steps. However, an interfering substance was removed by the second Sephadex LH-20 column (Table 2, scheme B), demonstrating that at least three successive column purifications are required before assaying 1α,25-(OH)$_2$-D$_3$ from human plasma by this method.

This radioreceptor assay has shown for the first time that the hormonal form of vitamin D$_3$, 1α,25-(OH)$_2$-D$_3$, is detectable in humans. The use of the intestinal chromatin receptor system to detect very low levels of 1α,25-(OH)$_2$-D$_3$ further confirms the high affinity, specificity, and physiologic importance of this receptor for 1α,25-(OH)$_2$-D$_3$. Application of the nuclear hormone receptor complex to the measurement of 1α,25-(OH)$_2$-D$_3$ represents a unique approach to the assay of sterol hormones. Moreover, the use of filters to adsorb the chromatin associated receptor during the washing away of unbound sterol is a novel method of separating free sterol from that which is bound to macromolecular components. This assay may be used to study the regulation of the metabolism of 25-OH-D$_3$ to 1α,25-(OH)$_2$-D$_3$ in humans and experimental animals. Finally, the utility of the assay for diagnosis of disorders in calcium homeostasis, such as hypoparathyroidism and renal osteodystrophy, prior to possible treatment with 1α,25-(OH)$_2$-D$_3$, is clear. Involvement of the hormone in hyperparathyroidism and neoplastic hypercalcemia may also be determined.

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13. A purified plasma sample was treated for 10 minutes with 10 ml of periodic acid (1 mg/ml) in a mixture of water and ethanol (67 : 33). This procedure converts 24,25-dihydroxyvitamin D$_3$ and 25,26-dihydroxyvitamin D$_3$ to the corresponding aldo- and keto- derivatives (unpublished observations).
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Evidence that Enzyme Polymorphisms Are Selectively Neutral, but Blood Group Polymorphisms Are Not

Abstract. *Data on enzyme polymorphism and blood group polymorphism were examined with special reference to molecular evolution, by using a statistic that depends on neither population structure nor other ecological factors. The data of the former are consistent with the hypothesis of neutral mutant and random genetic drift, whereas the latter are in accord with the hypothesis of balanced selection.*

In our previous report (*1*), published polymorphism data were used in conjunction with an invariant principle to conclude that the available data are consistent with the "neutral" theory of Kimura and Ohta (*2*). The principle is that when we assess the amount of heterozygosity, given that the gene frequency of the whole population is specified, the total amount is independent of the population structure (*3*). The data then available were some 400 proteins. We compared the data with four distinct hypotheses: all mutants are (i) neutral, (ii) advantageous, (ii) deleterious, and (iv) overdominant. They were consistent with (i) and (ii). The report had its reverberation in population genetics and molecular evolution (*4, 5*). Since then more data have been published, and therefore we made the same survey of all available data, which amount to 1045 proteins (*6*). The present result turned out to confirm the previous conclusion, and therefore our claim was greatly reinforced. The result is presented in Fig. 1A. We note that the distribution of the data presented in Fig. 1A is considerably flatter than that in our previous report (*1*). The flatness of the distribution is characteristic of neutral or advantageous mutants.

We have applied the same analysis to polymorphism data of human blood groups alone (*7*). Although the num-

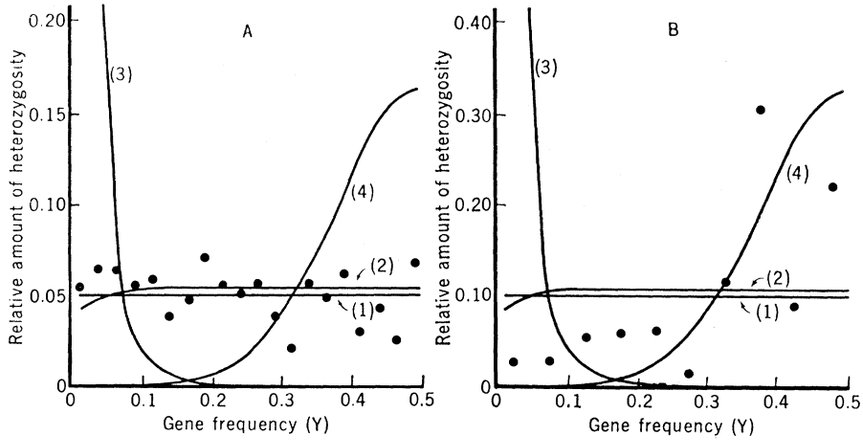

Fig. 1. Distribution patterns of heterozygosity. (A) Enzyme polymorphisms; (B) blood group polymorphisms. The curves indicate the theoretical expectations: (1) neutral, (2) advantageous, (3) deleterious, and (4) overdominance. The dots indicate the observed results. (The total area under each curve and the dots is unity.) Y is the global gene frequency.

ber of available data (26 loci) is undoubtedly insufficient to draw any strong conclusion, the result indicates that the distribution of heterozygosity deviates from that of neutrality and it is shifted toward a balanced selection. The result is presented in Fig. 1B (*8*). It seems quite certain that the heterozygote distributions of protein polymorphisms and of blood group polymorphisms are essentially different; see also (*4*).

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8. The scale in Fig. 1B is different from that of Fig. 1A because the amount of heterozygosity is normalized so that the total amount of heterozygosity is one. Only 10 points are plotted in Fig. 1B, while 20 points appear in Fig. 1A, because of a large difference in the number of loci examined (over 400 loci for Fig. 1A, but only 26 loci for Fig. 1B).
9. Contribution No. 962 from the National Institute of Genetics, Mishima, Shizuoka-ken 411, Japan. Aided in part by a grant-in-aid from the Ministry of Education, Japan. We are grateful to Dr. J. F. Crow for the suggestion of analyzing human blood group data separately and for many valuable discussions.

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Calcium Absorption and Calcium-Binding Protein Synthesis: Solanum malacoxylon Reverses Strontium Inhibition

Abstract. *The ingestion of diets containing high concentrations of stable strontium inhibits calcium absorption and intestinal calcium-binding protein synthesis and, as shown by others, does so by inhibiting the conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, the active form of vitamin D. The addition of the South American plant* Solanum malacoxylon *to strontium-containing diets counteracts the inhibitory action of dietary strontium, thereby indicating that the plant contains a factor which can mimic the action of 1,25-dihydroxycholecalciferol and representing the first such factor identified in a botanical source.*

A disease of cattle in the Argentine known as "Enteque seco" is characterized by extensive soft tissue calcification and is due to the ingestion of the plant *Solanum malacoxylon* by the grazing animal (*1*). Controlled studies with different species, including cattle (*2*), rabbits (*3*), sheep (*4*), and guinea pigs (*4*), verified the etiology of the disease and showed that the plant or an extract of the plant caused an increased absorption of calcium and phosphorus. Analogy between the active principle in *S. malacoxylon* and vitamin D has been made, and it was shown that the unknown factor seems to act faster and fade faster than a massive dose of vitamin D (*3*) and appeared to cause an increase in calcium-binding activity in rabbit ileum (*5*). The similarity between the activity of vitamin D and *S. malacoxylon* was confirmed by the report by O'Donnell and Smith (*6*). However, from the reported symptoms and signs of Enteque seco, it was more likely that the active principle in *S. malacoxylon* might have biological properties similar to the active form of vitamin D, 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$]. 1,25-$(OH)_2D_3$ is formed in the kidney by the 1-hydroxylation of the precursor metabolite, 25-hydroxycholecalciferol (25-OHD_3), the latter resulting from the 25-hydroxylation of vitamin D_3 (cholecalciferol) in the liver (*7*).

To test the hypothesis that the *S. malacoxylon* factor had activity similar to that of 1,25-$(OH)_2D_3$, use was made of the established observation that a high stable strontium intake by chicks blocks the conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$ by the kidney enzymes (*8*). The lack of 1,25-$(OH)_2D_3$ production in the presence of strontium results in a depressed absorption of calcium and the inhibition of synthesis of the intestinal vitamin D–dependent calcium-binding protein (*9*). The inhibitory action of strontium, as would be predicted, can be overcome by the administration of 1,25-$(OH)_2D_3$ per se but not by 25-OHD_3 or cholecalciferol (*8*), and we now report here that the inhibitory effect of dietary strontium can also be reversed by *S. malacoxylon*—evidence that the *S. malacoxylon* factor does, indeed, have activity similar to that of the active metabolite of vitamin D_3, 1,25-$(OH)_2D_3$.

Day-old White Leghorn cockerels were fed a commercial chick diet (Agway) for 23 days; at this time (designated day 0), they were placed on either a control diet containing 1.3 percent Ca, 0.65 percent P, and 1200 international units (I.U.) of vitamin D_3 per kilogram or a diet containing 2.5 percent Sr, 0.2 percent Ca, 0.65 percent P, and 1200 I.U. of vitamin D_3 per kilogram. On day 4, some of the chicks on the high strontium diet were continued on this diet, and the others were fed the same high strontium diet without vitamin D_3 but supplemented with either 0.2 or 0.5 percent of *S. malacoxylon* powder. The basal diet is the rachitogenic diet (*9*). On days 0, 4, and 7, six chicks from the appropriate groups were anesthetized with ether, and the degree of absorption of ^{47}Ca from a ligated duodenal segment in situ was determined (*10*). The 1-ml test dose contained NaCl, 150 m*M*; $CaCl_2$, 25 m*M*; and ^{47}Ca, 0.05 μc/ml, *p*H 7.2. After an absorption period of 15 minutes, the chicks were bled by cardiac puncture and killed with an overdose of sodium pentabarbitol. The duodenum was excised and the radioactivity therein was counted immediately in a well-type scintillation detector against reference ^{47}Ca. The ^{47}Sc contribution was excluded by the use of a single channel analyzer. The segment, after counting, was thoroughly rinsed and slit open lengthwise; the mucosa was removed from the underlying muscle coats (*11*). The duodenal mucosa was further processed and the supernatant