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Bilirubin Binding by Variant Albumins in Yanomama Indians

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SUMMARY

Sera of Yanomama Indians homozygous for the common albumin allele exhibited greater total effective-binding capacities for bilirubin than did sera of individuals homozygous for the *Yan-2* albumin variant in the in vitro experiments described herein. Total effective binding capacities of heterozygous samples were close to those of homozygotes for Al^A. Individuals homozygous for *Yan-2* might experience a higher risk of bilirubin toxicity and related disorders during the neonatal period. Further studies of binding and displacement of bilirubin by competitors, such as dietary or medicinal coumarins, might help explain the existence of these polymorphisms and the significance of phenotypic differences in binding to bilirubin.

INTRODUCTION

Although more than 2 dozen human albumin variants are now known, the genes associated with those reported to reach polymorphic frequencies are, with one exception, restricted to certain American Indian populations [1–4]. One of the most frequently studied of these polymorphisms is the *Yan-2* variant of the Yanomama Indians of southern Venezuela and northern Brazil [5–7]. Elsewhere in the primate order, albumin polymorphisms are rare except in the genus *Macaca*. In most macaques, irrespective of species affiliation, the frequency of the less common albumin allele (Al_{mac}^B) never exceeds .05. However, in India, several of these same macaque species have a much higher frequency of Al_{mac}^B, and an

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identical phenotype is, in fact, fixed in some species [8]. $Al_{mac}{}^{A}$ is electrophoretically identical to the albumin of all members of five species of genus *Papio* that we have studied. Since macaques diverged from the genus *Papio* some 7 million years ago [9], $Al_{mac}{}^{B}$ may be a variant allele, unique to genus *Macaca,* that has largely replaced $Al_{mac}{}^{A}$ in macaques from India, but not elsewhere. However, given the many instances in which the same protein electromorph has been shown to be due to different amino acid substitutions, this hypothesis must remain unproven until the necessary detailed biochemical studies on albumin have been carried out.

The evolutionary mechanisms responsible for the presence of these albumin polymorphisms are unknown. The paucity of albumin polymorphisms in both human and macaque populations suggest that evolution of albumin in primates has been conservative. The numerous, apparently independent, mutations resulting in a proliferation of human variants predominantly in the New World, and the restriction of $Al_{mac}{}^{B}$ to Indian macaques, irrespective of species affiliation, are inconsistent with the hypothesis that random genetic drift alone is responsible for their distribution. Although complete sequencing of monkey albumin has not been performed, the numbers and distributions of residues varies little from the albumin of humans [10]. The evolution of tertiary structure of albumin has been very conservative in mammals, with even the tertiary structure of bovine albumin being almost identical to that of human albumin [11]. Since albumin is known to have important roles in the maintenance of osmotic pressure as well as in the binding and transport of endogenous metabolites (such as bilirubin) and exogenous agents (such as drugs), it is possible that these albumin functions have influenced the frequency and distribution of this polymorphism in both species.

Bilirubin, a toxic byproduct of normal red blood cell destruction, is bound by the albumin molecule, carried to the liver where it is conjugated to a water-soluble form, then excreted. Circulating free (unbound) bilirubin, due to either poor binding to albumin or displacement of bilirubin from albumin by competitive binders [12], infiltrates and severely damages the central nervous system during the first week of life. Since the newborn infant is at risk of bilirubin toxicity during the first few days of life when the binding (by albumin) and conjugation of bilirubin are transiently inefficient, the albumin phenotype might influence neonatal bilirubin toxicity [13, 14]. Newborn macaques exhibit a pattern of physiological jaundice [13] and hyperbilirubinemia [15] similar to, but more accelerated than, that in humans. $Al_{mac}{}^{B}$ has a lower effective capacity for binding bilirubin than does $Al_{mac}{}^{A}$ [16]. If selection has favored similar functional properties of albumin in both human and macaque variants, albumin variants in American Indians might not bind bilirubin as effectively as the common form: albumin A (Al^{A}). While this condition would favor low, rather than high, frequencies of the variant, we will consider later the circumstances under which this trait might actually be a selective advantage. To clarify the possible role of bilirubin binding in the persistence of the albumin polymorphism in humans, we compared the binding of bilirubin by human sera from individuals heterozygous and homozygous for the albumin variant *Yan-2* with that of sera homozygous for the common form, Al^{A}. Lower effective-bilirubin-binding capacities by the *Yan-2* variant, as

found for the Al_{mac}^{B} variant in rhesus macaques, might have both clinical and theoretical significance in an environment in which a newborn is already at high risk for developing unconjugated hyperbilirubinemia.

MATERIALS AND METHODS

Serum samples from 41 Yanomama Indians of southern Venezuela and northern Brazil were studied. These included six samples from Indians homozygous for the *Yan-2* variant (*Yan-2/Yan-2*), 17 samples from Indians homozygous for the common form of human albumin (*A/A*), and 18 samples from heterozygous (*Yan-2/A*) Indians. Phenotypes of these samples had been previously electrophoretically identified for purposes unrelated to the present study, and all samples have been stored at −70°C prior to analyses described here. After thawing, aliquots of these samples were diluted 1:100 and analyzed by polyacrylamide gel electrophoresis [16] to reconfirm the albumin phenotypes.

The albumin concentration (A) of each sample was determined by the BCG method [17] and checked against total protein determinations following serum electrophoresis of the different phenotypes. Each sample was then diluted with 0.055 M phosphate buffer, pH 7.4, to give a final albumin concentration of approximately 100 μM, and the peroxidase assay was employed to determine binding constants [18]. The samples were enriched with bilirubin to give final bilirubin/albumin molar ratios (MR) of 0.3 and 0.9. An aliquot of the 0.3 MR sample was also enriched with biliverdin to give a biliverdin/albumin MR of 0.4. Unbound bilirubin in the samples was determined with and without biliverdin present and at two enzyme concentrations to correct for steady-state errors in the determination of bilirubin-albumin-binding parameters by enzyme oxidation [12, 19]. Since biliverdin is known to displace bilirubin only from the primary bilirubin-binding site, comparisons of free bilirubin data with and without biliverdin present are used in computation of n, the molar-binding capacity [18]. The biological-binding capacity of each sample was then computed as $[n \times A]$. The unbound bilirubin concentration and molar-binding capacity were then used to calculate the equilibrium-association constant (K) from the law of mass action [16]. The binding constants determined at the 0.3 MR were used to calculate the expected unbound bilirubin concentration at the 0.9 MR. These data were then compared with the experimentally determined unbound bilirubin concentration at the 0.9 MR. In cases of large discrepancies, secondary-site bilirubin binding is suspected, which may lead to erroneous estimates of the primary bilirubin-binding-site capacity when the biliverdin displacement method is used [18, 19]. Since the values of n and K cannot be estimated independently, and are usually inversely proportional, values of $[n \times K]$ were also estimated to ensure that no phenotypic differences observed for one of these parameters are due to systematic errors in estimates of the other. Finally, the total effective-binding capacity was estimated as $[n \times K \times A]$. The binding parameters for the samples representing each of the two homozygous phenotypes were compared using an approximation to the Student's t-test to account for unequal variances [20].

Binding was also assessed in two randomly selected homozygous samples using fluorescence quenching [21–23] to confirm qualitative differences in binding among phenotypes. For these experiments, the fluorescence quenching, by bilirubin, of a mixture of equal parts of samples of *Yan-2/Yan-2* serum and *A/A* serum was compared with that of the serum of each homozygote. Sera were diluted to a final albumin concentration of 10 μM using a 0.055 phosphate buffer at pH 7.4 and analyzed using a Perkin-Elmer 204A spectrofluorometer thermostated at 37°C.

RESULTS

Results of the binding studies are given in table 1. When samples were pooled by phenotype, the mean values of n (the binding capacity) of sera from individuals homozygous and heterozygous for the variant and sera from normal homozygotes

TABLE 1

PARAMETERS FOR BILIRUBIN BINDING OF YANOMAMA INDIAN SERUM BY ALBUMIN PHENOTYPE

	VARIANT (*Yan-2*)		COMMON (*A*)	COMPARISON OF HOMOZYGOTES		
	Homozygotes	Heterozygotes	Homozygotes	*t*	df	*P*
No. samples	6	18	17	...	...	...
n	1.17 ± .199	1.18 ± .29	1.52 ± .72	1.87	22.2	< .05
K	76.27 ± 13.72	96.57 ± 40.05	90.55 ± 58.88	0.93	...	n.s.*
n × *K*	86.94 ± 5.45	107.33 ± 34.17	108.26 ± 42.52	2.02	18.5	< .03
A	476.2 ± 47.6	523.6 ± 70.8	509.8 ± 59.1	1.39	13.0	n.s.
n × *A*	559 ± 128.61	614.3 ± 156.82	789.03 ± 403.81	2.07	22.8	< .02
n × *K* × *A*	41,340 ± 4,396	55,805 ± 17,652	55,396 ± 22,247	2.47	20.0	< .02

NOTE: *n* is the no. of high-affinity binding sites (capacity) per albumin molecule, *K* (affinity) is the bilirubin-binding-association constant ($\times 10^6\ M^{-1}$), and *A* is the total albumin concentration in μM/l.

* n.s. = not significant.

were 1.17, 1.18, and 1.52, respectively, and binding constants for these three phenotypes were 76.3, 96.6, and 90.6, respectively. The value of *n* was statistically significantly lower in sera from persons homozygous for the variant than in sera from homozygous normal persons ($t = 1.87$, $P < .05$). The values of *K* and *A* for sera homozygous for the *Yan-2* variant were not statistically significantly different from those of sera homozygous for Al^A at the .05 level of probability. All other binding parameters given in table 1 that are derived from these three estimates: [*n* × *K*], [*n* × *A*], and [*n* × *K* × *A*], were statistically significantly lower in the variant homozygotes than in the normal homozygotes ($t = 2.02$, $P < .03$; $t = 2.07$, $P < .02$ and $t = 2.47$, $P < .02$, respectively).

The effect of Al^A upon [*n* × *K*] and [*n* × *K* × *A*] was nonlinear, with the sera from heterozygotes exhibiting binding properties more similar to sera from *A/A* than from *Yan-2/Yan-2* persons. This nonlinearity was confirmed by the fluorescence quenching behavior of the mixture of the two homozygous sera, shown in figure 1, which fell between that of both homozygotes but qualitatively much closer to the normal homozygote.

DISCUSSION

As found in previous studies of human [24, 25] and macaque [16] serum, values of *n* for the Yanomama sera were not integral but fell between 1.0 and 2.0. This suggests that there is more than one bilirubin-binding site and that allosteric interactions occur between the binding properties at these sites [26].

Sera homozygous for the *Yan-2* variant exhibited a lower molar capacity for binding bilirubin (*n*) and lower values of [*n* × *K*], molar-binding efficiency, than did sera homozygous for Al^A. Since the number of samples homozygous for *Yan-2* studied was only six, this difference could be due to sampling error. We believe this is unlikely, however, since the value of *n* for *Yan-2* homozygotes was almost identical to that for heterozygotes, for whom the sample size was three times as large. The average value of [*n* × *K*] for sera from heterozygotes was not intermediate between the values for the *A/A* and *Yan-2/Yan-2* persons, but, rather,

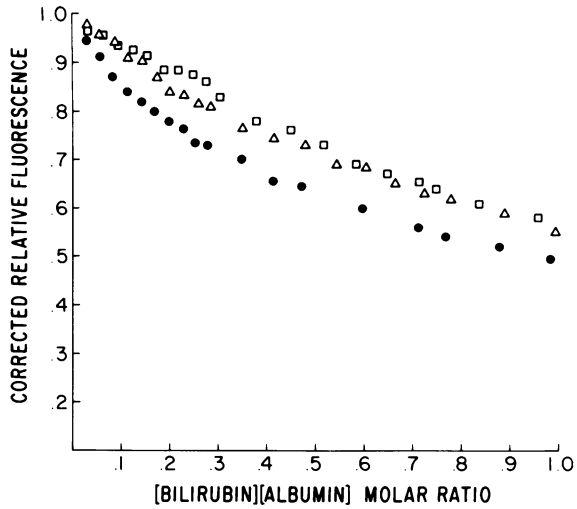

FIG. 1.—Fluorescence quenching of *Yan 2* and *A/A* homozygotes, and 50:50 mix (10 μm albumin). ● = homozygote variant (*Yan-2*), 10 μM; □ = homozygote normal (*A/A*), 10 μM; △ = heterozygote mix (½ *Yan-2*, ½ *A/A*), 10 μM.

was only slightly lower than that of the *A/A* sera. This outcome was confirmed by the results of fluorescence quenching of the sample constructed of equal parts of *A/A* and *Yan-2/Yan-2* sera. Both sets of data, therefore, indicate a nonlinear (recessive) effect of the allele associated with the *Yan-2* variant upon bilirubin binding. This effect, which is negative in homozygotes, suggests interaction effects upon binding.

The phenotypic differences in values of n, K, and $n \times K$ discussed above are consistent with those previously reported for a smaller number (four homozygous variants, nine heterozygotes, and 10 common homozygotes) of American Indian serum samples [27]. These few samples represent five different albumin variants other than *Yan-2* (albumins *Mexico-1*, *Mexico-2*, *Naskapi*, *Makiritare*, and *Maku*) and four different ethnic groups (Pima, Apache, Makiritare, and Yanomama). Thus, while valid controlled comparisons among the sera homozygous and heterozygous for an albumin variant and those homozygous for Al^A cannot be made and sample sizes are inadequate for statistical tests, these additional data suggest that other albumin variants may exhibit bilirubin-binding properties similar to those described here for *Yan-2*.

Values of $[n \times A]$ may be more meaningful indicators of functional differences in binding capacity among albumin phenotypes than is n because they reflect the biologically functional capacities for binding bilirubin. Although albumin concentrations in serum fluctuate with changes in diet and health status and are probably predominantly under nongenetic control, differences in total albumin concentration should be random (and, in fact, did not differ significantly) among the albumin phenotypes studied. The estimate of $[n \times A]$ for the *Yan-2/Yan-2* samples was statistically significantly lower than that of the *A/A* samples, using the more robust t-test for unequal variances. The value of K is an important

indicator of binding effectiveness because bilirubin is bound reversibly to albumin and can be displaced by competitors, especially if the value of K is low. Values of $[n \times K \times A]$, therefore, might provide the best estimate of biological effectiveness with which bilirubin is bound. Although values of K did not differ significantly between the two homozygous phenotypes at the .05 level of probability, the value of $[n \times K \times A]$ was statistically significantly lower in sera from persons homozygous for the *Yan-2* variant than in those homozygous for the common albumin allele.

The distribution of total effective-binding capacities, $[n \times K \times A]$, by phenotype, is given in figure 2. The variances of the *Yan-2/A* and *A/A* samples are statistically significantly larger than that of the *Yan-2/Yan-2* samples ($F_{(17,5)} = 16.1$, $P << .001$; and $F_{(16,5)} = 25.6$, $P << .001$, respectively). The coefficients of variation of this and other binding parameters for the *A/A* (and/or *A/Yan-2*) samples (but not the *Yan-2/Yan-2* samples) were somewhat higher than those reported elsewhere for freshly drawn samples of normal human sera, or 25%–30% [25]. Heterogeneity was probably not increased by lengthy storage or frequent thawing and refreezing of the *A/A* and heterozygous samples, because the *Yan-2/Yan-2* samples, which were subjected to identical storage conditions, were not equally affected. We do not believe that sampling error is responsible for this difference in variance, because, in that circumstance, the variance of the phenotype group comprised of the fewest number of samples should be greater, not less than, that of the other phenotype groups represented by a much larger number of samples. What factors, submolecular or otherwise, might contribute to this heterogeneity in the *A/A* and *Yan-2/A* sera are not clear at this time. Nongenetic heterogeneity in Al^A [28], including heterogeneity of binding characteristics [29], has been previously reported. However, additional genetic variation in Al^A, which is absent in *Yan-2* and which either cannot be or has not previously been identified electrophoretically, could conceivably be responsible for this heterogeneity.

The Amerindian tribes of Central and South America have been found to possess a series of electrophoretic "private polymorphisms," alleles in polymorphic pro-

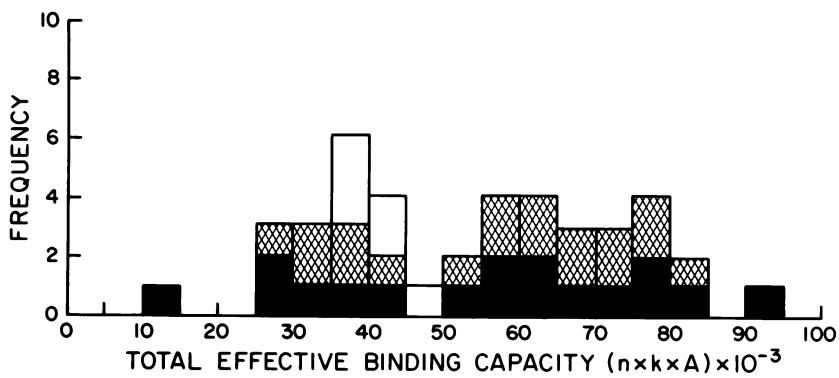

FIG. 2.—Frequency distribution of effective-binding capacities ($n \times k \times A$) of Yanomama Indians by albumin phenotype. ■ = homozygous *A/A* samples; ▩ = heterozygous samples; □ = homozygous *Yan-2/Yan-2* samples.

portions restricted to a single or adjacent tribe. Albumin *Yan-2* is one of the most striking of these. Elsewhere we have argued that the array of frequencies that these private polymorphisms present is consistent with the action of genetic drift upon alleles whose phenotypes are neutral in selective value, under the pressure of a mutation rate of the order of 0.7×10^{-5} [30]. This interpretation for the specific case of *Yan-2* is now complicated by the demonstration of what at first thought would seem to be a selective disadvantage, especially for homozygotes.

Previous work has indicated that the bilirubin-binding site lies between loops 3 and 4 of the albumin molecule [31]. Amino acid analysis, which would determine the proximity of the substitution site to the bilirubin-binding site, might clarify the role of protein structural variation on bilirubin binding. While the outcome of this study is consistent with the hypothesis that selection influences the non-random geographic distribution of human albumin variants, it is certainly not immediately clear why the *Yan-2* variant whose bilirubin-binding effectiveness is lower than that of Al^A should attain such a frequency, if selection is responsible. Were selection pressure against the *Yan-2* homozygote low enough to be overcome by genetic drift, it is not likely that statistically significant differences in bilirubin-binding properties could have been detected.

Wilding et al. demonstrated reduced warfarin binding by one human albumin variant [32]. Warfarin is a derivative form of dicoumarol, which, at physiological doses, binds human albumin competitively at the primary bilirubin-binding site [33]. We have found (our unpublished data, 1983) that dicoumarol is also a competitive displacer of bilirubin from macaque albumin and that the common form, $Al_{mac}{}^A$, which binds bilirubin more effectively than $Al_{mac}{}^B$, experiences greater displacement of bilirubin by dicoumarol in homozygous, but not in heterozygous, sera than does $Al_{mac}{}^B$. Numerous coumarin-containing plants have long been among the staple foods in the diet of at least some American Indians with both an albumin polymorphism [34] and an atypically high incidence of hyperbilirubinemia [35]. Tonka beans (*Dipteryx odorata*), from which coumarin was first isolated [36], contain up to 3% (dry weight) coumarin, are indigenous to the forested river banks of southern Venezuela and northern Brazil, and are reported to have been employed for medicinal purposes by the natives [37]. Moreover, coumarins are known to be excreted in human breast milk [38] and are, therefore, potentially available to neonates during the period of transient inefficiency of bilirubin metabolism. Both temporal and geographic variation in the availability of competitive binders of albumin could influence the direction of selection at the albumin locus.

REFERENCES

1. JOHNSTON F, BLUMBERG B, AGARWALL SS, MELARTIN L, BURCH T: Alloalbuminemia in southwestern U.S. Indians: polymorphism of albumin Naskapi and albumin Mexico. *Hum Biol* 41:263–270, 1969
2. POLESKY H, ROKALA D, BURCH T: Serum albumin polymorphism in Indians of southwestern United States. *Nature* 220:175–176, 1968
3. LISKER R, COBO L, MORA G: Distribution of albumin variants in Indians and non-Indians of Mexico. *Am J Phys Anthropol* 35:119–124, 1971

4. SCHELL L, BLUMBERG BS: The genetics of human serum albumin, in *Albumin: Its Structure, Function and Uses in Man,* edited by ROSENOER V, ROTHCHILD M, ORATZ M, New York, Pergamon Press, 1977, pp 113–141
5. TANIS R, FERRELL R, NEEL J, MORROW M: Albumin Yanomama-2, a 'private' polymorphism of serum albumin. *Ann Hum Genet* 38:179–190, 1974
6. WARD R, NEEL J: The genetic structure of a tribal population, the Yanomama Indians. XIV. Clines and their interpretation. *Genetics* 82:103–121, 1976
7. WEITKAMP L, MCDERMID E, NEEL J, ET AL.: Additional data on the population distribution of human serum albumin genes; three new variants. *Ann Hum Genet* 37:219–226, 1973
8. SHOTAKE T: Serum albumin and erythrocyte adenosine deaminase polymorphisms in Asian Macaques with special reference to taxonomic relationships among *Macaca assamensis, M. radiata* and *M. mulatta. Primates* 20:443–451, 1979
9. SZALAY FS, DELSON E: *Evolutionary History of the Primates.* New York, Academic Press, 1979
10. PETERS T JR: Serum albumin. *Adv Clin Chem* 13:37–111, 1970
11. BROWN J: Serum albumin: amino acid sequence, in *Albumin Structure, Function, and Uses,* edited by ROSENOER V, New York, Pergamon Press, 1977, pp 27–52
12. BRODERSEN R: Competitive binding of bilirubin and drugs to human albumin studied by enzymatic oxidation. *J Clin Invest* 54:1353–1364, 1974
13. GARTNER L, LEE K, VAISMAN S, LANE D, ZARAFU I: Development of bilirubin transport and metabolism in the newborn rhesus monkey. *J Pediatr* 90:513–531, 1977
14. WENNBURG R, AHLFORS C, RASMUSSEN L: The pathochemistry of kernicterus. *Early Hum Dev* 3:353–372, 1979
15. BURBACHER TM, SACKETT GP: Serum bilirubin levels in full term and premature *Macaca* nemestrina, in *Nursery Care of Nonhuman Primates,* edited by RUPPENTHAL GC, New York, Plenum Press, 1973, pp 117–124
16. SMITH DG, AHLFORS CE: The albumin polymorphism and bilirubin binding in rhesus monkeys (*Macaca mulatta*). *Am J Phys Anthropol* 54:37–41, 1981
17. DOUMAS B, WATSON W, BIGGS H: Albumin standards and the measurement of serum albumin with bromcresol green. *Clin Chim Acta* 31:87–96, 1971
18. AHLFORS CE: Competitive interaction of bilirubin and biliverdin only at the primary binding site on human serum albumin. *Anal Biochem* 110:295–307, 1981
19. AHLFORS CE: Effect of serum dilution on apparent unbound bilirubin concentration as measured by the peroxidase method. *Clin Chem* 27:692–696, 1981
20. DIXON WJ, MASSEY FJ: *Introduction to Statistical Analysis.* New York, McGraw-Hill, 1969, p 119
21. CHEN RF: The fluorescence of bilirubin-albumin complexes, in *Fluorescence Techniques on Cell Biology,* edited by THAER AA, SERNETZ M, New York, Springer-Verlag, 1973, pp 273–282
22. LEVINE R: Fluorescence-quenching studies of the binding of bilirubin to albumin. *Clin Chem* 23/12:2292–2310, 1977
23. PARSONS DL: Analysis of fluorescence quenching data by the method of Levine. *Clin Chem* 26:1869–1871, 1980
24. NORBY JG, OTTOLENGHI P, JENSEN J: Scatchard plot: common misinterpretation of binding experiment. *Anal Biochem* 102:318–320, 1980
25. WENNBERG R, LAU M, RASMUSSEN LF: Quantitative assessment of albumin-bilirubin binding in newborns. *Clin Res* 25:175A, 1977
26. JACOBSON J: The binding of bilirubin to human serum albumin-determination of the dissociation constants. *FEBS Lett* 5:112–114, 1969
27. LOREY FW, AHLFORS CE, SMITH DG: Effect of albumin polymorphism on the binding of bilirubin by human sera. Annual meeting, Western Society for Pediatric Research, Carmel, Calif., February, 1983
28. FINLAYSON JS: Physical and biochemical properties of human albumin, in *Workshop on Albumin,* Washington, D.C., DHEW Publication (HIH 76-925), 1975, pp 31–56

29. JANATOVA J: On the heterogeneity of serum albumin. A critical review. *J Med* 5:149–216, 1974
30. NEEL JV, THOMPSON EA: Founder effect and number of private polymorphisms observed in Amerindian tribes. *Proc Natl Acad Sci USA* 75:1904–1908, 1978
31. PETERS T JR: Serum albumin, in *The Plasma Proteins,* vol 1, edited by PUTNAM F, New York, Academic Press, 1975, p 159
32. WILDING G, BLUMBERG BS, VESSELL E: Reduced warfarin binding on albumin variants. *Science* 195:991–994, 1977
33. BRODERSEN R: Free bilirubin in blood plasma of the newborn: effects of albumin, fatty acids, pH, displacing drugs, and phototherapy, in *Intensive Care in the Newborn,* vol II, edited by STERN L, OH W, FRIIS-HANSEN B, New York, Masson, 1978, pp 331–345
34. RAICHELSON RM: The use of plants containing coumarins among native Americans of the Southwest and their biomedical relationship to serum albumin polymorphisms. *Med Anthropol* 3:477–502, 1979
35. SALAND J, MCNAMARA M, COHEN M: Navajo jaundice: a variant of neonatal hyperbilirubinemia associated with breast feeding. *J Pediatr* 85:271–275, 1974
36. WOOD GB, BACHE F: *The Dispensatory of the United States of America,* 12th ed. Philadelphia, J.B. Lippincott, 1865
37. POUND FJ: History and cultivation of the tonka bean (*Dipteryx odorata*) with analysis of Trinidad, Venezuelan and Brazilian samples. *Trop Agric* 15:4–9, 28–32, 1938
38. BRAMBEL CE, HUNTER RE: Effect of dicoumarol on the nursing infant. *Am J Obstet Gynecol* 59:1153, 1950