



The Origin of Man: A Chromosomal Pictorial Legacy

Author(s): Jorge J. Yunis and Om Prakash

Source: *Science*, New Series, Vol. 215, No. 4539 (Mar. 19, 1982), pp. 1525-1530

Published by: American Association for the Advancement of Science

Stable URL: <http://www.jstor.org/stable/1688162>

Accessed: 10/12/2009 06:19

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/action/showPublisher?publisherCode=aaas>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.



American Association for the Advancement of Science is collaborating with JSTOR to digitize, preserve and extend access to *Science*.

<http://www.jstor.org>

- R. J. Miller, D. J. Reis, *Soc. Neurosci. Abstr.* 6, 353 (1980); N. Aronin, M. DiFiglia, A. Liotta, J. B. Martin, *ibid.*, p. 353; E. J. Glazer and A. I. Basbaum, *ibid.*, p. 523.
10. E. Carstens and D. L. Trevino, *J. Comp. Neurol.* 182, 151 (1978); G. J. Giesler, Jr., D. Menétrey, A. I. Basbaum, *ibid.* 184, 107 (1979); W. D. Willis, D. R. Kenshalo, Jr., R. B. Leonard, *ibid.* 188, 543 (1979); S. Hockfield and S. Gobel, *Brain Res.* 139, 333 (1978); H. Burton and A. D. Craig, Jr., *ibid.* 161, 515 (1979).
 11. D. L. Trevino, R. A. Maunz, R. N. Bryan, W. D. Willis, *Exp. Neurol.* 34, 64 (1972); W. D. Willis, D. L. Trevino, J. D. Coulter, R. A. Maunz, *J. Neurophysiol.* 37, 358 (1974); D. D. Price, R. Dubner, J. W. Hu, *ibid.* 39, 936 (1976); D. D. Price, R. L. Hayes, M. Ruda, R. Dubner, *ibid.* 41, 933 (1978); D. R. Kenshalo, Jr., R. B. Leonard, J. M. Chung, W. D. Willis, *ibid.* 42, 1370 (1979); D. D. Price, H. Hayashi, R. Dubner, M. A. Ruda, *ibid.*, p. 1590; G. J. Giesler, Jr., D. Menétrey, G. Guilbaud, J.-M. Besson, *Brain Res.* 118, 320 (1976).
 12. J. C. Adams, *Neuroscience* 2, 141 (1977).
 13. L. A. Sternberger, *Immunocytochemistry* (Wiley, New York, ed. 2, 1979).
 14. J. H. LaVail and M. M. LaVail, *J. Comp. Neurol.* 157, 303 (1975).
 15. T. Hokfelt, L. Terenius, H. G. J. M. Kuypers, O. Dann, *Neurosci. Lett.* 14, 55 (1979); R. M. Bowker, H. W. M. Steinbusch, J. D. Coulter, *Brain Res.* 211, 412 (1981).
 16. J. H. Neale, J. L. Barker, G. R. Uhl, S. H. Snyder, *Science* 201, 467 (1978).
 17. I thank J. Coffield and E. Humphrey for their expert technical assistance; L. Ostby for light microscopic photography; M. Abdelmoumene, G. Bennett, P. J. P. Brickfield, R. Dubner, S. Gobel, and M. Hoffert for reading the manuscript; and E. Welty for typing it.

29 June 1981; revised 24 August 1981

The Origin of Man: A Chromosomal Pictorial Legacy

Abstract. *Man, gorilla, and chimpanzee likely shared an ancestor in whom the fine genetic organization of chromosomes was similar to that of present man. A comparative analysis of high-resolution chromosomes from orangutan, gorilla, chimpanzee, and man suggests that 18 of 23 pairs of chromosomes of modern man are virtually identical to those of our "common hominoid ancestor," with the remaining pairs slightly different. From this lineage, gorilla separated first, and three major chromosomal rearrangements presumably occurred in a progenitor of chimpanzee and man before the final divergence of these two species. A precursor of the hominoid ancestor and orangutan is also assumed.*

Comparisons of banded metaphase chromosomes (320 to 500 bands per haploid set) of man, chimpanzee, gorilla, and orangutan have revealed a general homology of chromosomal bands in the four species and suggested a common ancestor for chimpanzee, gorilla, and man (1, 2). Using high-resolution G-banded chromosomes from late prophase (1000 bands per haploid set) (3), we can now account for every nonheterochromatic G-positive and G-negative band in the four primates. Furthermore, by comparing chromosomes of humans, apes, and some Old World monkeys, we have been able to work backward in evolution to suggest likely karyotypes for three presumed common ancestors of apes and man. This study was based on the remarkable similarity of chromosomes of man, chimpanzee, gorilla, and orangutan, the few changes needed to explain their differences, and the use of ancestral chromosomal patterns to derive the general sequence of events that might have taken place in primate evolution prior to man's emergence. Such an approach suggests (i) the existence of a precursor to orangutan and a hominoid ancestor of gorilla, chimpanzee, and man; (ii) the emergence of the hominoid ancestor; and (iii) the existence of a progenitor of chimpanzee and man after the divergence of gorilla.

Cultured lymphocytes from two male and three female orangutans (*Pongo pyg-*

maeus), one male and four female gorillas (*Gorilla gorilla*), four male and five female chimpanzees (*Pan troglodytes*), and ten women and 21 men (*Homo sapiens*) were examined by use of a high-resolution chromosome-methotrexate cell synchronization technique (3). To test for equivalence between chromosomes and bands in the four species, we photographed 20 relatively straight, G-banded, late-prophase examples of each chromosome from the four species ($\times 1600$). The photographs were enlarged twice and matched side by side for a detailed analysis of reproducibility of banding patterns, band thickness, and staining intensity (Fig. 1). Additional chromosome preparations were stained with the C-banding technique (4) to determine to what extent the banding patterns observed were related to heterochromatin. Descriptions were simplified by ascribing the new international human high-resolution chromosome nomenclature (5) to the chromosomes of the great apes. Occasionally, when a question arose regarding chromosomal ancestry of the four species, individual chromosomes from more primitive species were studied. These included two male and two female rhesus monkeys (*Macaca mulatta*) and one male baboon (*Papio papio*). The results illustrate a remarkable similarity in the banding patterns of most chromosomes. Except for differences in nongenic constitutive het-

erochromatin (6), chromosomes 6, 13, 19, 21, 22, and X appear to be identical in all four species; chromosomes 3, 11, 14, 15, 18, 20, and Y look the same in three species; and chromosomes 1, 2p, 2q (7), 5, 7 to 10, 12, and 16 are alike in two species (Figs. 1 and 2).

Most chromosomal differences in the four species consist of inversions of chromosomal segments and variations in constitutive heterochromatin. The most common inversions are of the pericentric type, although a few are paracentric. Chromosomes 4, 5, 9, 12, 15, and 16 of man and chimpanzee differ by a pericentric inversion, whereas chromosome 7 of chimpanzee and gorilla differ by a paracentric inversion. Occasionally, both peri- and paracentric inversions appear to be involved, as shown by comparisons of chromosome 16 of man and gorilla and chromosomes 3 and 17 of man and orangutan.

Differences in constitutive heterochromatin among the four species are caused by (i) variations in amount for the centromeric and paracentromeric regions (particularly in chromosomes 1, 9, 16, and the short arm of acrocentric chromosomes 13 to 15, 21, and 22) (6); (ii) the presence of intercalary bands in chimpanzee (added to subbands 7q22.2 and 13q14.2) and orangutan (additional to the distal end of band 4q12); and (iii) differences in size of the Y chromosome, which tend to obscure the basic homology of the nonheterochromatic segment (p11.32q11.23) in the four species (Fig. 2). In addition, telomeric or terminal bands are found in approximately half of all the chromosome arms from chimpanzee and in nearly all of those from gorilla, but they are conspicuously absent in chromosomes from man and orangutan (Fig. 2). Polymorphic variations of heterochromatin were commonly found in the four species and were particularly dramatic in the telomere of the short arm of gorilla chromosomes 2q, 13 to 15, and 18.

In addition to inversions and variations in heterochromatin, a few chromosomes of the four species showed reciprocal translocation (5; 17 in gorilla), band insertion (terminal band 20p13 on centromeric band 8q11.2 in orangutan), differences in the number and position of nucleolar organizers (8), and telomeric fusion (chromosomes 2p and 2q, with inactivation of the 2q centromere in man) (1, 2). Humans have nucleolar organizers on chromosomes 13 to 15, 21, and 22; in chimpanzee, they are on chromosomes 13, 14, 18, 21, and 22; in gorilla on chromosomes 13, 21, 22; and in orang-

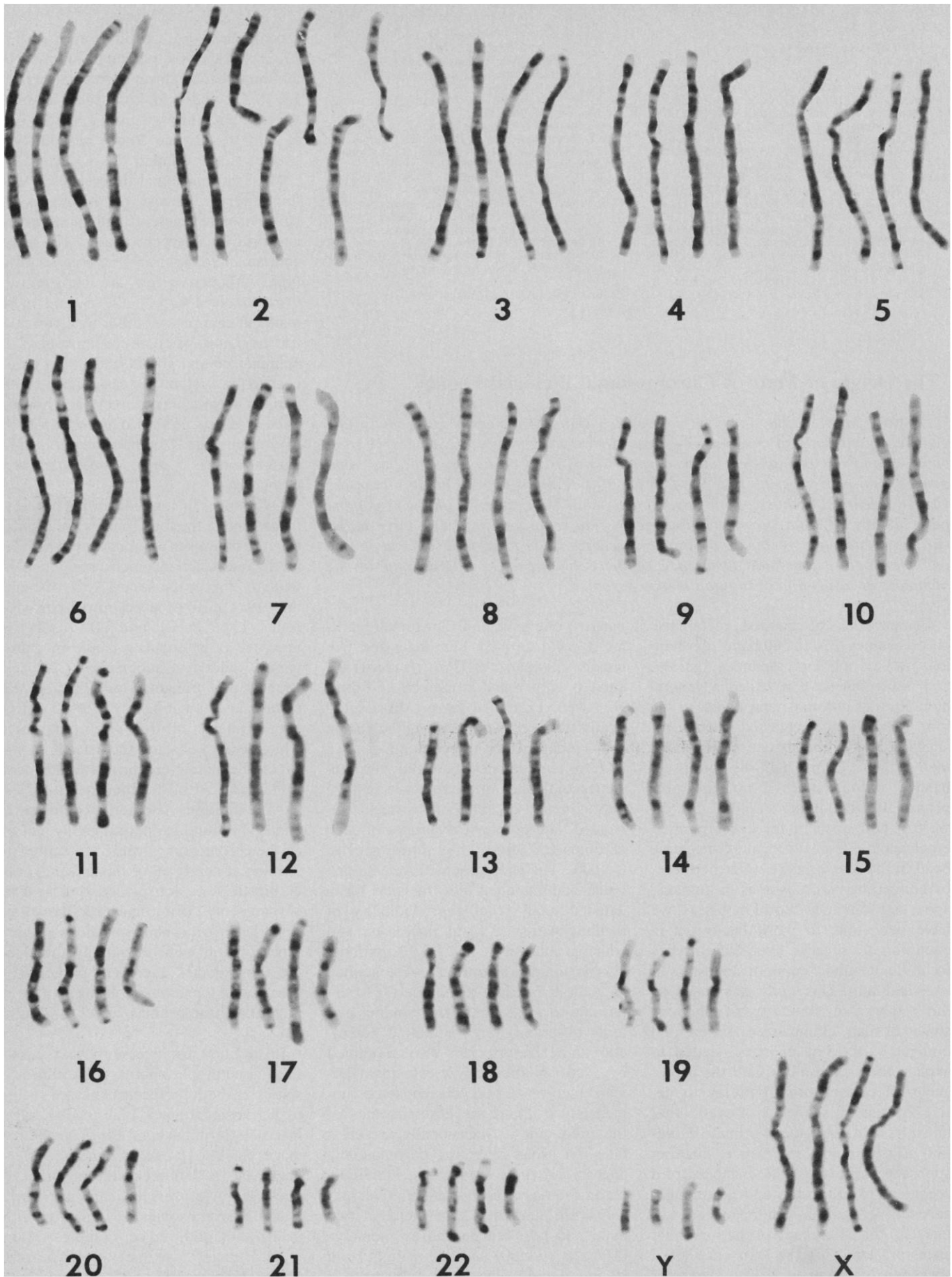


Fig. 1. G-banded late-prophase chromosomes (1000-band stage) from man, chimpanzee, gorilla, and orangutan, arranged from left to right, respectively, to better visualize the extensive homology that exists among them. Heterochromatin is variable and particularly abundant in the paracentromeric region of human chromosomes 1, 9, and 16, the telomeres of chimpanzee and gorilla, the short arm of chromosomes 13 to 15 of gorilla, and the Y chromosome.

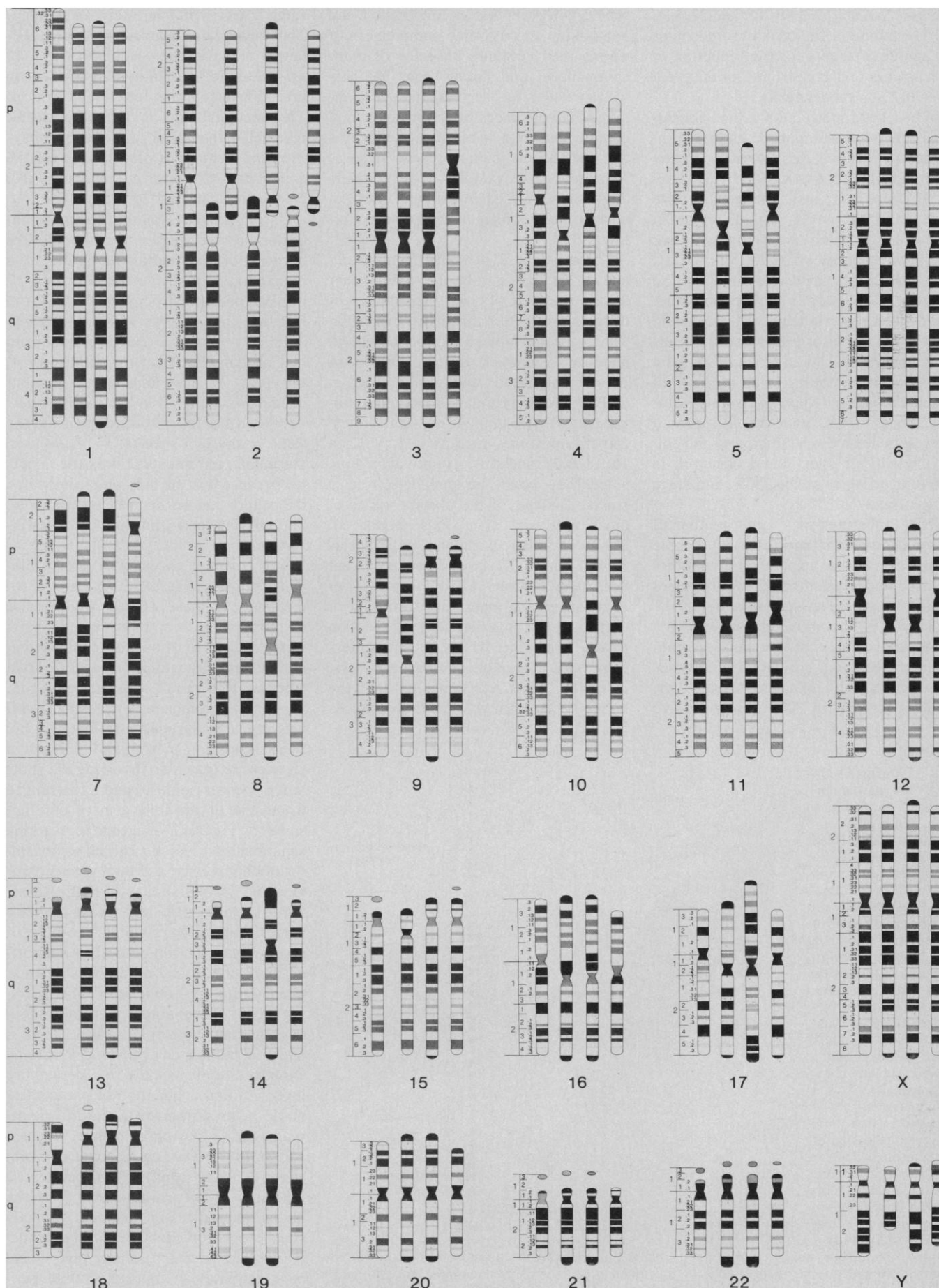


Fig. 2. Schematic representation of late-prophase chromosomes (1000-band stage) of man, chimpanzee, gorilla, and orangutan, arranged from left to right, respectively, to better visualize homology between the chromosomes of the great apes and the human complement.

utan on 2p, 2q, 7, 9, 13 to 15, and 22 (Fig. 2). The telomeric fusion of chromosomes 2p and 2q accounts for the reduction of the 24 pairs of chromosomes of the great apes to 23 in modern man.

When heterochromatin is not considered, man and chimpanzee have 13 presumably identical chromosome pairs (chromosomes 3, 6 to 8, 10, 11, 13, 14, 19 to 22, and XY); man and gorilla have nine (chromosomes 3, 6, 11, 13, 19 to 22, and XY); and man and orangutan have eight (chromosomes 5, 6, 12 to 14, 19, 21, and 22). Furthermore, reversal of the somewhat numerous inversions that have taken place in the four species and the few instances of translocation, insertion, and fusion, would result in virtually 100 percent homology of all of the non-heterochromatic G-positive and G-negative bands. This homology includes a correspondence in the thickness and color intensity of every band observed in the four primates at the 1000-band stage (Figs. 1 and 2).

The similarities of a large number of chromosomes and the relatively simple steps needed to explain the chromosomal differences among the four species make it possible to speculate about the existence of common ancestors of the great apes and man. Because human, chimpanzee, and gorilla chromosomes are very closely related to each other, and the orangutan is a more primitive

species (6), we first reconstructed the most likely chromosome complement of a presumed common ancestor of man, chimpanzee, and gorilla (Fig. 3). This was arrived at by assigning to our "common hominoid ancestor" a chromosomal complement that most easily explains the ones found in the present species. Representative examples of the comparative analysis used in deriving such an ancestral chromosomal complement follow.

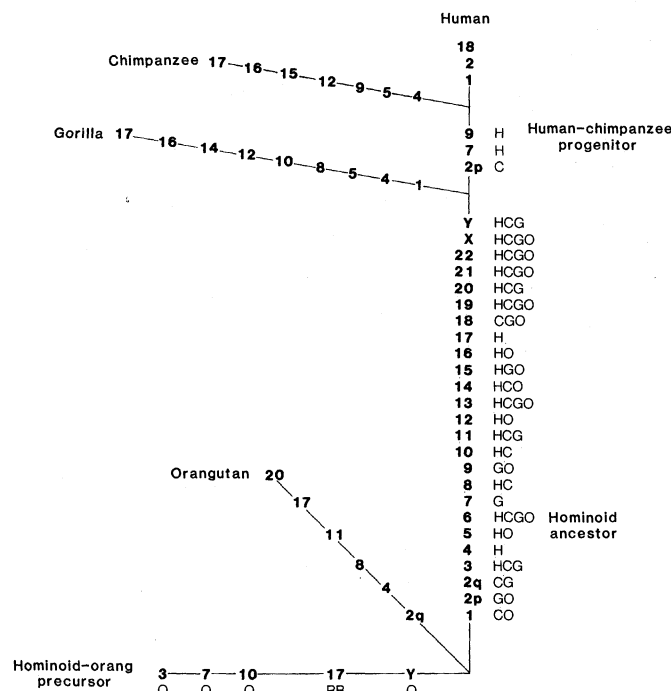
Chromosome 3 of man, chimpanzee, and gorilla appear to be the same except for the presence of a very small amount of telomeric heterochromatin in gorilla. Thus, chromosome 3 of the hominoid ancestor resembled chromosome 3 of man, chimpanzee, and gorilla (Fig. 3) and more closely that of man and chimpanzee (9). Assignment of other ancestral chromosomes such as 1, 5, 6, 12 to 16, 18 to 22, and X was relatively simple since they could be deduced from a similar analysis of the chromosomes of great apes and man (Figs. 2 and 3). However, other chromosomes, such as 2, 4, 7, and 17, show differences that need explanation. Chromosome 7 of chimpanzee and man differ from that of gorilla mainly by a paracentric inversion of the long arm, which in turn differs from that of orangutan by a pericentric inversion. Thus, chromosome 7 of the hominoid ancestor was like that of gorilla

since it represents an intermediate step. The ancestral chromosome 2p is believed to have been similar to that of orangutan and gorilla, with a pericentric inversion accounting for chimpanzee 2p. The ancestral 2q, on the other hand, resembled that of gorilla and chimpanzee, and human chromosome 2 can be explained by fusion of a chimpanzee-like 2p and the ancestral 2q (Fig. 2).

Chromosome 17 of the hominoid ancestor was ascertained to be human-like because of the finding in gorilla of a human-like long arm of chromosome 17 translocated to chromosome 5 and the fact that chromosome 17 in chimpanzee differs by a pericentric inversion from that of man (Fig. 2). In chromosome 4, the bulk of the long arm (segment q22qter) is identical in the four species, but the rest of the chromosome is not the same in any two species. We have used the human chromosome 4 as the ancestor because it is the only one from which the others can be derived by a simple, but differing, pericentric inversion or insertion (Fig. 2).

The common ancestor of man, chimpanzee, and gorilla had 24 pairs of chromosomes. Of these, 18 pairs were similar to those of present man, and 15 pairs were similar to those of chimpanzee, gorilla, and orangutan (Fig. 3). Gorilla emerged as a result of a pericentric inversion in chromosomes 4, 8, 10, 12, 14, and 16, a reciprocal translocation between chromosomes 5 and 17, and a paracentric inversion involving the short arm of chromosome 16 and a rearranged distal end of the long arm of chromosome 1. Figure 3 suggests that gorilla separated first and left behind a progenitor of chimpanzee and man whose chromosome 2p was similar to that of present-day chimpanzee and whose chromosomes 7 and 9 were similar to those of modern man. This hypothesis is supported by chromosomal differences best explained through "obligatory" intermediary steps. For example, chromosome 9 of orangutan and gorilla are acrocentrics, and the human chromosome 9 can be explained more simply by pericentric inversion and acquisition of paracentromeric heterochromatin from an original orang-gorilla-like chromosome (Figs. 1 and 2). In contrast, chimpanzee chromosome 9 likely arose by a pericentric inversion of the chromosome 9 of the human-like ancestor and not directly from the chromosome 9 of the orang-gorilla-like ancestor, since this would have required an unusual double pericentric inversion, with two of four break-points being the same as those giving rise to the human chromosome 9. Man and

Fig. 3. Presumed ancestors of man, chimpanzee, gorilla, and orangutan. In the middle are illustrated the chromosomes of a presumed hominoid ancestor whose chromosomes were largely similar to those of human (H) and to a great extent to chimpanzee (C), gorilla (G), and orangutan (O). Before the divergence of man and chimpanzee took place, their ancestor shared similar chromosomes 2p, 7, and 9 (human-chimpanzee ancestor). Man then diverged by fusion of chromosomes 2p and 2q into chromosome 2 and by a small pericentric inversion in chromosomes 1 and 18. By contrast, seven and nine major nonheterochromatic changes in individual chromosomes occurred in chimpanzee and gorilla, respectively. A presumed precursor of the hominoid ancestor and orangutan had the same chromosomes as the hominoid ancestor except for chromosomes 3, 7, 10, and Y, which were similar to those of orangutan, and chromosome 17, which was like that of rhesus (R) and baboon (B). From this precursor, orangutan diverged with changes in chromosomes 2q, 4, 8, 11, 17, and 20.



chimpanzee have basically the same chromosome 7, whereas the gorilla-like ancestral chromosome differs from them by a paracentric inversion. Evidence for a common ancestor of man and chimpanzee also comes from chromosome 2, since human chromosome 2 is most simply explained by telomeric fusion of a chimpanzee-like 2p chromosome and a 2q chromosome similar to that of chimpanzee and gorilla (Figs. 1 and 2). These findings on chromosomes 2, 7, and 9 together suggest a common ancestor of human and chimpanzee. From this forefather, man emerged after the formation of a small pericentric inversion in chromosomes 1 and 18 and the fusion of chromosomes 2p and 2q to form the characteristic human chromosome 2. For present-day chimpanzee to appear, however, seven major nonheterochromatic changes had to occur (Fig. 3).

Once chromosome complements had been deduced for common ancestors of man and chimpanzee, and of man, chimpanzee, and gorilla, it became possible to determine the likely chromosome complement of a presumed progenitor of our hominoid ancestor and orangutan. This was accomplished by comparison of chromosomes of the hominoid ancestor with those of orangutan and related primitive apes (baboon and rhesus). As deduced from Figs. 2 and 3, such a progenitor of orangutan and our hominoid ancestor had 24 chromosome pairs, 14½ of which were like those of our hominoid ancestor and orangutan (chromosomes 1, 2p, 5, 6, 9, 12 to 16, 18, 19, 21, 22, and X). Three and one-half of the remaining 9½ were the same as those of present-day orangutan (chromosomes 3, 7, 10, and Y); five were like those of our hominoid ancestor (chromosomes 2q, 4, 8, 11, and 20); and one was like that of rhesus and baboon (chromosome 17) (Figs. 2 and 3). Chromosomes 3, 7, 10, and Y were considered orangutan-like and 2q and 8 as hominoid-like, since they were the same in the more primitive rhesus and baboon, except that the centromere of orangutan chromosome 3 is at 3p21.33 and those of rhesus and baboon are at 3q23. Chromosome 17 in the orangutan differs from the human-like hominoid chromosome 17 by a pericentric and a paracentric inversion. Since baboon and rhesus have a chromosome 17 with the same long arm inversion as that of orangutan, and the rest of the chromosome is like that of man, the progenitor was assigned a rhesus-baboon-like chromosome 17. Chromosome 11 of orangutan has no known counterpart in other primates. However, since chromosome 11 of man, chimpanzee,

and gorilla are similar, and since they differ from those of rhesus and baboon by a simple pericentric inversion (p11.2q13.5), the orangutan chromosome 11 is believed to have arisen after speciation through a large pericentric inversion, followed by translocation of the centromeric region. Finally, chromosome 20 of orangutan probably arose after speciation by a paracentric inversion in the long arm and insertion of the terminal band p13 into q11.21 of chromosome 8.

Earlier work with metaphase chromosomes had shown a basic similarity among New and Old World monkeys, apes, and man, making it possible to construct a chromosomal phylogeny from prosimians to man (10). Although the karyotype of the ancestor to man, chimpanzee, and gorilla had been deduced (2), several uncertainties remained. The hominoid ancestral chromosome 1 had been classified as being the same in the four species instead of being like that of chimpanzee and orangutan because with contracted chromosomes it had not been possible to determine a rearrangement of part of band q42 at the telomeric end of the long arm of the gorilla chromosome or to note a small pericentric inversion in that of man. Chromosome 4 had been classified as being like that of gorilla and orangutan, when indeed it is different in all four species. Chromosome 8 had been classified as being like that of man, chimpanzee, and orangutan rather than like that of man and chimpanzee, since an extra large G-negative band near the centromeric region of the long arm of orangutan chromosome 8 was not detected. Finally, the Y chromosome was believed to differ in each species, and no homology could be found. Yet when finely banded chromosomes are used and only the noncentromeric and nontelomeric heterochromatic regions of the Y chromosome are considered (segment p11.32q11.23), a basic homology is observed in man, chimpanzee, and gorilla (Figs. 1 and 2). The orangutan chromosome Y can then be explained as possibly differing by a pericentric inversion (p11.2q11.23).

It was not possible earlier to derive a complete ancestral karyotype of the progenitor of the hominoid ancestor and orangutan because of technical difficulties in tracing evolutionary changes that occurred in chromosomes 3, 4, 8, 11, 17, 20, and Y (2, 9). Also, because of apparent similarities in chromosomes 12 and 16 and in telomeric heterochromatin of chimpanzee and gorilla, no ancestor of man and chimpanzee was considered (2,

9), and a hybrid zone was believed to have existed for gorilla and chimpanzee (2). Our refined banding technique shows that chromosomes 12 and 16 of chimpanzee and gorilla were formed by different inversions (breakpoints in bands p11.23 and q14.2 in gorilla and p12.2 and q15 in chimpanzee), and there are differences in amount, color intensity, and location of telomeric heterochromatin in the two species (Figs. 1 and 2).

With the use of high-resolution chromosome technology, we can account for every nonheterochromatic band of the three great apes and man and have a more precise delineation of the structural chromosomal rearrangements found in the four species. The 100 percent homology of nonheterochromatic bands of man and the great apes is not surprising, since more than 50 genes have been located on homologous chromosomes and chromosome bands of the four species (11).

Studies of DNA reassociation kinetics (12), protein structure and antigenicity (13), and histocompatibility antigens and blood groups (14) all indicate that chimpanzee, gorilla, and man share a substantial common ancestry and that orangutan diverged earlier from this lineage. Furthermore, almost total homology of single-copy DNA (12) and amino acid sequence of proteins (15) has been found in man and chimpanzee, suggesting a very close evolutionary relationship between the two. These findings conflict with the view, based primarily on anatomic and behavioral data, that man (placed in Hominidae family) diverged separately from the evolutionary line leading to the great apes (placed in Pongidae family) (16). Our detailed comparative analysis of high-resolution chromosomes supports molecular evidence that the great apes and man belong to the Hominidae family, which separates into the Ponginae (orangutan) and Homininae (gorilla, chimpanzee, and man) subfamilies (16). It also provides evidence in favor of the existence of three ancestors to the great apes and man from which first orangutan, then gorilla, and finally chimpanzee and man diverged.

JORGE J. YUNIS

OM PRAKASH

*Department of Laboratory Medicine
and Pathology, University of
Minnesota Medical School,
Minneapolis 55455*

References and Notes

1. J. de Grouchy *et al.*, *Ann. Genet.* **15**, 79 (1972); B. Dutrillaux, M. O. Rethoré, J. Lejeune, *ibid.* **18**, 153 (1975); Paris Conference (1971), supplement (1975); *Cytogenet. Cell Genet.* **15**, 201 (1975).
2. B. Dutrillaux, "Sur la nature et l'origine des chromosomes humains," *Monogr. Ann. Genet. Expansion Sci. Fr.* (1975), pp. 41-71.

3. J. J. Yunis, *Science* **191**, 1268 (1976).
4. ———, L. Roldan, W. G. Yasmin, J. C. Lee, *Nature (London)* **231**, 532 (1971).
5. J. J. Yunis, *J. Hum. Pathol.* **12**, 494 (1981).
6. ——— and W. G. Yasmin, *Science* **174**, 1200 (1971); J. R. Gosden, A. R. Mitchell, N. Seunanez II, C. M. Gosden, *Chromosoma* **63**, 253 (1977).
7. The short arm (p) and long arm (q) of human chromosome 2 are represented by two acrocentric chromosomes (2p and 2q) in the great apes.
8. R. Tantravahi, D. A. Miller, V. G. Dev, O. J. Miller, *Chromosoma* **56**, 273 (1978).
9. Since constitutive heterochromatin represents nongenic material that can be acquired after speciation and often varies from species to species (6), it was not included in the analysis illustrated in Fig. 3.
10. B. Dutrillaux, *Humangenetik* **48**, 251 (1979).
11. Human Gene Mapping 5, *Cytogenet. Cell Genet.* **25**, 82 (1979).
12. P. L. Deininger and C. W. Schmid, *Science* **194**, 846 (1976); R. E. Benveniste and G. T. Todaro, *Nature (London)* **261**, 101 (1976).
13. M. Goodman and R. E. Tashian, Eds, *Molecular Anthropology* (Plenum, New York, 1976).
14. W. W. Socha and J. Moor-Jankowski, *J. Hum. Evol.* **8**, 453 (1979); J. J. Garver, A. M. Estop, P. Meera Kahn, H. Balner, P. O. Pearson, *Cytogenet. Cell Genet.* **27**, 238 (1980).
15. M.-C. King and A. C. Wilson, *Science* **188**, 107 (1975).
16. A. L. Zihlman, J. E. Cronin, D. L. Cramer, V. M. Zarich, *Nature (London)* **275**, 744 (1978); M. Goodman, *Prog. Biophys. Mol. Biol.*, in press.
17. Supported in part by NIH grant 26800. We thank the Yerkes Primate Center of Georgia for gorilla and orangutan blood samples, K. Dunham of Ames, Iowa, for chimpanzee blood samples, and A. Beacom for reviewing the manuscript.

23 July 1981; revised 10 December 1981

Corticosterone: A Critical Factor in an Opioid Form of Stress-Induced Analgesia

Abstract. *The finding that some opioid-mediated forms of stress-induced analgesia are antagonized by hypophysectomy and dexamethasone has led to the suggestion that β -endorphin, released from the pituitary, may mediate these analgesic reactions. "Long-term analgesia" (an opioid-mediated form of stress-induced analgesia), which is blocked by dexamethasone and hypophysectomy, was also blocked by adrenalectomy and reinstated with corticosterone therapy. Corticosterone is proposed to play a permissive role in long-term analgesia and to be a critical hormone mediating this phenomenon.*

Exposure to a variety of stressors produces a subsequent decrease in pain responsiveness (1). This stress-induced analgesia has received considerable recent attention, largely because of its potential for providing insight into a possible functional role for endogenous opioids in behavioral and adaptive phenomena. The brain has pain-inhibiting systems in which endogenous opioids may play a role (2). The phenomenon of stress-induced analgesia suggested that endogenous opioids might be released by stress, thereby inhibiting pain and perhaps protecting the organism in some way (3). More recent work, however, has suggested that some forms of stress-induced analgesia are mediated by opioid systems [animals develop a cross-tolerance between the analgesic effects of morphine and the stress, and the analgesia can be reversed by opiate antagonists (4)], whereas others are mediated by nonopioid mechanisms (5).

Since multiple opioid systems exist in both the brain and the pituitary (6), recent research has been directed at determining which system mediates the opioid form of stress-induced analgesia. The discovery that hypophysectomy (removal of the pituitary) reduces an opiate-mediated stress-induced analgesia has supported the proposal that pituitary β -endorphin may mediate this type of analgesia (1). In further support of this view, the synthetic glucocorticoid dexametha-

sone has been shown to block both the stress-induced rise in plasma β -endorphin (7) and opioid stress-induced analgesia (5).

These and other findings led Baizman *et al.* (8) and Lewis *et al.* (5) to propose that pituitary β -endorphin might be mobilized by stress transported to the brain by retrograde flow through the portal system, and thus decrease responsiveness to painful stimuli by interacting with central structures. Such an action by pituitary β -endorphin is possible, but difficult to reconcile with reports that the hypothalamic content of β -endorphin decreases rather than increases after 30 minutes of footshock, and that even large doses of intravenous β -endorphin have little effect on pain responsiveness (9). Both hypophysectomy and dexamethasone treatment either eliminate or reduce the stress-induced release of pituitary adrenocorticotrophic hormone (ACTH) as well as β -endorphin (7). This fact is noteworthy because corticosterone is under anterior lobe ACTH regulation, and corticosterone affects central processes associated with pain inhibition (10, 11). This line of reasoning suggests that the manipulations which seem to implicate pituitary β -endorphin may actually produce their effects by altering pituitary-adrenocortical interaction.

The purpose of our studies was to examine the role of the pituitary-adrenal axis in the production of the opioid form

of stress-induced analgesia. A number of different procedures result in an opioid stress-induced analgesia. We used a procedure in which subjects are not tested until 24 hours after the stress session, but the pain responsiveness test is preceded by a brief reinstatement procedure in which the subject is again exposed to the stressor (4). This allows for the dissipation of nonspecific factors such as fatigue and local anesthetic effects and may result in a "purer" opioid form of analgesia. The long-term effect is completely reversed by opiate antagonists and completely cross-tolerant with morphine, whereas these outcomes are not always complete under short-term testing soon after the stress session, even if the stress session is prolonged (5).

We first examined the effects of hypophysectomy on long-term analgesia. Eight hypophysectomized rats and eight that had been subjected to sham surgery (12) were restrained and treated with 80 5-second 1-mA shocks delivered through fixed tail electrodes on the average of one per minute. Eight more rats of each type were restrained for an equivalent period, but not shocked. Twenty-four hours later all rats received a shock reexposure procedure which consisted of five single-crossing shuttlebox escape trials. Immediately afterward, subjects were given three analgesia test trials (at 4-minute intervals) in a tail-flick apparatus in which latency to flick the tail from radiant heat served as the measure of pain sensitivity. These procedures are described elsewhere (4). Hypophysectomy completely blocked the long-term analgesia effect (Fig. 1A). A 2 by 2 analysis of variance confirmed this conclusion by showing significant shock [$F(1, 28) = 13.24, P < .01$] and hypophysectomy [$F(1, 28) = 12.13, P < .01$] main effects, and, most important, a significant interaction of hypophysectomy with shock [$F(1, 28) = 10.66, P < .01$]. Newman-Keuls individual group comparisons ($\alpha = .05$) indicated that the inescapably shocked sham rats were more analgesic than the other three groups.

In a second experiment, a 2 by 4 factorial design was used with rats restrained or inescapably shocked 2 hours after receiving an intraperitoneal injection of dexamethasone [0 mg per kilogram of body weight (saline), 0.25 mg/kg, 0.5 mg/kg, or 1.0 mg/kg]. As in the first experiment, 24 hours later all rats were given five shuttlebox trials and three analgesia test trials.

Dexamethasone prevented the inescapable shock-induced analgesia (Fig. 1B), with blockage complete at 0.5 and 1.0 mg/kg. Newman-Keuls individual