Table 1. Cosynthetase activities (expressed in units per milligram of protein) in extracts of organs from four fox squirrels and four gray squirrels. The values for gray squirrels are corrected for the activity due to sequestered blood.

	Fox squirrels				Gray squirrels			
	1	2	3	4	1	2	3	4
Heart	< 1.0	0.8	1.0	0.6	2.8	4.5	5.0	3.9
Liver	< 0.5	0.5	< 0.5	< 0.5	2.7	2.2	4.0	2.6
Spleen	1.0	< 0.5	< 0.5	< 0.5	15.5	10.9	4.8	3.8
Kidney	< 0.6	1.8	< 0.8	2.6	1.2	3.5	3.2	0.8

respectively, and one fox squirrel that had a count of 8.0 percent. Two clinical hematologists were not able to distinguish blood samples of the fox squirrel from those of the gray squirrel by microscopic examination of coded smears stained by Wright's method.

Even though overproduction of uroporphyrinogen I in human congenital porphyria seems to occur only in erythropoietic tissue (*6*), the genetically determined defect in cosynthetase activity extends to other tissues as well (*7*). To test whether cosynthetase activity is also low in nonerythropoietic tissues of the fox squirrel, four of these animals and four gray squirrels were exsanguinated under methoxyfluorane anesthesia, and the heart, kidneys, liver, and spleen were removed, washed, and homogenized separately in three volumes of 0.05*M* potassium phosphate buffer, *p*H 7.9. Crude extracts prepared by centrifuging the homogenates for 45 minutes at 15,000*g* at 4°C were assayed for cosynthetase activity by the same method used for the hemolyzates. As with blood, it was difficult to measure cosynthetase activity of most fox squirrel tissues with the maximum amount of extract that could practicably be added to the reaction mixture. Table 1 shows that in all the extracts from fox squirrel organs, except for two from kidneys, cosynthetase activity was present at a concentration of 1.0 unit per milligram of protein or less. On the other hand, cosynthetase activity was easily demonstrated in extracts of all 16 organs from gray squirrels. Although some of this activity in tissues from gray squirrels was due to the presence of residual blood pooled in the organs at the time of homogenization, a correction could be made for this on the basis of a hemoglobin determination carried out on the extract and the measured activity in blood from the animal. The data for gray squirrels shown in Table 1 have been corrected for this activity due to sequestered blood; the magnitude of the correction ranged from 7 percent of the total activity (spleen from squirrel No. 2) to 53 percent of the total activity (spleen from squirrel No. 4). The cosynthetase activities in organ extracts from gray squirrels are comparable to those previously measured in extracts of mouse organs (*4*). The results indicate that the low activity of cosynthetase in fox squirrels extends to nonerythropoietic tissue; the presence of relatively high activities in kidney tissue from some fox squirrels warrants further investigation.

Fox squirrels may be assumed to use a synthetase-cosynthetase system for uroporphyrinogen III biosynthesis, since this mechanism is common to bacteria and green plants, and to other mammals. A complete absence of activity of uroporphyrinogen III cosynthetase would not be expected, since uroporphyrinogen III is an essential intermediate in heme biosynthesis. Evidently the residual activity of the enzyme suffices for the needs of the animals for protoporphyrin IX. Nevertheless, the evidence that cosynthetase activity is much less in blood and tissue extracts from *S. niger* than it is in similar extracts from the closely related *S. carolinensis* suggests that a partial deficiency of this enzyme accounts for the formation of uroporphyrin I by the former species. Since the enzyme defect in the heme biosynthetic pathway is the same in the fox squirrel as in the porphyric cattle and human beings, the fox squirrel provides an inexpensive, widely available, easily maintained laboratory model for experimental studies of the cosynthetase deficiency of congenital erythropoietic porphyria.

EPHRAIM Y. LEVIN
Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
VAGN FLYGER
Natural Resources Institute, University of Maryland, College Park 20742

References and Notes

1. W. J. Turner, *J. Biol. Chem.* **118**, 519 (1937).
2. R. Schmid, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, Eds. (McGraw-Hill, ed. 2, New York, 1966), p. 813.
3. E. Y. Levin, *Science* **161**, 907 (1968); G. Romeo and E. Y. Levin, *Proc. Nat. Acad. Sci. U.S.* **63**, 856 (1969).
4. E. Y. Levin, *Biochemistry* **7**, 3781 (1968).
5. G. Romeo, B. L. Glenn, E. Y. Levin, *Biochem. Genet.* **4**, 719 (1970).
6. A. Neuberger, H. M. Muir, C. H. Gray, *Nature* **165**, 948 (1950).
7. G. Romeo, M. M. Kaback, E. Y. Levin, *Biochem. Genet.* **4**, 659 (1970).
8. We acknowledge the priority of Dr. W. J. Turner in proposing this experimental model for congenital porphyria, and thank him for access to his notebooks and correspondence. Drs. W. H. Zinkham and J. D. Dickerman examined the blood smears. Mrs. P. J. Hathaway provided technical assistance. Supported in part by grant NB-05367 from NIH.

15 April 1971; revised 1 June 1971

Linkage Groups II and XII of the Mouse: Cytological Localization by Fluorochrome Staining

Abstract. *The* T(2;12) 163H *translocation involves two chromosomes which carry the two groups of genes known as linkage groups II and XII. Staining with quinacrine mustard reveals that these chromosomes are numbers 10 and 19 of the fluorescent karyotype of the normal mouse. Linkage group II is assigned to chromosome 10, linkage group XII to chromosome 19.*

Until recently it has been generally impossible to identify individual mitotic metaphase chromosomes of the mouse because of their lack of distinguishing morphological characteristics. However, the technique of staining chromosomes with quinacrine mustard and related compounds (*1*) has recently been applied to the mouse and has permitted specific identification of all chromosome pairs in the mouse complement (*2*). With the identification of the X chromosome (*2*), the first assignment of a linkage group (XX) to a specific chromosome of the fluorescent karyotype was made. We have applied quinacrine mustard staining to metaphase material from mice homozygous or heterozygous for the translocation designated *T(2;12)163H* and have identified the chromosomes involved with specific chromosomes of the fluorescent karyotype of the normal mouse (*2*). We are now able to assign linkage groups II and XII to specific metaphase pairs.

The cytological localization of all linkage groups should now be possible through the use of appropriate trans-

locations. In the *T163H* translocation, a submetacentric marker chromosome has been formed from two of the normal mouse telocentrics, apparently by a mechanism of centric fusion. The chromosomes involved in the translocation are known to be the smallest autosome and a medium-sized autosome (*3*). Linkage group II is carried on the medium-sized chromosome, since it is also present in another translocation, *T(2;9)138Ca*, which does not include the smallest autosome pair (*4*). Linkage group XII is, therefore, on the smallest pair (*5*).

Female *T163H* homozygotes were mated either to male homozygotes or to chromosomally normal males and were killed on about the fourteenth day of pregnancy. The fetuses were minced and cultured in Eagle's minimum essential medium supplemented with 10 percent fetal calf serum, nonessential amino acids, and antibiotics.

For chromosome preparation, cultures were harvested by treatment with trypsin after exposure for 14 hours to $5 \times 10^{-8}M$ colchicine. The cells were exposed for 20 minutes at 37°C to 0.7 percent sodium citrate, then fixed three times in a mixture of absolute methanol and glacial acetic acid (3 : 1). Air-dried slides were then prepared. Slides were stained with quinacrine mustard as described by Caspersson *et al.* (*6*). A Zeiss standard fluorescence microscope was used with the exciter filter BG 12 and the barrier filter combination 53 and 44. Suitable metaphases were photographed through a 100× planapochromat objective with an iris. Kodak Tri-X 35-mm film was used and developed in Microdol X. Prints were made on Agfa-Gevaert paper No. 4. Karyotypes were prepared for *T163H* homozygotes and heterozygotes, and Polaroid transparencies were made of the translocated chromosomes. The transparencies were scanned on a Joyce-Loebl microdensitometer to obtain fluorescence distribution profiles (*2*).

All examined cells from *T163H* homozygotes contained 38 chromosomes, including a pair of large submetacentrics, each consisting of a chromosome No. 10 and a No. 19 (the smallest autosomal pair) of the normal fluorescent karyotype (Fig. 1A) fused at their centromeric ends. Cells from translocation heterozygotes all had 39 chromosomes with one large submetacentric and one member each of pairs No. 10 and No. 19. A karyotype prepared from a *T163H* heterozygous cell is shown in Fig. 1B.

Pair No. 10 of the normal karyotype shows similarities in its fluorescence pattern to pairs No. 13 and No. 14, but it can be distinguished from them by the fact that No. 13 has a darker distal region and a narrower proximal bright region, whereas No. 14 has its medial dark band displaced somewhat more distally and has the brightest distal region. The narrower dark band transecting the distal bright region is less pronounced in No. 14 than in No. 10. (Compare these pairs to the long arms of the translocated chromosomes in Fig. 1.) Distribution profiles of fluorescent intensity for the translocation chromosome and for normal No. 10's and No. 19's are shown in Fig. 2.

Since many translocations are available in the mouse, which taken together involve almost all known linkage groups (*7*), it should now be

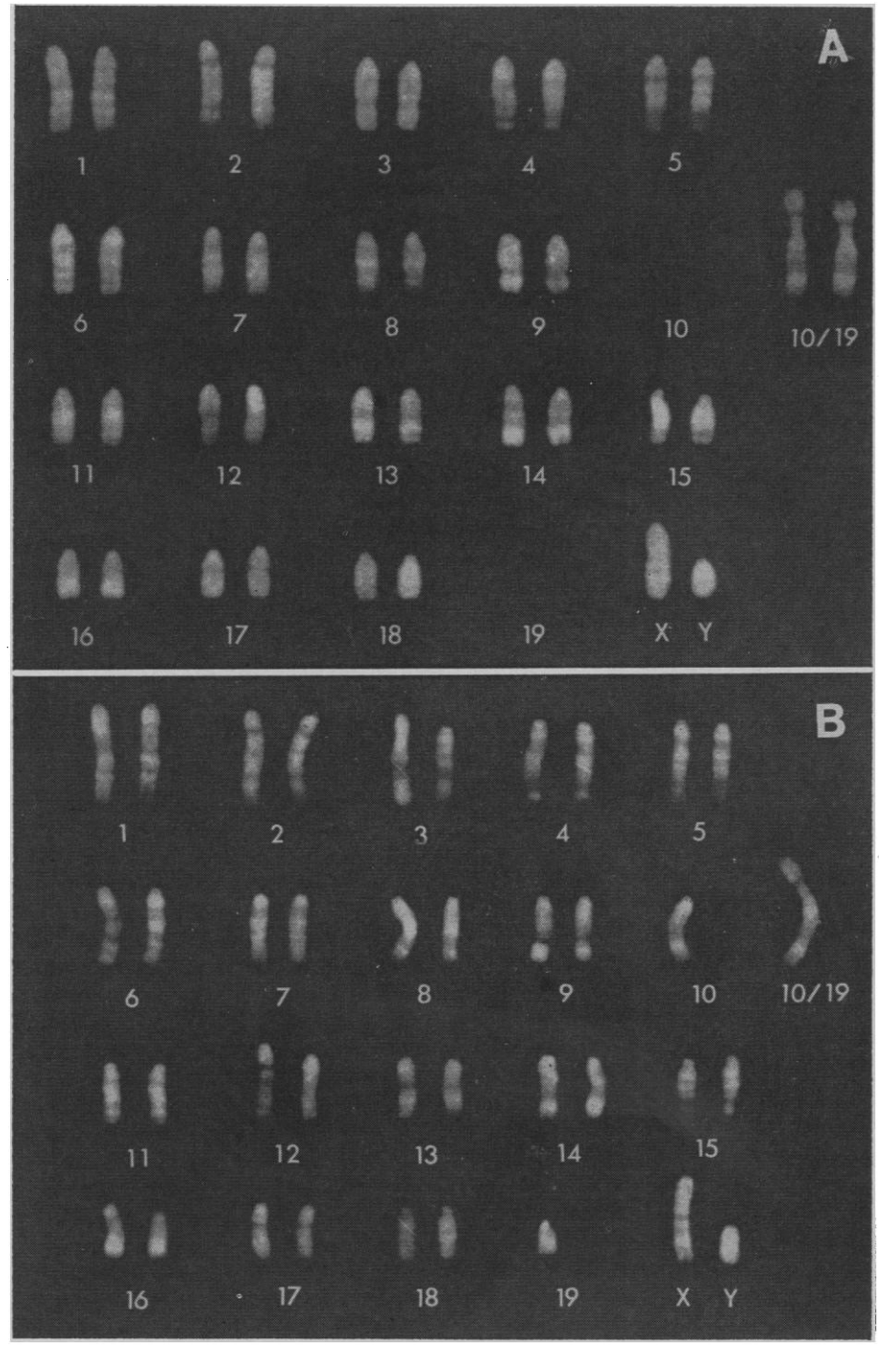

Fig. 1. Karyotypes prepared for cells from mice carrying the *T163H* translocation. (A) Homozygous cell; note the two large submetacentric chromosomes and the lack of pairs No. 10 and No. 19. (B) Heterozygous cell; note the single submetacentric and the presence of one member each of pairs No. 10 and No. 19.

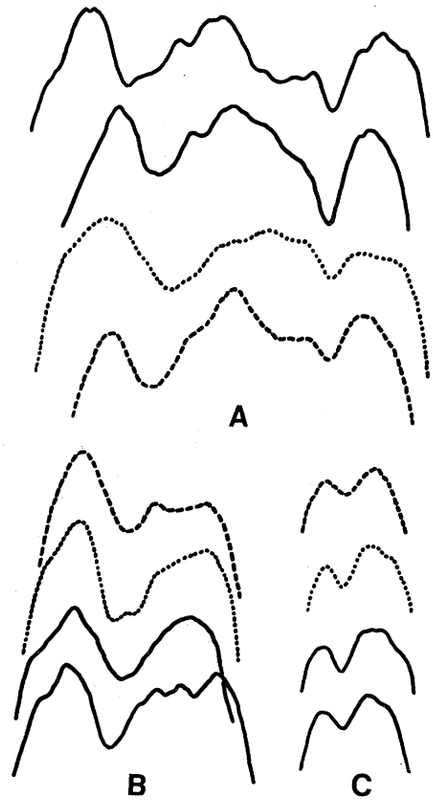

Fig. 2. Distribution profiles of fluorescent intensity for submetacentric marker chromosomes (A) and for normal No. 10's (B) and No. 19's (C). Maximums on the curves represent bright bands on the chromosomes, and minimums represent dark bands. Unbroken lines represent chromosomes from mice homozygous for the translocation (A) or from normal mice (B and C). Dotted and dashed lines represent chromosomes from two different heterozygous cells. The curves for the submetacentrics (A) have been arranged with the region corresponding to the short arms on the right. Curves for No. 10's (B) are arranged with the centromere region to the right, whereas the curves for the No. 19's (C) have the centromere regions to the left.

possible to assign each linkage group to the metaphase chromosome carrying it by applying fluorescent staining to metaphase material from stocks carrying various translocations. Other chromosomal rearrangements, such as inversions (*8*), may also be useful for this purpose. When more linkage groups have been assigned to chromosomes identified by fluorescent staining and a generally accepted standard fluorescent karyotype for the mouse has come into existence, it may prove desirable to adjust the numbering system for mouse linkage groups so that each linkage group has the same number as its chromosome, the chromosomes being numbered according to their relative length within the standard fluorescent karyotype.

MURIEL NESBITT
UTA FRANCKE

Department of Psychiatry, University of California at Los Angeles, Los Angeles 90024

References and Notes

1. T. Caspersson, L. Zech, E. J. Modest, G. E. Foley, U. Wagh, E. Simmonsson, *Exp. Cell Res.* **58**, 128 (1969).
2. U. Francke and M. Nesbitt, *Cytogenetics*, in press.
3. E. P. Evans, M. F. Lyon, M. Daglish, *ibid.* **6**, 105 (1967).
4. B. M. Slizynski, *J. Genet.* **55**, 122 (1957).
5. M. F. Lyon, *Mouse News Lett.* **40**, 26 (1969).
6. T. Caspersson, L. Zech, C. Johansson, E. J. Modest, *Chromosoma* **30**, 215 (1970).
7. T. C. Carter, M. F. Lyon, R. J. S. Phillip, *J. Genet.* **54**, 462 (1956).
8. T. H. Roderick and N. L. Hawes, *Proc. Nat. Acad. Sci. U.S.* **67**, 961 (1970).
9. Supported by California State Department of Mental Hygiene; Mental Retardation Program, Neuropsychiatric Institute, University of California at Los Angeles; and PHS grants HD-04612-02 and HD-00345-01.

1 July 1971; revised 28 July 1971

Persistent Increase in Brain Serotonin Turnover after Chronic Administration of LSD in the Rat

Abstract. *Lysergic acid diethylamide at doses of 20 micrograms per kilogram per day was administered orally to rats for 1 month. Eighteen hours after the final dose a 25 to 30 percent increase in the synthesis and turnover of serotonin was noted, as well as a moderate but significant increase in the concentration of tryptophan (18 percent) and serotonin (13 percent) in the brain.*

Several kinds of psychiatric disorders have been found among the chronic users of lysergic acid diethylamide (LSD-25), ranging from mild anxiety to panic reactions and long-lasting schizophreniform psychoses (*1*). Since the half-life of an acute dose of LSD-25 in man is reported to be very short (*2*), the behavioral alterations among the chronic users of the drug might be associated with some persistent metabolic effects of the chronic use of LSD-25.

Therefore, we have studied possible persistent effects on the cerebral metabolism of serotonin in the rat, produced by the chronic administration of LSD-25 in small doses similar to those encountered in man. Serotonin metabolism was selected as an object of study because some current hypotheses suggest that altered metabolism of indoleamines may be important in the mechanism of action of LSD-25 (*3*) and may also occur in various behavioral disorders in man (*4*).

Male Sprague-Dawley rats, weighing 150 to 160 g at the beginning of the experiment, were used. Lysergic acid diethylamide (20 μg/kg dissolved in 1.0 ml of water) was given to the rats through an oro-gastric cannula once a day at 5:00 to 7:00 p.m. for 1 month. The dose of LSD-25 was increased daily according to weight gain. Control animals received the same volume of water only. The weight gain of LSD-treated rats was 97 percent of that of the control group at the moment of death. There were no grossly evident behavioral changes in the LSD-treated animals in any period of the treatment. Sixteen to 20 hours after the last dose of LSD-25, 10 μc of uniformly labeled L-[^{3}H]tryptophan (New England Nuclear Co., 5 c/mmole) in 25 μl of a physiological saline solution were injected intracisternally (*5*) under light ether anesthesia. Fifteen minutes later the rats were decapitated. The brains were removed, weighed, and homogenized in 6.0 ml of 1.0*N* HCl in 0.5 percent (wt/vol) ascorbic acid. After centrifugation at 30,000*g* for 10 minutes, the resulting pellets were resuspended in 3.0 ml of the homogenization medium, recentrifuged, and discarded. After the addition of a drop of 2 percent (wt/vol) ethylenediaminetetraacetic acid the *p*H of the combined supernatants was adjusted to 6.9 with 1.0*N* NaOH. After centrifugation at 30,000*g* for 10 minutes, the tissue extracts were poured over Amberlite CG-50 columns at *p*H 6.1 (H^+ form) (*6*). Serotonin that adhered to the column was eluted with 3.5*N* HCl; its concentration in the eluate was measured fluorometrically (*7*). The *p*H of the material from the Amberlite columns' effluent was adjusted to 1.5 with 1.0*N* HCl, and passed through a Sephadex G-15 column (0.5 g of dry Sephadex in 0.1*N* HCl) (*6*). The effluent of the Sephadex column was then poured over a Dowex 50 column (200 to 400 mesh, H^+ form). The 5-hydroxyindoleacetic acid (5HIAA) retained by the Sephadex column was eluted with 2.5 ml of 0.02*N* NH_4OH and extracted, and its concentration was then mea-