amphetamine (30 mg/kg) are equipotent in releasing insulin in vivo (62 ± 1 versus 66 ± 15 μunit/ml at 15 minutes) and that methamphetamine (10 mg/kg) is effective in releasing insulin, with a three- to fourfold increase over control values at 15 minutes. Although the studies presented here demonstrate that methamphetamine can cause insulin release through a direct effect on the pancreas, additional mechanisms are certainly not ruled out. The possibility that the release of insulin by methamphetamine is partly mediated through a neural mechanism should be investigated.

The hypoglycemic effect of amphetamine described by Moore *et al.* (*1*) was also noted in our studies. As shown in Fig. 2, no significant change in blood glucose concentrations was seen until 15 minutes after intravenous injection of methamphetamine, at which time a significant decline of 40 percent was seen in the experimental animals when compared with their controls injected with saline. This hypoglycemia was even more marked at 30 minutes after injection and was still present at 45 minutes.

While methamphetamine administered to mice was followed consistently by severe hypoglycemia, this response in the rat was observed only in certain experiments. The factors precipitating such a reaction are unclear at this time, but recent data from our laboratory (*6*) suggest that the hyperinsulinemia combined with certain hormonal and environmental conditions may produce severe hypoglycemia in animals treated with methamphetamine.

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Kinetic Path of Genes Undergoing Selection

Abstract. *As natural populations approach genetic equilibrium, the various genes in the population are capable of assuming intermediate distributions that might not be anticipated from either the rate of the process or the final distribution of the genes. Since it is possible that many populations have not reached genetic equilibrium, the distribution of genes in natural populations may be a reflection of the kinetic path by which the genes approach equilibrium. Attention to kinetic path provides an explanation for an apparent discrepancy in recent studies of selection in man.*

Each large, natural population usually includes individuals of dissimilar genetic makeup. Two questions arise concerning the genetic characteristics of the population: (i) What will be the ultimate frequency of the various genes? and (ii) How rapidly will the change, if any, occur? The answer to either question may have considerable practical importance. Soon after Mendelian principles of heredity began to receive general recognition, an assertion was made that genetically dominant traits, such as the hereditary hand deformity known as brachydactyly, will increase in prevalence until a majority of the population has the dominant characteristic. To refute this assertion, the mathematician G. H. Hardy introduced calculations to show that the frequency of either of two alternative genes (alleles) at a single genetic locus remains unchanged when the population consists of a large number of randomly mating individuals who have neither a selective advantage nor disadvantage. Moreover, within one generation, the alleles distribute themselves in a predictable ratio among homozygotes and heterozygotes (*1*). His calculations serve as a basis for the Hardy-Weinberg equilibrium, one of the fundamental principles of genetics. Similarly, the successful medical treatment of rare, recessive disorders has generated recent concern that the prevalence of these disorders will increase with each generation until they become a major problem. Available prediction equations (*2*) and techniques of computer simulation (*3*) lead to the conclusion that the frequency of the abnormal genes may, indeed, increase in the population to some extent, but only after a lag of many millennia. These two aspects of genetic systems, equilibrium distribution and the rate of approach to equilibrium, are similar to those encountered in thermodynamics and chemical kinetics, fields in which both the extent and rapidity of a reaction are significant.

I now wish to call attention to the importance of a third characteristic of genetic systems: intermediate gene distribution. As genes approach equilibrium, they can distribute themselves in a manner which may not be anticipated from either the rate of the process or the ultimate fate of the genes. The intermediate distribution of the genes may be characteristic of the system and yet dissimilar to the ultimate distribution they will have when the system reaches equilibrium or fixation. Intermediate distributions of genes have received attention in numerous earlier studies of genetic systems. Examples are Lewontin and White's analysis of the adaptive surface for inversion in the grasshopper *Moraba scurra* (*4*) and Livingstone's use of the computer to simulate the approach to equilibrium of human populations with abnormalities of hemoglobin or deficiencies of glucose-6-phosphate dehydrogenase (*3*). The following is an example of a genetic process in which attention to the intermediate distribution of the genes provides an explanation for an apparent discrepancy in studies of selection in man.

Geographic (*5, 6*) and recent cellular (*7*) studies suggest that hemoglobin S (Hb S), hemoglobin C (Hb C), and β-thalassemia (β-th), which are determined by alleles at an autosomal locus, and sex-linked deficiency of human red cell glucose-6-phosphate dehydrogenase all provide a selective advantage against falciparum malaria. In support of this theory, a highly positive geographic correlation is found between the frequency of genes for glucose-6-phosphate dehydrogenase deficiency and those for either β-th or Hb S (*5*). When the frequency of Hb S in numerous villages is plotted against the frequency of either Hb C (*5*) or β-th (*8, 9*), however, a positive correlation is not seen (Fig. 1). The points tend to distribute themselves in a triangular area bounded by the major axes and a diagonal line of negative slope. This triangular area is much

smaller than the triangular area imposed by the condition that the sum of the frequencies for the abnormal alleles cannot exceed a value of one. This lack of positive correlation is puzzling, for all three alleles are thought to provide selective advantages against the same environmental agent. Allison (*5*) and Siniscalco *et al.* (*10*) have suggested that this inverse or reciprocal correlation results from the reduced genetic fitness of the individual who has the alleles for both Hb S and either β-th or Hb C. They suggest that this reduced fitness tends to allow the normal allele, Hb A, and one abnormal allele, but not both abnormal alleles, to exist. For a system of three alleles, however, mutual exclusion between the two abnormal alleles does not occur unless the subject who is heterozygous for both abnormal alleles has a fitness even lower than that of the two homozygous abnormal subjects (*11*). This is certainly not true for Hb S/β-th subjects and is probably not the case for Hb S/Hb C subjects. Mutual exclusion between the two abnormal alleles, therefore, does not exist in systems triallelic for Hb A, Hb S, and β-th.

An explanation for the distribution seen in Fig. 1 can be found in a study of the kinetic path by which the frequency of three alleles approaches equilibrium. The results of computer simulation of the approach to equilibrium are presented in Fig. 2. The homozygous state for the normal allele is assumed to be slightly disadvantageous; the heterozygous state for both abnormal alleles, moderately disadvantageous; and the homozygous state for the abnormal alleles, highly disadvantageous. In a natural population, one would expect the second abnormal allele to be introduced into the population at a frequency which is at least slightly different from the simultaneous frequency for the first abnormal allele. When this occurs, the system tends to approach equilibrium by moving along one of the two major axes, then reversing its direction and moving very slowly along the line of negative slope to the final equilibrium position (Fig. 2). The approach to equilibrium along the line of negative slope requires many millennia. In the face of migration and changing selective pressure, equilibrium may never be achieved. The frequencies will move directly and more quickly to the equilibrium position along a diagonal line of positive slope if the two abnormal alleles are introduced into the population at identical frequencies, but this is a very unlikely event.

This indirect approach to equilibrium should cause the points of Fig. 1 to be not only negatively correlated but also peripherally distributed about a triangular area. The findings confirm this prediction. All but 2 of the 60 points of Fig. 1 (including two coincidental points) fall within a triangle formed by the axes and a diagonal line with intercepts of 0.18, 0.0 and 0.0, 0.18; yet only 12 of the 58 points fall within a concentric triangle having half this area and lying in the center of the scattergram. The difference between this peripheral distribution and a uniform distribution is statistically highly significant (χ^2, 19.9; d.f., 1; $P < .005$).

Other examples also emphasize the importance of the kinetic path of genes under selection. For sex-linked genes, the frequency of an abnormal gene can be distributed between the two sexes and different age groups in a manner suggesting that the frequency of the abnormal gene is declining when, in fact, it is actually increasing (*12*). Another, more familiar, example is the manner in which alleles can distribute themselves between closely linked loci. The alleles may show coupling or repulsion for considerable periods of time during the approach to equilibrium. The necessity for these considerations has increased as recent dis-

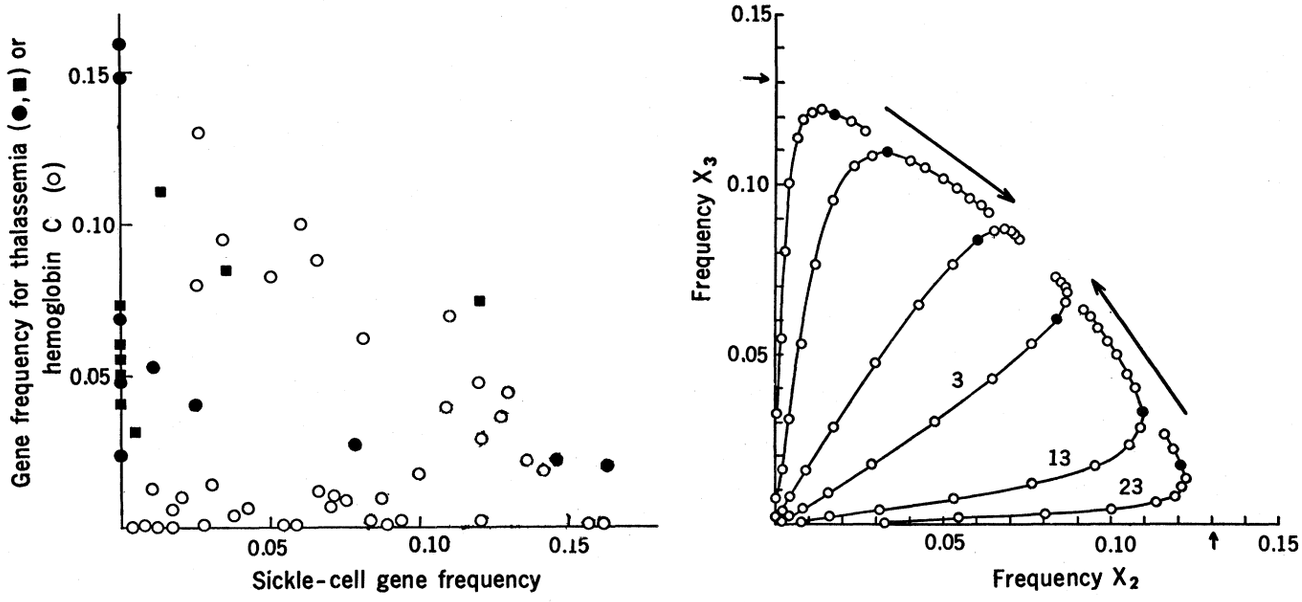

Fig. 1 (left). Gene frequencies for sickle-cell disease (Hb S), Hb C, and β-th in various villages. The data are from the studies of the following. ○, Allison (*5*); ●, Barnicot *et al.* (*8*); ■, Stamatoyannopoulos and Fessas (*9*). Fig. 2 (right). Computer simulation of a population having one normal allele (A_1), with frequency X_1, and two abnormal alleles (A_2,A_3), with frequencies of X_2 and X_3. One abnormal allele was introduced at a frequency of 0.001. After 3, 13, or 23 generations, the other abnormal allele was introduced at a frequency of 0.001. Relative fitnesses (W) were $W_{11} = 0.85$; $W_{12} = W_{13} = 1.00$; $W_{23} = 0.4$; and $W_{22} = W_{33} = 0.0$; where W_{ij} is the fitness of the individual with genotype A_iA_j. The points represent values at intervals of five generations. The darkened symbols represent values which are present 40 generations (approximately 1000 years) after the introduction of the second abnormal allele. All paths approach an equilibrium value of $X_2 = X_3 = 0.079$. A frequency of 0.13 is achieved by either abnormal allele, if only one abnormal allele is introduced.

coveries of many genetic variants of proteins and antigens have facilitated a determination of how the frequencies of various genes are distributed relative to each other and according to such classes as sex, age, linkage groups, and geographic location.

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Immunochemical Detection of Minor Bases in Nucleic Acids

Abstract. *Rabbits immunized with bovine serum albumin conjugates of 5-bromouracil, 5-iodouracil, and 6-methyladenosine produced antibodies specific for the bases. These antibodies were used to detect immunochemically 5-bromouracil and 6-methyladenosine in denatured DNA.*

Methylated derivatives of the four major bases may occur in minor amounts in DNA and RNA where they play a role in the metabolism and function of the nucleic acid. The pattern of this methylation is species-specific and depends on the recognition of specific oligonucleotide sequences by the methylating enzymes (*1*). The detection and quantification of these methylated bases has been accomplished chemically after suitable degradation of the nucleic acid (*2*). Purine- and pyrimidine-specific antibodies of unusually high specificity can be obtained by immunization with ribonucleoside-protein conjugates (*3*). For example, it has been possible to obtain antibodies so specific for the purine or pyrimidine nucleoside used for immunization that no cross-reaction with other purine or pyrimidine bases could be detected. We have, therefore, attempted to detect minor bases in nucleic acids by immunological procedures, using antibodies prepared by immunization with conjugates of the appropriate nucleosides. In the first experiments, DNA containing 5-bromouracil was used as a model system.

Bovine serum albumin (BSA) conjugates of 5-iodouridine (5-IU), 5-bromouridine (5-BU), and 6-methyladenosine (6-MeA) were prepared by the periodate oxidation method (*4*). The conjugates in complete Freund's adjuvant (0.4 mg of conjugate per rabbit per week) were injected into the toe pads of rabbits once a week for 3 weeks. The rabbits were bled 1 week after the last immunization and the serum globulins were precipitated by sodium sulfate.

The specificities of antibody globulins to 5-IU and antibody globulins to 5-BU were examined by the Ouchterlony gel diffusion method. Both antibodies showed strong reactivity with 5-IU–BSA, 5-BU–BSA, and the BSA conjugate of 1-β-D-ribofuranosyl thymine (T-BSA). Little or no cross-reaction with uridine-BSA (U-BSA) or with BSA itself was observed, and no reaction was seen with adenosine-BSA (A-BSA), cytidine-BSA (C-BSA), guanosine-BSA (G-BSA), or 6-MeA–BSA. Serums from bleedings before immunization were negative when tested against all conjugates. Absorption of antibody globulin to 5-BU with T-BSA, to remove cross-reacting antibodies, abolished essentially all reactivity with 5-BU–BSA, probably because the bromine atom and the methyl group have the same van der Waals radius (*5*). However, absorption of antibody globulin to 5-IU with T-BSA, which removed all reactivity with T-BSA, produced an antibody preparation still reactive with 5-BU–BSA and 5-IU–BSA. The specificity of the absorbed antibody to 5-iodouridine was then studied by complement fixation (Fig. 1a). There was fixation with 5-IU–BSA and with 5-BU–BSA but no reaction with T-BSA, U-BSA, or BSA. Complement fixation inhibition studies confirmed these results in that 5-IU and 5-BU were better inhibitors than thymidine by more than three orders of magnitude; no inhibition by uridine could be detected.

The capacity of these antibodies to detect 5-BU in DNA could be studied because it is possible to substitute 5-BU for as much as 50 to 100 percent of the thymidine in the DNA of certain thymidine-requiring strains of organisms (*5*). A thymidine-requiring strain of *Escherichia coli,* namely *E. coli* 15T$^-$, was grown for 100 minutes and 300 minutes in the presence of 5-bromouracil. DNA was isolated from each culture, and the buoyant densities were determined. According to a method of calculation reported by Hackett and Hanawalt (*5*), *E. coli* DNA (300 minutes) had 100 percent of the thymidine in the newly synthesized strand replaced by BU; *E. coli* DNA (100 minutes) had

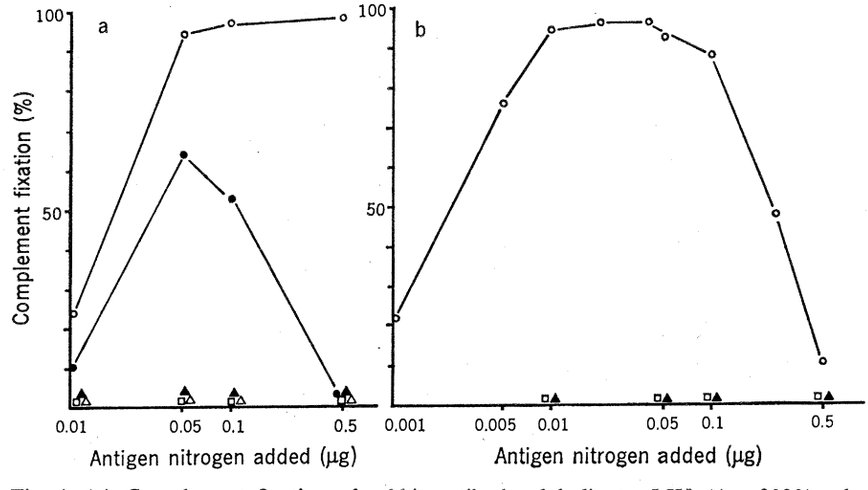

Fig. 1. (a) Complement fixation of rabbit antibody globulin to 5-IU (1 : 2000), absorbed with T-BSA. ○, 5-IU–BSA; ●, 5-BU–BSA; △, T-BSA; ▲, U-BSA; □, BSA. (b) Complement fixation of rabbit antibody globulin to 6-MeA (1 : 2000), absorbed with A-BSA. ○, 6-MeA–BSA; ▲, A-BSA; □, BSA.