between $MgSiO_3$ (ilmenite) and Al_2O_3 (corundum) at high pressure.

In the present work, I have made eight experimental studies of pyrope glass in the loading pressure region between 100 and 300 kbar and temperature of about 1000° to 1400°C. Very fine powder of the glass form of $3MgSiO_3 \cdot Al_2O_3$, which was provided by A. Major (Australian National University), was intimately mixed with a few percent of graphite as the starting material. The graphite serves to absorb the laser irradiation and thus heats the sample while under compression. The sample so prepared was compressed in a diamond-anvil press fitted with a lever-and-spring type assembly and heated by a continuous YAG laser while the sample was maintained at pressure. The recovered samples were studied by a powder x-ray diffraction technique with a modified 57.3-mm Debye-Scherrer camera. Details of the experimental procedure have been described elsewhere (*9*).

In the pressure region between 100 and 240 kbar (inclusive), the glass crystallized completely to pyrope garnet. However, at 250 kbar, the x-ray diffraction film showed the presence of about 30 percent of a new phase possessing the ilmenite-type structure, the remaining 70 percent consisting of pyrope. At 290 kbar, a nearly pure ilmenite-type phase of $MgSiO_3 \cdot \frac{1}{3}Al_2O_3$ or $(Mg_{.75}Al_{.25})(Si_{.75}Al_{.25})O_3$ has been observed. The aluminum is probably distributed between the magnesium and the silicon sites equally, unless there is magnesium silicon disorder, which seems unlikely. The x-ray diffraction data for the ilmenite phase of $MgSiO_3 \cdot \frac{1}{3}Al_2O_3$ obtained at about 300 kbar are listed in Table 1. A least-squares fit of *d*-spacings yields lattice parameters of $a_0 = 4.755 \pm 0.002$ and $c_0 = 13.360 \pm 0.005$ Å for the hexagonal cell of the ilmenite structure. The relative intensities listed in Table 1 are very similar to those of the ilmenite-type phase of $MgSiO_3$ and Al_2O_3, and the characteristics of reflection extinctions are consistent with the space group ($R\bar{3}$) of the ilmenite structure. The molar volume for the ilmenite phase is thus calculated to be 26.26 ± 0.03 cm³/mole. It is inferred that the garnet-ilmenite transition of $Mg_3Al_2Si_3O_{12}$ occurs at loading pressures between 240 and 250 kbar and at 1000° to 1400°C. The zero-pressure volume change associated with the garnet-ilmenite transition of $3MgSiO_3 \cdot Al_2O_3$ is -8.08 cm³/mole, or -7.1 percent.

It would be of great interest to compare the observed lattice parameters and the molar volume of the ilmenite-type phase of $MgSiO_3 \cdot \frac{1}{3}Al_2O_3$ with those of the ilmenite-type phase of $MgSiO_3$ and Al_2O_3. The *a*-axis for the ilmenite-type phase in this work is 0.47 percent greater than the value for an ideal solid solution between $MgSiO_3$ (ilmenite) and Al_2O_3 (corundum), the *c*-axis is 0.45 percent smaller than ideal, and the volume is 0.48 percent greater than ideal.

From Table 1, it is also seen that the orthorhombic perovskite modification of pyrope-garnet starts to appear at a loading pressure of about 300 kbar. In an earlier study (*6*), I have reported that pyrope-garnet disproportionates into a mixture of $MgSiO_3$ (perovskite modification) plus Al_2O_3 (corundum) at pressures greater than 300 kbar. In view of the similarity between the x-ray diffraction patterns of Al_2O_3 (corundum) and $MgSiO_3 \cdot \frac{1}{3}Al_2O_3$ (ilmenite), the previously observed Al_2O_3 (corundum) in pyrope-garnet might be residual ilmenite ($MgSiO_3 \cdot \frac{1}{3}Al_2O_3$) reported in this work. The evidence that the *d*-spacings and the characteristics of reflection extinctions for the perovskite phase observed in pyrope-garnet differ from those for the pure $MgSiO_3$ (*10*) supports the conclusion that a substantial amount of Al_2O_3, if not all of that of the pyrope composition, is accommodated in the orthorhombic perovskite lattice. The synthesis of the orthorhombic perovskite phase of $ScAlO_3$ at high pressure (*11*) also lends considerable support to the above conclusion. Thus, the ilmenite phase of $MgSiO_3 \cdot \frac{1}{3}Al_2O_3$ may transform directly to a single phase possessing the orthorhombic perovskite structure, instead of a mixture of $MgSiO_3$ (perovskite modification) plus Al_2O_3 (corundum).

Recovered samples from shock-wave studies on iron-rich garnets have been reported to be an orthorhombic modification of the ilmenite structure (*12*). However, the x-ray diffraction pattern of that work was interpreted by me (*13*) as a mixture of several intermediate high-pressure phases of iron-rich garnets on the basis of static high-pressure studies. Furthermore, the ilmenite-like phase for iron-rich silicates has not yet been observed in any of the static high-pressure experiments (*14*).

Results of this study indicate that pyrope garnet in the upper mantle would persist through the transition zone (400 to 650 km), and then transform to the ilmenite structure in the upper part of the lower mantle, and ultimately transform to the orthorhombic perovskite phase. In conjunction with the orthorhombic perovskite phases developed by olivine and pyroxene (*10, 15*) these results strongly suggest that the earth's lower mantle comprises mainly perovskite phases of ferromagnesian silicates.

LIN-GUN LIU*
Research School of Earth Sciences, Australian National University, Canberra, A.C.T., Australia

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* Present address: Seismological Laboratory, California Institute of Technology, Pasadena 91125.

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Reduced Warfarin Binding of Albumin Variants

Abstract. *Binding studies of albumins A/A and A/Me from human plasma and isolated albumin revealed small, but statistically significant, reductions in warfarin binding of albumin A/Me compared to albumin A/A. Such differences in binding in vitro could result in altered pharmacological responses to warfarin administered to individuals possessing albumin A/Me. To determine if clinically significant differences in warfarin distribution exist, observations should be made in vivo on patients of different albumin phenotype who are receiving warfarin therapeutically.*

Normal subjects exhibit large interindividual variations in the disposition of several commonly used drugs, due mainly to genetically controlled differences in rates of drug metabolism (*1*). This conclusion, based on twin and family studies performed under basal conditions, revealed that environmental factors contribute surprisingly little to the maintenance of these large variations between individuals that range in magnitude from 3- to 30-fold, depending on the drug and the population. However, basal rates of drug disposition are readily altered by nu-

Table 1. Warfarin binding to albumin variants. Summary of present and prior results on serum collected from various Indian groups. The number of specimens studied in each group is shown in parentheses. In general, binding measurements were repeated twice on each sample. For comparison of all variants in column 3, $P = .005$; A/A compared to A/Na, $P = .021$, and A/A compared to A/Me, $P = .0025$ by the Mann-Whitney U test.

Phenotype	Study 1* (% bound)‡	Study 2* (% bound)‡	Study 3* (% bound)‡	Study 4† (K)§	Study 5* (% bound)‖
A/B			72.6 (1)		
A/A	52.6 (5)	59.1 (1)	61.2 (7)	1.62 (14)	97.8 (13)
A/Me			58.2 (2)	1.26 (15)	97.2 (14)
Me/Me				1.34 (1)	97.3 (1)
A/Na	52.0 (3)	56.9 (2)	55.7 (5)		
Na/Na	46.9 (1)	55.5 (1)	54.7 (1)		

*Binding studies performed on serum. †Binding studies performed on isolated, charcoal-treated albumin. ‡Warfarin = concentration, 300 μg/ml. §K is the binding association constant $\times 10^{-5}M^{-1}$. ‖Warfarin = concentration, 50 μg/ml.

merous environmental factors that can affect drug absorption, distribution, biotransformation, excretion, or combinations of these (*2*).

With respect to drug distribution, genetically controlled variations in binding were suggested by greater similarity within identical as compared to fraternal twins with respect to nortriptyline binding to albumin (*3*). Since endogenous ligands were not removed from albumin before these binding studies were conducted, dietary or other environmental differences that alter the concentration of tightly bound endogenous ligands (*4*) could have produced the observed interindividual variations in nortriptyline binding. Interindividual differences in the binding of a drug can arise from structural (genetic) differences in the albumin molecule, from changes in the concentration of albumin (*5*), from dietary (environmental) differences affecting the concentrations of endogenous ligands such as fatty acids, or from combinations of these and other factors.

Our study was undertaken to determine whether genetically controlled, structural alterations of the albumin molecule in previously described albumin variants (*6*) change the capacity of the albumin molecule to bind drugs. This possibility has been mentioned (*7, 8*) in earlier studies where a difference in the binding of bromphenol blue to the Naskapi albumin variants (*7*) was suggested. If changes in binding significantly affect the concentration of the free form of certain drugs, the optimal dose of these drugs could differ in such individuals. It is, however, important to emphasize that our binding studies were performed in vitro and that in order to determine if there are pharmacological effects of clinical importance, observations should be made on patients of different albumin phenotype who are receiving warfarin therapeutically.

Polymorphic variants of albumin are distributed in northern and southwestern American Indians (Albumin Naskapi) (*6*), southwestern and middle American Indians (Albumin Mexico) (*6*), and northern South American Indians (Albumin Makiritare and Yanomana) (*6*), but have not been reported in populations other than American Indians with the exception of some populations in New Guinea (*9*). Rare variants occur in many populations with the possible exception of Africans.

Table 1 (columns 1 and 2) summarizes several early, previously unpublished studies on the binding of warfarin to albumin variants with the use of whole serum. The results indicate a small but detectable difference in the percentage of warfarin bound; in some cases these differences were statistically significant. In 1972, more extensive studies, also summarized in Table 1 (column 3), were performed on several phenotypes, again with the use of whole serum. As was observed in the earlier studies (columns 1 and 2), binding of warfarin to the A/Me, A/Na, and Na/Na phenotypes was less than to A/A. On the basis of these results, a more detailed study was undertaken in 1975 on the binding of warfarin to whole serum and to isolated albumin, with Albumin Mexico variants from the Pima tribe of Arizona. Each of the studies revealed that, compared to normal albumin, warfarin binding to the variants was reduced (columns 4 and 5).

The specimens for the 1975 study (Table 1, columns 4 and 5) were collected from Pima Indians in Arizona (A/A, A/Me, and Me/Me). The specimens for the earlier studies (Table 1, columns 1, 2, and 3) were collected at intervals, beginning in 1968, from the following sources: A/A, A/Na, and Na/Na specimens were obtained from Naskapi and Montagnais Indians in Schefferville, Quebec, Canada; A/Me and Me/Me specimens were obtained from residential students at the Sherman School in California; and A/B specimen was obtained from an American of European descent.

Routine screening of the albumin variants was performed on starch gel electrophoresis in Ashton and Braden's discontinuous buffer system at *p*H 8.6 (*7*). Albumin for the binding studies was isolated from serum by affinity chromatography (*10*) after having been subjected to the charcoal treatment of Chen (*11*). The isolated albumin concentration was determined spectrophotometrically at 280 nm (extinction, 1 percent solution, 1-cm path length, 5.3). This gave consistent results with those obtained with the methods of Meites and Faulkner (*12*) and Lowry *et al.* (*13*).

Equilibrium dialysis was used to measure the binding of warfarin to isolated albumin. Dialysis was performed at 37°C and *p*H 7.4 for 5 hours, at which time it was determined that equilibrium had been attained. In each determination the albumin concentration was $0.96 \times 10^5 M^{-1}$ to $1.15 \times 10^5 M^{-1}$, and the warfarin concentration ranged from 1 to 10 μg/ml, determined through the use of ^{14}C-labeled warfarin (Amersham-Searle), where the ^{14}C-labeled warfarin represented 12.5 percent of the total warfarin present. Association constants and the number of binding sites per albumin molecule were calculated by the method of Scatchard (*14*).

The binding of warfarin to albumin in serum was measured at 37°C and *p*H 7.4 by equilibrium dialysis. Serum total protein and albumin concentrations were determined by the method of Meites and Faulkner (*12*).

In serums from individuals with normal (A/A) and variant (A/Me) albumins, a significant difference ($P = .015$, one-tail) in warfarin binding was observed between the A/A and A/Me populations by Mann-Whitney U test analysis (Table 2, columns 3 and 8). The mean percentages of bound warfarin for the A/A and A/Me populations were 97.8 and 97.2 percent, respectively. However, since the free form of a drug is the pharmacologically active form, a more appropriate comparison may be between the free fractions of warfarin for the two populations. The 2.8 percent free fraction of the A/Me population represents a 27 percent increase over the 2.2 percent free fraction of the A/A population. The mean concentrations of serum albumin of 4.5 and 4.4 g/100 ml for the A/A and A/Me populations, respectively, were within normal limits and did not differ from each other significantly (Table 2, columns 5 and 10).

These results are relevant, since decreased albumin concentrations are associated with reduced binding of prednisone (*5*), diphenylhydantoin (*5*), chlordiazepoxide (*5*), and diazepam (*5*).

Studies on chromatographically isolated, charcoal-treated albumin disclosed that the warfarin binding association constant of the A/Me population was significantly different ($P = .004$, one-tail) from the warfarin binding association constant of the A/A population by Mann-Whitney U test analysis (Table 2, columns 2 and 7). As expected from the serum data, the A/Me albumin had less affinity for warfarin than the A/A albumin. The mean warfarin binding association constants for the A/A and A-Me populations were $1.62 \times 10^5 M^{-1}$ and $1.26 \times 10^5 M^{-1}$, respectively; the mean number of warfarin binding sites per albumin molecule was 1.23 ± 17 and 1.40 ± 0.18, respectively. Despite the fact that the A/Me variants bound warfarin to a significantly lower extent than did A/A albumin in both whole serum and isolated albumin preparations, no significant correlation ($r = .339$) occurred between K_a values (Table 2, columns 2 and 7) from isolated albumin preparations and values obtained from whole serum for the percentage of warfarin bound (Table 2, columns 3 and 8).

Several studies performed over the past 7 years on six albumin variants disclosed that, compared to the common albumin A, the variants A/Me and A/Na had reduced capacity to bind warfarin (Table 1); a single A/B variant exhibited greater warfarin binding. In our study with whole serum and isolated albumin, significant differences occurred between the A/A and A/Me heterozygotes.

The free, but not the protein bound, fraction of a drug is pharmacologically active since only the free form is available to receptor sites. Moreover, only the free form can be metabolized or excreted. Therefore, the extent to which a drug binds albumin is an important pharmacologic variable, depending on the drug, from 0 to 100 percent. For a drug whose binding to albumin is 50 percent, a 1 percent change in the amount of drug bound alters the free fraction by only 2 percent. This small change would be unlikely to alter significantly such pharmacological characteristics of the drug as its distribution, excretion, metabolism, or interaction with receptor sites. By contrast, for a highly bound drug such as warfarin, whose binding to albumin at therapeutic plasma concentrations is 99 percent, an increase of only 1 percent in the free fraction doubles the amount of drug available for pharmacological activity. Therefore, the same quantitative change of 1 percent in binding that exerted a negligible effect on a drug that was 50 percent bound can exert a profound effect on the disposition of a drug bound 99 percent. For example, small changes in the binding of warfarin cause marked differences in the elimination rate constant, metabolism, and the apparent volume of distribution of warfarin (*15, 16*). However, an initial increase in the free fraction of a highly bound drug need not be accompanied by altered metabolism or pharmacological response to the drug if it is compensated for by an increase in the volume of distribution of the drug in the body. This increase in distribution could prevent a net change in the free concentration of drug. Thus, it is important to ascertain whether an alteration in the distribution of a drug accompanies a change in its free fraction.

Pharmacological responses of an individual to a given dose of drug may be enhanced by increasing the ratio of free to bound drug. Increases in the free fraction of a drug can result from displacement of drug from albumin by other compounds, from a decrease in the concentration of albumin (*5*), from administration of very high doses of the drug, or from decreased affinity of albumin for a drug produced either by genetically determined alterations in the structure of the albumin molecule or by disease states such as uremia (*17*). With respect to the third mechanism mentioned above for increasing the free fraction of a drug, the slightly higher free fraction of warfarin in our study compared to those of Levy and associates (*15*) is probably due to the fact that we used warfarin concentrations ten times greater than theirs. Decreased capacity of variant albumins to bind warfarin in vitro could lead to clinically significant increases in the pharmacologically active, free warfarin concentrations (*15, 16*), since this drug is normally highly bound to albumin. Thus, for individuals possessing variant albumins, administration of apparently normal load-

Table 2. Binding of warfarin to albumin A/A and albumin A/Me variants; S.D., standard deviation.

A/A					A/Me				
1	2	3	4	5	6	7	8	9	10
Individual	K_a* ($10^5 M^{-1}$)	Bound†‡ (%)	Total plasma protein† (g/100 ml)	Plasma albumin† (g/100 ml)	Individual	K_a* ($10^5 M^{-1}$)	Bound†‡ (%)	Total plasma protein† (g/100 ml)	Plasma albumin (g/100 ml)
C-203-220	2.04	98.7	8.0	4.3	C-203-221	1.69	98.3	10.8	4.1
C-203-222	1.70	98.2	8.3	4.4	C-203-228	1.00	97.8	8.8	3.5
C-203-223	1.72	98.5	9.1	5.4	C-203-230	1.16	97.5	8.7	6.7
C-203-224	1.01	98.5	10.5	4.6	C-203-231	1.18	97.7	8.6	4.9
C-203-225	1.21	98.2	10.3	4.4	C-203-282	0.92	96.7	9.2	3.7
C-203-226	1.22	98.1	9.5	3.8	C-203-233	1.06	96.7	8.2	4.1
C-203-227	2.05				C-203-234	1.24	96.3	9.5	3.4
C-203-229	1.69	97.2	7.0	2.8	C-203-235	1.05	96.9	9.3	4.0
C-203-242	1.40	96.7	10.7	4.0	C-203-236	0.68	97.6		
C-203-244	1.88	97.2		4.7	C-203-238	1.69	97.4	9.1	4.1
C-203-245	1.92	97.6	8.8	4.2	C-203-239	1.68	97.7	8.7	4.7
C-203-246	2.07	97.8	8.4	5.3	C-203-240	1.38	96.8	9.3	4.7
C-203-249	1.41	97.9	9.1	5.8	C-203-241	0.87	96.9	8.0	4.2
C-203-251	1.42	97.2	11.1	4.7	C-203-243	1.61			
					C-203-248	1.76	97.7	10.3	5.1
Mean ± S.D.	1.62§± 0.35	97.8‖ ± 0.6	9.2 ± 1.2	4.5 ± 0.8		1.26§ ± 0.35	97.2‖± 0.6	9.2± 0.6	4.4 ± 0.7

*Binding association constant obtained from isolated, charcoal-treated albumin. †Determined on whole plasma. ‡Warfarin concentration, 50 μg/ml. §Mann-Whitney U Test. $P = .015$ (one-tail). ‖Mann-Whitney U test, $P = .004$ (one-tail).

ing doses of warfarin might be followed by enhanced pharmacological responses and toxicity. To investigate this possibility the pharmacokinetic properties of warfarin administered in vivo would have to be compared in individuals of different albumin phenotype who are receiving warfarin therapeutically. Other drugs may differ from warfarin in showing either no change or enhanced binding in vitro to variant albumins.

GEORGE WILDING
Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey 17033
BARACH S. BLUMBERG
Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111
ELLIOT S. VESELL
Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey 17033

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25-Hydroxycholecalciferol to 1,25-Dihydroxycholecalciferol: Conversion Impaired by Systemic Metabolic Acidosis

Abstract. *An acute systemic acidosis in vitamin D depleted rats that was induced by ammonium chloride feeding resulted in defective biological hydroxylation of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. Impaired enzymatic hydroxylation occurred despite the presence of either hypophosphatemia or hypocalcemia. The data suggest that acidosis interferes with the adaptive enzymatic control of 25-hydroxycholecalciferol metabolism in the vitamin D depleted state.*

Renal tubular acidosis is a syndrome characterized by abnormalities in tubular function, although the glomerular filtration rate remains normal. Hypophosphatemia, hypercalciuria, secondary hyperparathyroidism, metabolic acidosis, calcium malabsorption, and growth retardation accompany the disease, as well as skeletal abnormalities which are diagnosed radiologically as rickets in children and osteomalacia in adults (*1–3*). Although the skeletal lesions and calcium malabsorption do respond to pharmacological doses of vitamin D (*3*), they are also reversed by alkali therapy alone (*2, 4, 5*). 1,25-Dihydroxycholecalciferol [1,25-$(OH)_2D_3$], the most potent biologically active metabolite of vitamin D_3, is enzymatically formed from 25-hydroxycholecalciferol (25-OH-D_3) within the mitochondria of renal cortical cells (*6, 7*). Although it has been established that 1,25-$(OH)_2D_3$ synthesis is defective in end-stage renal disease (*8*), is suppressed by hyperphosphatemia (*9*), and is stimulated by parathyroid hormone (PTH) and hypophosphatemia (*9, 10*), the effect of a systemic acidosis on 1,25-$(OH)_2D_3$ production is still unknown. Our data herein support the hypothesis that the conversion of 25-OH-D_3 to 1,25-$(OH)_2D_3$ is defective in acidotic nonuremic vitamin D depleted animals despite an associated hypophosphatemia or hypocalcemia, both of which are reported to lead to stimulated 1-hydroxylase activity.

Vitamin D deficiency was produced in weanling male rats by feeding them a vitamin D deficient diet for 7 weeks. During this interval the animals were divided into two separate groups: group 1 received a vitamin D deficient diet containing 0.2 percent phosphorus and 0.06 percent calcium; group 2 received a vitamin D deficient diet containing 0.2 percent phosphorus and 1.3 percent calcium. After the rats became vitamin D deficient, both groups were subdivided. For groups 1B and 2B, distilled water containing 1.8 percent NH_4Cl was substituted for the regular distilled drinking water. Groups 1A and 2A continued to receive the regular distilled water. Three days later, 62.5 pmole of 25-OH-[26,27-^{3}H]D_3 (specific activity, 9.3 c/mmole) dissolved in 0.1 ml of ethanol was injected intrajugularly into all the animals; 18 hours after the injection all animals were killed; blood was obtained from the abdominal aorta of each animal and the calcium and phosphorus content and the *p*H were measured. Measured portions of serum as well as portions of small intestinal mucosa with identical protein concentration were then pooled for each of the four groups. The pooled samples were homogenized and extracted as reported previously (*11*), and were chromatographed on columns of Sephadex LH-20 according to the method of Holick *et al.* (*12*). The radioactivity recovered as 1,25-$(OH)_2$-[26,27-^{3}H]D_3 in the eluates was rechromatographed by means of Celite liquid-liquid partition chromatography as described by Haussler and Rasmussen (*13*). Portions of individual fractions obtained from either the LH-20 or Celite chromatographic procedures were dissolved in a solution containing 3.0 g of PPO (2,5-diphenyloxazole) and 100 mg of diethyl POPOP (1,4-bis-[5-phenyl-2-oxazolyl]-benzene) per liter of toluene and counted in a Tri-Carb liquid scintillation counter with a 2 percent counting error.

As shown in Table 1, NH_4Cl decreased the *p*H of the blood in both group 1B and group 2B. The serum calcium concentrations of the rats in groups 1A and 1B were lower and the phosphorus higher than the values obtained from groups 2A and 2B. Whereas acidosis resulted in a decrease in the serum calcium and an increase in phosphorus in group 2B, it produced an increase in serum calcium and a fall in phosphorus in group 1B animals. As noted in Table 2, serum and intestinal concentrations of ^{3}H-labeled 1,25-$(OH)_2D_3$ in the hypocalcemic animals of group 1A was greater than that observed in the normocalcemic animals of group 2A despite a significantly higher amount of circulating phosphorus in group 1A (Table 1). Since hypocalcemia is a potent secretagogue