2). The suggestion (*8, 9*) that destruction of target cells by lymphocytes depends upon the number of lymphocyte–target cell interactions is further supported by other experiments which demonstrated that the extent of target cell destruction also depended on the concentration of lymphocytes (*3, 8–12*) and that rocking facilitated lysis (*6*).

In the second series of experiments, examination of the fate of lymphocytes after lytic interaction with tumor cells showed that the lymphocytes involved were not inactivated during the process but were capable of destroying additional tumor cells. For these experiments, 1×10^5 lymphocytes were mixed with 8×10^5 ^{51}Cr-EL4 cells, and the number of tumor cells destroyed was determined as a function of time. It was known from previous experiments (Fig. 1) that 8×10^5 represented a considerable excess of tumor cells. The number of tumor cells destroyed in 5 hours was twice the number of lymphocytes present, and there was no decay in the rate of destruction (Fig. 2). If the reacting lymphocytes were "consumed" or inactivated, there would have been a significant decrease in the rate of destruction, and the total number of tumor cells lysed would have been no greater than 1×10^5 (*13*). Although previous experiments led to the suggestion that the lymphocyte is not inactivated as a result of its cytolytic activity (*8, 12, 14*), this conclusion has recently been questioned by Henney (*13*). Under the conditions of our experiments, the lymphoid cell kills and continues to kill (*15*).

GIDEON BERKE
KAREN A. SULLIVAN
D. BERNARD AMOS
Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710

References and Notes

1. W. Rosenau and H. D. Moon, *J. Nat. Cancer Inst.* **27**, 471 (1961); D. B. Wilson and R. E. Billingham, *Advan. Immunol.* **7**, 189 (1967); P. Perlmann and G. Holm, *ibid.* **11**, 117 (1969); B. R. Bloom, *ibid.* **13**, 102 (1971).
2. G. Berke and R. H. Levey, *J. Exp. Med.* **135**, 972 (1972).
3. G. Berke, K. A. Sullivan, D. B. Amos, *ibid.*, p. 1334; K. A. Sullivan, G. Berke, D. B. Amos, *Transplantation* **13**, 627 (1972).
4. Tumor EL4, obtained from the National Cancer Institute, Bethesda, Maryland, is an ascites leukosis carried in C57BL/Spr mice [P. A. Gorer, *Brit. J. Cancer* **4**, 372 (1950)]. The rejection by BALB/c hosts is usually complete by 11 days after transplantation.
5. Medium RPMI-1640 or phosphate-buffered saline (Grand Island Biological Co., Grand Island, New York) serves equally well for short-term experiments. Sodium [^{51}Cr]chromate (Amersham-Searle Co., Des Plaines, Illinois) was used for labeling as previously described (*2, 3, 6*).
6. T. G. Canty and J. R. Wunderlich, *J. Nat. Cancer Inst.* **45**, 761 (1970). In our experiments in which rocking was interrupted after 30 minutes, ^{51}Cr release continued for approximately another 30 minutes. Acceleration of killing began within 10 minutes of initiating rocking in previously stationary cultures.
7. A higher concentration of lymphocytes (2×10^6 ml^{-1}) at this concentration of tumor cells (3×10^5 ml^{-1}) resulted in more lysis.
8. G. Berke, G. Yagil, H. Ginsburg, M. Feldman, *Immunology* **17**, 723 (1969).
9. The principle that the destruction of target cells is a function of their concentration as well as of the concentration of lymphocytes can be extended to systems (for instance, human) where the number of effector lymphocytes is restricted.
10. D. B. Wilson, *J. Exp. Med.* **122**, 143 (1965).
11. K. T. Brunner, J. Mauel, J.-C. Cerottini, B. Chapuis, *Immunology* **14**, 181 (1968).
12. G. Berke, W. Ax, H. Ginsburg, M. Feldman, *ibid.* **16**, 643 (1969).
13. It is believed, on the basis of quantitative kinetic analysis and microcinematography, that one lymphocyte is required and sufficient to destroy one target cell [Wilson (*10*); Berke *et al.* (*8*); H. Ginsburg, W. Ax, G. Berke, in *Pharmacological Treatment in Organ and Tissue Transplantation*, A. Bertelli and A. P. Monaco, Eds. (Williams & Wilkins, Baltimore, 1970), p. 85; C. S. Henney, *J. Immunol.* **107**, 1558 (1971)].
14. K. T. Brunner, J. Mauel, H. Rudolf, B. Chapuis, *Immunology* **18**, 501 (1970).
15. By using ^{51}Cr-labeled lymphocytes, we recently obtained direct evidence that lymphocytes are not killed during the reaction.
16. Supported by PHS research grants KO-AI18399, SO4-RR06148, and TO1-AI00285. We thank K. Alter for technical help.

11 April 1972

Gene Differences between Caucasian, Negro, and Japanese Populations

Abstract. *The numbers of gene (codon) differences per locus between two randomly chosen genomes within and between Caucasian, Negro, and Japanese populations have been estimated from gene frequency data for protein loci. The estimated number of gene differences between individuals from different populations is only slightly greater than the number between individuals from the same population.*

Nei (*1, 2*) developed a statistical method by which the number of gene (codon) differences per locus between two different populations can be estimated from gene frequency data. He also indicated that the number of codon differences per locus is an ideal measure of gene differences between populations. We have used this method to investigate the gene differences between the three major ethnic groups of man, Caucasoids, Negroids, and Mongoloids (Japanese). The results obtained indicate that the gene differences between individuals from different ethnic groups are only slightly greater than those between individuals from the same group.

The method used is based on the identity of genes within and between populations. Thus if x_i and y_i are frequencies of the ith allele at a locus in populations X and Y, respectively, the probability of identity of two randomly chosen genes is $j_X = \Sigma x_i^2$ in population X and $j_Y = \Sigma y_i^2$ in population Y. The probability of identity of two genes, chosen at random, one from each of the two populations, is $j_{XY} = \Sigma x_i y_i$. We designate by J_X, J_Y, and J_{XY} the arithmetic means of j_X, j_Y, and j_{XY} over all loci, including monomorphic ones, respectively. Then, a minimum estimate of the number of net codon differences per locus (D) between X and Y can be obtained by

$$D = D_{XY} - (D_X + D_Y)/2 \qquad (1)$$

where $D_X = 1 - J_X$ and $D_Y = 1 - J_Y$ are minimum estimates of the number of codon differences per locus between two randomly chosen genomes (intrapopulational codon differences) in populations X and Y, respectively, while $D_{XY} = 1 - J_{XY}$ is a minimum estimate of the number of codon differences per locus between two genomes, one from each of the two populations (*3*). Note that D_X, D_Y, and D_{XY} are equal to the expected proportions of different genes between two randomly chosen genomes from the respective populations. On the other hand, a maximum estimate of the number of net codon differences per locus is obtained by using $D_{XY} = -\log_e J_{XY}$, $D_X = -\log_e J_X$, and $D_Y = -\log_e J_Y$ in Eq. 1, where J_{XY}, J_X, and J_Y are the geometric means of j_{XY}, j_X, and j_Y, respectively (*4*). The real number of codon differences is expected to be somewhere

Table 1. Minimum and maximum estimates of the number of codon differences* per locus within and between Caucasian and Negro populations. The subscripts C and N refer to the Caucasian and Negro populations, respectively; D stands for the number of net codon differences between the two populations. These estimates are based on gene frequency data for 44 protein loci.

	D_C	D_N	D_{CN}	D
Minimum†	0.107	0.092	0.110	0.011
Maximum	.139	.112	.142	.017

* Codon differences that are detectable by electrophoresis. † Minimum estimate of codon differences per locus is equal to the expected proportion of different genes between two randomly chosen genomes.

between minimum and maximum estimates, but ordinarily there is not much difference between the two estimates when populations in the same species are compared (*2*). In practice, of course, both minimum and maximum estimates refer to those codon differences that are detectable by available techniques (for example, electrophoresis).

For the present purpose, gene frequency data for many loci, which are ideally a random sample of the genome, are required. Surveying literature, we collected data for 44 protein loci for Caucasian and Negro populations (*5*). For the Japanese population, gene frequencies were available only for 28 loci for which the Caucasian and Negro data existed (*6*). The gene frequencies for these protein loci were all studied by electrophoresis. The majority of the data for Caucasians and Negroes were from the American Caucasians (25 loci) and the American Negroes (26 loci). The average proportion of polymorphic loci for the three populations was 44 percent (*7*). The Japanese population had a slightly higher proportion of polymorphic loci (50 percent) than the other two populations, but this was apparently due to the property of the loci used for this population. When only those loci for which the Japanese data existed were used, the proportion for Caucasian and Negro populations increased and the racial differences virtually disappeared. The allele fixed for a monomorphic locus was always the same for all three populations; there was no complete gene substitution at any locus.

There are some reasons to believe that the loci used here do not deviate greatly from a random sample of the genome. The weighted mean of average heterozygosities ($1 - J_X$, where J_X is the arithmetic mean) of the three populations is about 10 percent. While this value is slightly higher than the estimate (7 percent) obtained by Harris (*8*) for 20 arbitrarily chosen protein loci in man, it agrees closely with the mean of average heterozygosities for six different species so far studied (*9*).

The estimate of the number of intrapopulational codon differences per locus is about 0.09 to 0.14 in Caucasian and Negro populations when all 44 loci are used (Table 1). The proportion of different genes between two randomly chosen genomes from the same population (D_C or D_N; minimum estimate) is about 10 percent. The value for Caucasians is slightly larger than that for Negroes. On the other hand, D_{CN} is slightly larger than D_C or D_N. The number of net codon differences (D) between the two populations is about 0.01 to 0.02. Thus, the interpopulational net codon differences relative to the intrapopulational codon differences $[R = 2D/(D_C + D_N)]$ are estimated to be about 11 to 14 percent. This indicates that the gene differences between the two populations are rather small compared with those within the same population. Electrophoresis is known to detect only a portion of all the existing codon differences, but this would not affect our estimate of R appreciably, since D, D_C, and D_N will be affected equally by the proportion of detectability.

Table 2. Minimum and maximum estimates of the number of codon differences* per locus within and between Caucasian, Negro, and Japanese populations when 28 common loci are used. The figures on the diagonal are intrapopulational codon differences (D_X), and those above the diagonal are interpopulational net codon differences (D).

Population	Caucasian	Negro	Japanese
Caucasian			
Minimum †	0.146	0.016	0.005
Maximum	.193	.025	.008
Negro			
Minimum †		.120	.015
Maximum		.148	.026
Japanese			
Minimum †			.120
Maximum			.150

* Codon differences that are detectable by electrophoresis. † Minimum estimate of codon differences per locus is equal to the expected proportion of different genes between two randomly chosen genomes.

As mentioned earlier, the majority of our Negro data came from the American Negroes. It is known that about 20 percent of American Negro genes are of Caucasian origin (*10*). Correction for the effect of interracial mixture, however, does not increase our estimates of D drastically (*11*). Furthermore, it is possible to estimate the value of D between Caucasians and African Negroes from Harris's (*8*) data for 20 protein loci. The minimum and maximum estimates obtained from these data are 0.01 and 0.02, respectively. Thus, the genes appear to be quite similar even between Caucasians and African Negroes.

The Japanese population can be compared with the other two populations by using 28 common loci (Table 2). The estimate of the number of intrapopulational codon differences (D_X) for Japanese is close to that for Negroes, but slightly smaller than that for Caucasians. The number of net codon differences (D) between Negroes and Japanese is almost the same as that between Negroes and Caucasians. The value between Caucasians and Japanese is about one-third of that between the other two pairs of populations. However, the absolute value of the difference is so small that not much significance can be given to it. In fact, our preliminary studies of blood group gene frequencies suggest that the genetic distance between Caucasians and Japanese is no closer than that between Caucasians and Negroes. Therefore, we tentatively conclude that the net codon differences between Caucasian, Negro, and Japanese populations are roughly the same and about 0.01 to 0.03 per locus. In any event, it is obvious that the interpopulational net codon differences are small compared with the intrapopulational codon differences, in all pairs of populations.

From the results mentioned above, it may be concluded that the genes in the three major ethnic groups of man are remarkably similar, although the phenotypic differences in such characters as pigmentation and facial structures are conspicuous. It seems likely that the genes controlling these morphological characters were subjected to stronger natural selection than "average genes" in the process of racial differentiation. The results of our preliminary studies of blood group gene frequencies also support this conclusion (*11*). Using a different method, Cavalli-Sforza (*12*) obtained a similar conclusion.

Nei (*2*) has studied the number of net codon differences between local populations of the house mouse and *Drosophila pseudoobscura* by using the above method. His results indicate that this number is 0.002 to about 0.03 per locus in most cases. On the other hand, the value between two semispecies of the house mouse has been estimated to be about 0.1 to 0.2 per locus. Furthermore, the number of net codon differences between different species of *Drosophila* appears to be about 0.2 to 2.5 per locus (*13*). All of these estimates refer to electrophoretically detectable codon differences. Thus, the gene differences between human ethnic groups are of the same order of magnitude as those between local populations of the house mouse and *D. pseudoobscura*.

MASATOSHI NEI
ARUN K. ROYCHOUDHURY
Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912

References and Notes

1. M. Nei, *Genetics* **68**, s47 (1971).
2. ———, in *Genetic Distance*, J. F. Crow, Ed. (Plenum, New York, in press); *Amer. Natur.* **106**, 283 (1972).
3. At the nucleotide level, there must be at least one codon difference between any two alleles (nucleotide sequences) at a locus. Thus, $d_X = 1 - J_X$ is a minimum estimate of the expected number of codon differences between two randomly chosen genes from population X, while D_X is the average value of d_X over all loci (genomes). Similarly, D_Y and D_{XY} are minimum estimates of the codon differences per locus. Therefore, $D = D_{XY} - (D_X + D_Y)/2$ may be regarded as a minimum estimate of net codon differences between X and Y when intrapopulational codon differences are subtracted. Note that in a randomly mating population D_X is equal to average heterozygosity.
4. If the individual codon changes are independent and follow a Poisson distribution, the mean number of net codon differences (D) is given by using $D_{XY} = -\log_e J_{XY}$, $D_X = -\log_e J_X$, and $D_Y = -\log_e J_Y$, where J_{XY}, J_X, and J_Y are the arithmetic means of j_{XY}, j_X, and j_Y, respectively. If the rate of codon changes varies from locus to locus, the geometric means of j_{XY}, j_X, and j_Y are better than the arithmetic means because the quantities being considered are the logarithms of these values. The geometric means always give a larger estimate of D than the value obtained from the arithmetic means. However, under certain circumstances the geometric means give an overestimate of D [see (*2*)].
5. The protein loci used are as follows: acid phosphatase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucomutase (PGM1, PGM2, PGM3), adenylate kinase, peptidase (A, B, C, and D)* [see (*6*)], NADH diaphorase, adenosine deaminase, glutamic-pyruvic transaminase, mitochondrial glutamic oxaloacetic transaminase*, soluble glutamic oxaloacetic transaminase, lactate dehydrogenase*, phosphoglycerate kinase, catalase, carbonic anhydrase*, malic dehydrogenase, hexokinase, citrate dehydrogenase, nucleoside phosphorylase, red-cell oxidase, red-cell pyrophosphatase*, pyruvate kinase*, triosephosphate isomerase*, phosphohexose isomerase, placental acid phosphatase*, placental alkaline phosphatase, urinary amylase*, hemoglobin (α and β), haptoglobin (α and β), transferrin, Gc-globin, α_1-acid glycoprotein, α_1-antitrypsin, ceruloplasmin*, cholinesterase (locus 2)*, albumin Naskapi*, and the third component of complement (C'3)*. For the gene frequency data and the sources, see (*11*).
6. Gene frequency data for the protein loci with asterisks in (*5*) were not available for the Japanese population.
7. A locus is defined as polymorphic if the frequency of the second most common allele is equal to or larger than 0.01.
8. H. Harris, *Brit. Med. Bull.* **25**, 5 (1969).
9. M. Kimura and T. Ohta, *Nature* **229**, 467 (1971).
10. T. E. Reed, *Science* **165**, 762 (1969).
11. A. K. Roychoudhury and M. Nei, in preparation.
12. L. L. Cavalli-Sforza, *Proc. Int. Congr. Genet. 12th* **3**, 405 (1969); in *Disadvantaged Child*, J. Hellmuth, Ed. (Brunner/Mazel, New York, 1970), vol. 3, p. 111.
13. M. Nei, *Amer. Natur.* **105**, 385 (1971).

14. We thank Drs. C. Stern, J. V. Neel, and P. L. Workman for their comments on an earlier draft of this report. Supported by PHS grant GM-17719 and NSF grant GB-21224.

6 January 1972; revised 24 April 1972

Adenosine 3′,5′-Monophosphate Is Localized in Cerebellar Neurons: Immunofluorescence Evidence

Abstract. *Adenosine 3′,5′-monophosphate is localized in specific cerebellar neurons, as shown by fluorescence immunocytochemistry with a specific rabbit immunoglobulin. Positive staining is exhibited by Purkinje neurons and granule cells. The increase in concentration of cyclic adenosine monophosphate in the cerebellum, which is known to follow decapitation, is represented by greatly increased fluorescence of Purkinje neurons only. These immunofluorescence data provide the first evidence for localization of cyclic adenosine monophosphate in specific neurons and may permit further exploration into the role of this cyclic nucleotide in neuronal function.*

An involvement of adenosine 3′,5′-monophosphate (cyclic AMP) in brain function is inferred from biochemical studies on brain slices (*1*) or homogenates (*2*); these studies show that central neurotransmitters, particularly norepinephrine (*1, 3*), can regulate the activity of adenylate cyclase (*3*). However, biochemical analysis of brain has not yet demonstrated intercellular or intracellular differences in cyclic AMP content among various types of neurons and glia. We report that localization of cyclic AMP within specific central neurons can be detected by a technique of fluorescence immunocytochemistry (*4*). Furthermore, the pattern of histochemical reactivity varies with experimental conditions that are known to produce marked alterations in the cyclic AMP content of brain extracts.

The indirect immunofluorescence technique has been described (*4*). In this technique, rabbit antiserums were prepared against the 2-*O*-succinyl derivative of cyclic AMP, which was conjugated to either human serum albumin or to keyhole limpet hemocyanin (*5*), and the immunoglobulin (Ig) G fractions of these antiserums were used. In the final step, a fluorescein isothiocyanate–conjugated goat immunoglobulin G (IgG) that was prepared against rabbit IgG (*6*) was used to stain the complex of cyclic AMP and rabbit Ig. Portions of the rabbit Ig used for the immunocytochemical staining were also used for a specific and sensitive assay for cyclic AMP in tissue extracts (*5*). Cryostat sections (10 to 14 μm thick) of unfixed rat cerebellum and brainstem, frozen in liquid nitrogen, were stained by the immunofluorescence method and examined by dark-field fluorescence microscopy. We selected rat cerebellum for this analysis because of its uniquely high endogenous concentrations of cyclic AMP (*7*).

Concentrations of cyclic AMP in the cerebellum climb rapidly after the animal is decapitated, plateau after 90 to 100 seconds, and remain elevated for at least 5 minutes; concentrations of adenosine triphosphate (ATP) fall during this period (*8, 9*). When cerebellums were frozen in liquid nitrogen 90 to 150 seconds after decapitation and cryostat sections were prepared for immunofluorescence staining of cyclic AMP, discrete cell staining of high intensity was observed only in specific cerebellar cortical neurons (Fig. 1, A and C). Approximately 80 to 90 percent of Purkinje (P) cells show positive staining under these conditions: Most reactive P cells are stained exclusively in the cytoplasm, but a few P cells are stained mainly in the nucleus or in both nucleus and cytoplasm. Positive staining is also observed in the granule cell layer (Fig. 1A); the source of reactivity in the granule cell layer is the granule cell, because the fluorescence is undiminished when mossy fibers degenerate after total unilateral cerebellar pedunculectomy.

Moderate fluorescence that is seen occasionally in the molecular layer is presumably from P cell dendrites; strongly reactive primary and secondary dendritic branches could occasionally be traced back to the perikaryon. Virtually no immunoreactivity could be seen within the cerebellar white matter (Fig. 1, A and C). The perikaryon and proximal dendritic branches of some large multipolar neurons of the pontine reticular formation and deep cerebellar nuclei also exhibited strong cytoplasmic reactivity (Fig. 1B).

Several immunological reactions establish the specificity of the indirect immunocytochemical staining and confirm that the staining observed in these neurons is attributable to their cyclic AMP content. No staining of cerebellar sections was observed when the Ig fraction of nonimmunized rabbits was used as the primary immunoreagent or when