that guinea pig RBP lacks the particular antigenic sites, present on rat but not on rabbit RBP, against which the anti-rat RBP antiserum was directed. In its overall structure, however, guinea pig RBP might be very similar to rat RBP. It should also be noted that the results reported here were obtained with those particular antisera that we have raised in rabbits and used in clinical and nutritional studies. Antisera raised in other animals, either other rabbits or other species, might react with different antigenic sites and might provide somewhat different results with regard to immunological cross-reactivity between different species.

These data suggest that retinol transport in plasma is mediated in primates by a transport system and by an RBP structurally very similar to human RBP and in rodents by an RBP structurally very similar to rat RBP. The existence of a comparable retinol transport system in a third species, from a third order of mammals, was also demonstrated by the isolation and partial characterization of RBP from dog plasma. These results suggest that mammals in general may have a retinol transport system similar to the human and rat systems, but with some immunologically significant differences in RBP among mammalian orders. Evidence against this suggestion was recently reported by Huang, Howarth, and Owen (17), who partially purified RBP from porcine serum and who estimated the molecular weight by gel filtration as approximately 47,000. It should be noted, however, that in this latter study (17) the porcine RBP was incompletely purified, that the gel filtration column was apparently not directly standardized, and that the molecular weight estimate was not fully consistent with the reported sedimentation velocity coefficient. We believe that further studies are necessary to determine whether a transport system comprising a low molecular weight RBP circulating as a protein–protein complex of higher apparent molecular weight is present in a variety of other mammalian species. Moreover, no definitive information is available on this point with regard to nonmammalian vertebrate species.

Gel filtration of sera from human, rat, dog, cow, swine, and chicken on the same calibrated column demonstrated that the retinol-containing proteins had similar apparent molecular weights (approx. 60,000–80,000) in whole serum in all six species. The presence of uncomplexed RBP (molecular weight approx. 20,000) in the sample of puppy serum studied (Fig. 3), and its virtual absence from two other samples of canine serum and plasma, suggests

that the wide fluctuation reported in both plasma and urinary vitamin A concentrations in dogs (18, 19) may be a function of the relative amount of uncomplexed RBP present. With its low molecular weight, uncomplexed RBP could be largely filtered in the renal glomerulus and subjected to renal catabolism or urinary excretion, or both (4, 20).

Purified canine RBP was generally similar to human and rat RBP with regard to molecular weight (approx. 20,000), electrophoretic mobility, microheterogeneity on disc gel electrophoresis, and with regard to its ultraviolet absorption and its fluorescence excitation and emission spectra. As indicated above, in whole dog plasma, RBP circulates mainly as a complex of higher apparent molecular weight. While proteins with prealbumin mobility that interact specifically with RBP have been identified and characterized for the human and the rat, the nature of the RBP-binding protein in the dog has not been established. In an earlier study, Farer et al. (21), utilizing standard electrophoretic techniques, identified proteins with prealbumin mobility in a variety of vertebrate species, including primates, birds, and fishes, but failed to observe prealbumin proteins in sera of cow, swine, and dog. However, the appearance of two species of prealbumin in the rat after partial purification (2), not evident in whole plasma, makes interpretation of electrophoretic results on whole serum difficult with respect to the presence or absence of prealbumin proteins. Further studies are needed in order to characterize fully the form in which RBP circulates, as a complex, in dog plasma.

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