brates, flatfish of the order Heterosomata are poorest in DNA (only 20 percent that of mammals). They are regarded as the retainer of the original diploid genetic content (*8*), and it is not surprising that they alone have only a single locus coding for LDH (*2, 3*). The hagfish, on the other hand, is rather rich in DNA (as much as 75 percent that of mammals) (*9*). In this light, the revelation that they already possess four gene loci for hemoglobin polypeptides (*4*) as well as two and possibly three gene loci for LDH subunits is not unexpected (*10*).

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References and Notes

1. C. L. Markert and H. Ursprung, *Develop. Biol.* **5**, 363 (1962).
2. C. L. Markert and I. Faulhauber, *J. Exp. Zool.* **159**, 319 (1965).
3. N. O. Kaplan, M. M. Ciotti, M. Hamolsky, R. E. Bieber, *Science* **131**, 392 (1960); R. D. Cahn, N. O. Kaplan, L. Levin, E. Zwilling, *ibid.* **136**, 962 (1962).
4. S. Ohno and M. Morrison, *ibid.* **154**, 1034 (1966).
5. A. Blanco and W. H. Zinkham, *ibid.* **139**, 601 (1963); W. H. Zinkham, *ibid.* **144**, 1353 (1964).
6. R. Ceppellini, *Biochemistry of Human Genetics* (Churchill, London, 1959), pp. 133–138.
7. H. G. Kunkel, "Myeloma proteins and antibodies," *Harvey Lecture* **59**, (1963–64); L. Herzenberg, *Cold Spring Harbor Symp. Quant. Biol.* **19**, 455 (1964).
8. S. Ohno and N. B. Atkin, *Chromosoma* **18**, 455 (1966).
9. N. B. Atkin, personal communication.
10. Supported in part by PHS grant CA-05138-07. Contribution No. 1-67, Department of Biology, City of Hope Medical Center. We thank L. Andersen for editorial assistance. We are also grateful for criticism offered by M. Cohn of the Salk Institute and by E. Beutler, Burroughs Hill, and E. Roberts of this institute.

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Transferrin Polymorphism and Population Differences in the Genetic Variability of Chimpanzees

Abstract. *Genetic divergencies between chimpanzee populations, not only between* Pan panicus *and* Pan troglodytes *but also between different groups of the latter, are revealed by typing of transferrin. In particular, differences in the incidence of polymorphic transferrins occur between the groups formed by subdividing a large captive chimpanzee colony of heterogeneous geographic origins into racial types solely on the basis of morphological traits. Genetic variability is extremely high in one of these groups, intermediate in another, and relatively low in a third, with the pattern of changing frequencies of allelic genes at the* T_f *locus following the pattern of geographic distribution of the actual conspecific populations or races for which the groups are named.*

Chimpanzees have a very broad geographic distribution that extends for thousands of miles through the rain forests of central Africa from the Atlantic coast of Gambia to the mountains of Uganda and Tanganyika. Noting that chimpanzees vary sharply in morphological appearance from one part of their range to another, Hill (*1*) distinguishes at least four types which he considers races of *Pan troglodytes: P. t. verus* (Ptv), *P. t. troglodytes* (Ptt), *P. t. koolokamba* (Ptk), and *P. t. schweinfurthi* (Pts), in addition to *Pan paniscus,* the pygmy chimpanzee which is found on the south bank of the Congo river. At the far western periphery of the genus and progressing eastward, the race of *P. t. verus* is followed, after the Dahomey gap, by that of *P. t. troglodytes* and by *P. t. koolokamba*; the latter in the Cameroons meets the race of *P. t. schweinfurthi* which extends for over 1600 km through the Congo to Uganda and Tanganyika (*1*). A study of the genetic structures of the natural chimpanzee populations throughout their geographic range would clarify the process of evolution in a primate group which, among nonhuman forms, provides the closest homolog of the genus *Homo*. The potentialities of such a study are indicated by the data being gathered at the Holloman Air Force Base in New Mexico, where over 170 captive chimpanzees were obtained from a variety of sources. Hill and Fineg, on the basis of morphological criteria, classified the members of the colony into the four racial types: Ptv, Ptt, Ptk, and Pts. Marked differences in gene frequencies were then found between the Ptv and Pts groups when the colony was typed for various erythrocyte isoantigens (*2*).

Our study, which is concerned with the transferrin polymorphism of chimpanzees, describes further genotypic differences between the Ptv and Pts groups of the Holloman colony. The most striking finding is that, whereas transferrin monomorphism or homozygosity at the T_f locus predominates in the Ptv group, heterozygosity at this locus predominates in Pts chimpanzees. The degree of such genetic variability appears to be higher in *Pan troglodytes* as a whole than in humans and may be especially intense in chimpanzees from particular geographic areas.

The different molecular forms of transferrin, the serum iron-binding protein, were detected by vertical starch-gel electrophoresis (*3*) and autoradiography of serum samples treated with Fe^{59}, by the procedure of Giblett *et al.* (*4*). Five molecular forms and eight phenotypes of transferrin were found when serums from 111 chimpanzees of the Holloman colony were analyzed (Fig. 1). From the transferrin of least anodic mobility to those of faster mobility, these transferrins are labeled D, C, E, B, and A. Boyer and Young (*5*) had previously discovered and named transferrins D, C, B, and A in 25 chimpanzees of another group. Goodman *et al.* (*6*) also previously

Table 1. Distribution of racial groups and transferrin phenotypes in the older animals (66) and newer animals (66) of the Holloman chimpanzee colony. In the parentheses, the first number is the total number of animals in each group, and the second number is the number of animals typed for transferrins.

Transferrin phenotypes	Animals (No.) classified into racial groups and typed for transferrins							
	Older				Newer			
	Ptv (26/20)	Ptt (13/9)	Ptk (1/0)	Pts (26/23)	Ptv (37/34)	Ptt 14/14)	Ptk (1/1)	Pts (14/10)
DD	1	1		4				
DC				9				
DE		1						
DA	1	2		2				
CC	17	3		1	30	11		9
CB	1			1	4	3		1
CA		2		4			1	
AA				2				

found these same four transferrins among 77 chimpanzees of the Yerkes Orange Park colony. A new transferrin (E) was discovered in this study (*7*). There is clear evidence (*8*) that transferrins of chimpanzees, like those of humans (*9*) and rhesus monkeys (*10*), are controlled by a series of codominant alleles at an autosomal locus. Thus the eight transferrin phenotypes (Fig. 1) found in the Holloman colony are represented as genotypes; we consider that DD, CC, and AA are controlled by homozygous alleles, and DC, DE, DA, CB, and CA by heterozygous alleles.

Serums from three pygmy chimpanzees (*11*) at the Frankfurt Zoo were also analyzed for transferrins. In this small sample of *Pan paniscus,* there were three molecular forms of transferrin, C, E′, and F, and three new phenotypes, CF, E′F, and FF (Fig. 2). Transferrin C has the same mobility in *Pan paniscus* and *Pan troglodytes*. But E′ and F are new transferrins from chimpanzees (*7*). Transferrin E′ is a little slower in mobility than E; and F, which migrates faster than A, has a mobility very slightly slower than that of the most common human transferrin.

The data on the distribution of transferrin phenotypes and on the frequencies of the transferrin alleles in the different racial groups of Holloman chimpanzees are presented in Tables 1 and 2. In contrast to the results obtained in the *Pan paniscus* group, in which the most frequently occurring transferrin was F, the most frequent one in the Holloman chimpanzees and other groups (*5, 6*) of *Pan troglodytes* was C transferrin. However, marked differences in the degree of the transferrin polymorphism and in the frequencies of the different transferrin alleles occur among the racial groups. Of the Ptv animals 87 percent were T_f^C homozygotes, in contrast to 61 percent of the Ptt animals and only 30 percent of the Pts animals. The one Ptk animal was a CA heterozygote. In addition to T_f^C, the T_f^D and T_f^A alleles occurred at appreciable frequencies in the Pts group. The difference in the distribution of T_f alleles between the Ptv and Pts groups is highly significant by chi-square analysis ($P < .001$). The difference between the Ptv and Ptt groups in the distribution of these alleles is also significant ($P < .01$), but the difference between the Ptt and Pts groups is not significant by chi-square analysis ($0.5 < P < .10$).

Table 2. Frequencies of transferrin alleles in 54 Ptv, 23 Ptt, and 33 Pts chimpanzees of the species *Pan troglodytes*.

Alleles	Racial groups		
	Ptv	Ptt	Pts
A	.009	.087	.152
B	.046	.065	.030
C	.917	.717	.530
D	.028	.109	.288
E	.000	.022	.000

The Holloman colony was built up to its present size during the past 6 years, and there is reason to believe that the geographic areas from which many of the earlier members of the colony came were different from the sources of most of the later members. Although we cannot accurately determine the original geographic source of any particular chimpanzee in the colony, we know that a dealer who supplied many of the earlier animals imported his chimpanzees from the Cameroons, an area containing several chimpanzee races (*P. t. troglodytes, P. t. koolokamba,* and *P. t. schweinfurthi*) (*1*). We also know that another dealer supplying many of the recent animals imports his chimpanzees from Sierra Leone and Liberia. These two countries are on the Atlantic coast not far from Gambia and thus are in the western part of the chimpanzees' range where Hill's subspecies *P. t. verus* predominates. The records show that of the 132 animals classified from morphological appearances for racial type the majority of the Pts group are among the first 66 chimpanzees received in the col-

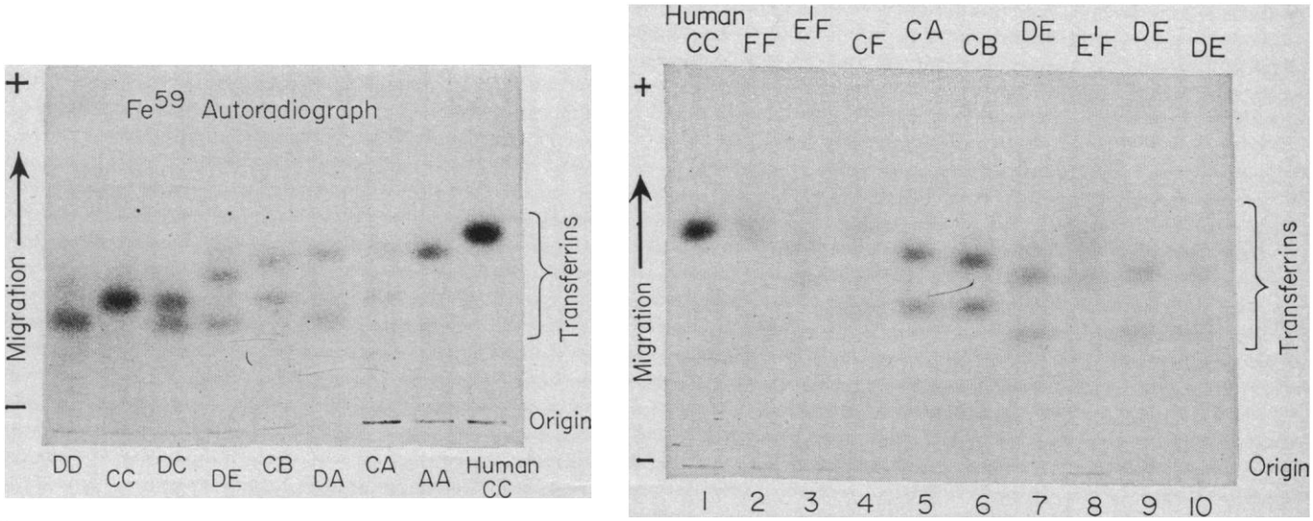

Fig. 1 (left). Comparison of the transferrin phenotypes of serums of nine chimpanzees from Holloman Base and one human serum by vertical starch-gel electrophoresis and autoradiography. The serums were chosen to demonstrate the eight phenotypes found in the Holloman colony in relation to the most common human phenotype. Fig. 2 (right). Autoradiograph (of samples treated with Fe^{59}) after vertical starch-gel electrophoresis comparing the relative mobilities of transferrins of seven chimpanzees to the mobility of the most common human transferrin, as shown. The serums separated in slots 2, 3, and 4 are from three pygmy chimpanzees. To demonstrate the difference in mobility between *E′ and E*, the serum used in slot 3 is repeated in slot 8 and run between two chimpanzee serums of *DE* phenotype. The serum in slot 7 which is repeated in slot 10 is from a Ptt Holloman chimpanzee, and the other *DE* serum in slot 9 is from a chimpanzee at the Delta Regional Primate Research Center. Thus the frequency of T_f^B may not be as uncommon as indicated by the Holloman results alone. (Although so far only 24 chimpanzees at the Delta Center have been typed for transferrins, five of these animals showed the *DE* phenotype.)

ony, whereas the majority of the Ptv group are among the last 66 chimpanzees added to the colony (Table 1). Furthermore, the frequencies of the variant transferrin types differ markedly when the older half of the colony is compared to the newer type. The tabulation of phenotypes in Table 1 reveals that in the older half of the colony the proportions of the different transferrins are 59C:27D:15A:-2B:1E, whereas in the newer half the proportions are 109C:8B:1A. Thus the transferrin polymorphism is not nearly as marked in the half of the colony containing the majority of Ptv chimpanzees (the newer half) as in the half containing the majority of the Pts animals (the older half).

The transferrin polymorphism in the Ptt group is stronger than in the Ptv group, but not as strong as in the Pts group. The three most common transferrin alleles in the colony have frequencies in the Ptt group which are close to the average of their frequencies in the Ptv and Pts groups. The pattern of intermediate gene frequencies in the Ptt group (Table 2) correlates with the intermediate geographic location ascribed by Hill (*1*) to *P. t. satyrus* (*P. t. troglodytes*), which lies between *P. t. verus* at the far western side of the chimpanzees' range and *P. t. schweinfurthi* at the center and eastern side of the range. This correlation suggests that the morphological traits used by Hill to classify the Holloman chimpanzees into racial types are good indicators of the different conspecific chimpanzee populations as they occur in their natural habitats.

Syner (*12*) discovered a variant of the lactate dehydrogenase B subunit and of glucose-6-phosphate dehydrogenase in two different individuals of the Pts group; Hoffman (*13*) found a variant of the hemoglobin alpha chain in a third Pts chimpanzee and a variant of the hemoglobin beta chain in another Pts chimpanzee. In addition to these rare variant alleles found only in the Pts group of the Holloman colony, polymorphisms of the red-cell enzymes, phosphoglucomutase and adenylatekinase, were found by Tashian (*14*) throughout the colony, with the frequency of the polymorphisms being greater in the Pts group than in the Ptv group. These results suggest that genetic variability is extremely high in the chimpanzee population from particular geographic areas (presumably in the eastern or central part of the range) and relatively low in the population from certain other areas (presumably at the western periphery of the species distribution). A survey of the incidence of polymorphic protein macromolecules in the native chimpanzee populations from localities of differing ecological and geographic characteristics would clarify the genetic and natural selective mechanisms operative in chimpanzee evolution and possibly also in human evolution.

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References and Notes

1. W. C. O. Hill, in *Encyclopaedia Britannica* (1964), vol. 4, p. 555. (The major species of chimpanzee, designated in this earlier article by Hill as *Pan satyrus*, is now officially known as *Pan troglodytes*.)
2. J. Moor-Jankowski, A. S. Wiener, C. H. Kratochvil, J. Fineg, *Science* **152**, 219 (1966).
3. O. Smithies and O. Hiller, *Biochem. J.* **72**, 121 (1959).
4. E. R. Giblett, C. G. Hickman, O. Smithies, *Nature* **183**, 1589 (1959).
5. S. H. Boyer and W. J. Young, *ibid.* **187**, 1035 (1960).
6. M. Goodman, R. McBride, E. Poulik, E. Reklys, *ibid.* **197**, 259 (1963).
7. A purely empirical system of lettering is employed for naming newly discovered transferrins of chimpanzees.
8. M. Goodman and A. J. Riopelle, *Nature* **197**, 261 (1963).
9. E. R. Giblett, *Progr. Med. Genet.* **2**, 34 (1962).
10. M. Goodman and R. C. Wolf, *Nature* **197**, 1128 (1963).
11. M. Goodman, *Human Biol.* **35**, 377 (1963). Serums were provided by H. Klinger.
12. F. Syner, personal communication.
13. H. A. Hoffman, personal communication.
14. R. Tashian, personal communication.
15. Supported by contract AF 29(600)-5587 and by NSF grant GB-4823.

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Circadian Periodicity in Susceptibility to Lidocaine Hydrochloride

Abstract. *Separate groups of mice standardized in an alternating 12-hour-light, 12-hour-dark regimen were treated with lidocaine hydrochloride every 3 hours over a 24-hour period. The results indicate a quantitative circadian periodicity with maximal convulsant activity at 2100 hours which was approximately a fourteen fold increase over the values observed at 1500 hours.*

Recent studies have elucidated the pharmacologic response to drugs as affected by the circadian (about 24-hours) rhythms of animals. Mice subjected to alternating light and dark regimens, a factor known to influence their rhythmical behavior, displayed a circadian response to several pharmacologic agents, namely, pentobarbital (*1*, *2*), nikethamide (*3*), methopyrapone (*4*), and aurothioglucose (*5*). The degree of response was found to vary quantitatively along a 24-hour time scale.

The experiment reported here was conducted to determine whether mice, standardized in constant environmental conditions with alternating 12-hour-light and 12-hour-dark cycles, would display a significantly circadian quantitative response to the central stimulant activity of the local-anesthetic drug lidocaine hydrochloride. In therapeutic doses lidocaine hydrochloride inhibits the conduction of impulses along a nerve fiber. However, central nervous system stimulation, even to the point of convulsions, is the most frequent and serious systemic toxicity response induced by lidocaine hydrochloride. The existence of a direct relationship between the degree of susceptibility to convulsions induced by lidocaine hydrochloride and mammalian 24-hour rhythms could be of considerable interest to clinicians in medical, dental, and veterinary practices.

Adult female albino mice (Carworth Farms, CF-1), 22.24 ± 0.23 g body weight, were housed 16 per cage with a total floor area of 1216 in.^{2} (7845 cm^2), or 7.6 in.^{2} per mouse; this eliminated the psychological variable of aggregation due to overcrowded housing. The mice were housed in a controlled environmental room maintained at a temperature of 23.3° ± 1.0°C, with a relative humidity of 65 ± 2.0 percent. The room was programmed so as to provide 12 hours of incandescent lighting (four 40-watt bulbs) from 0605 to 1805 hours and a totally darkened phase from 1805 to 0605 hours. Testing during the dark phase was conducted in an adjacent room having similarly controlled conditions, except that in order to make observations possible a very low level of illumination was maintained by means of a 20-watt photographic safelight. During the light phase animals were tested under conditions com-