Cytogenetic Effects of LSD 25 Therapy in Man

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Chromosomes have been studied in leukocytes cultured from patients undergoing experimental therapy with lysergic acid diethylamide (LSD 25). Aberration frequencies prior to therapy were established for three of the four patients; these were comparable to frequencies in control subjects. Following each of three doses given intravenously (usually 200μg per dose), each patient's chromosomes were reexamined. Some increase in aberration frequency was observed, along with the appearance of some types of aberration not present before treatment. However, a return to control levels occurred in follow-up samples taken one to six months after the final dose was administered. We feel therefore that continued experimental therapy is not strongly contraindicated. On the other hand, these results can not be construed to minimize the possible cytogenetic hazards suggested by other studies concerned primarily with drug abuse.

In April 1967, shortly after the publication by Cohen et al[1] of some striking data on the effects of lysergic acid diethylamide (LSD 25) on human chromosomes, an opportunity arose for us to study, in prospective fashion, the possibility of in vivo effects of LSD 25 therapy on the chromosomes of patients.

Since 1963, one of us (C.S.) has been investigating the effects of LSD 25 on the behavior of individuals with conduct disorders, and has obtained some encouraging results.[2,3] In view of the disturbing nature of the observations of Cohen et al who described a significantly increased frequency of chromosome breakage in leukocytes cultured with LSD 25 and in one psychiatric patient extensively treated with LSD 25 among other drugs, we felt that it was important to learn whether there were cytogenetically demonstrable consequences of experimental therapy with LSD 25. Accordingly, four patients were studied, three of them entirely prospectively.

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Patients and Control Subjects

Individual subjects are designated in three ways in the Table: by letter for easy reference in the text, by international subject identification code,[4] and parenthetically by our accession number. All patients had requested hospital admission for LSD 25 therapy for behavioral problems resistant to conventional psychiatric treatment. Control subjects were matched to patients according to age and sex.

The case histories of the patients were unremarkable in the context of the chromosome studies, except for that of patient A, who had been employed for three years in a radiological laboratory. Among the controls, only one provided information bearing on exposure to agents known to produce chromosome change: control B had multiple diagnostic roentgenograms of the lower extremities during the years 1952 to 1954 and again in 1960.

Methods

LSD 25 was administered intravenously, in a dose of 2.5μg/kg of body weight per dose or 200μg total, whichever was smaller. Each dose was thus 200μg/kg dose for patients A, B, and E, and 187μg for patient C. Usually, three treatments were given with intervals of one or two weeks between doses. The procedure for treatment has been described elsewhere.[2,3] Each LSD 25 dose was preceded by a tab-

let containing chlorpromazine (CPZ), usually 100 mg, or by a placebo. This step was designed to show whether the psychotherapeutic effects of LSD 25 would be affected by CPZ-induced modification of the first few hours of the LSD 25 experience. Doses of CPZ are given in Fig 1 for each treatment of each patient.

All chromosome studies were carried out on metaphases from leukocyte cultures.[5] Blood samples were taken from all patients, except patient A, before any LSD 25 therapy, and in general 1 hour before and 1 and 24 hours after each dose. Upon completion of therapy, follow-up samples were taken at intervals ranging from one to six months. Three blood samples were taken from each control; in all cases, a one-week interval elasped between samples 1 and 2, and a two-week interval between samples 2 and 3.

Leukocytes are most often harvested after three days in culture, at which time there may be a mixture of the first and second mitosis in vitro.[6] Thus, unstable chromosome abnormalities present in those cells which divide early may be lost. For this reason, some cultures were harvested at intervals of 46 to 52 hours after explanation. However, in this study the incidence of abnormalities in those of the "early" cultures which thrived was almost identical, in paired cultures from single blood samples, with the incidence in those harvested after three days.

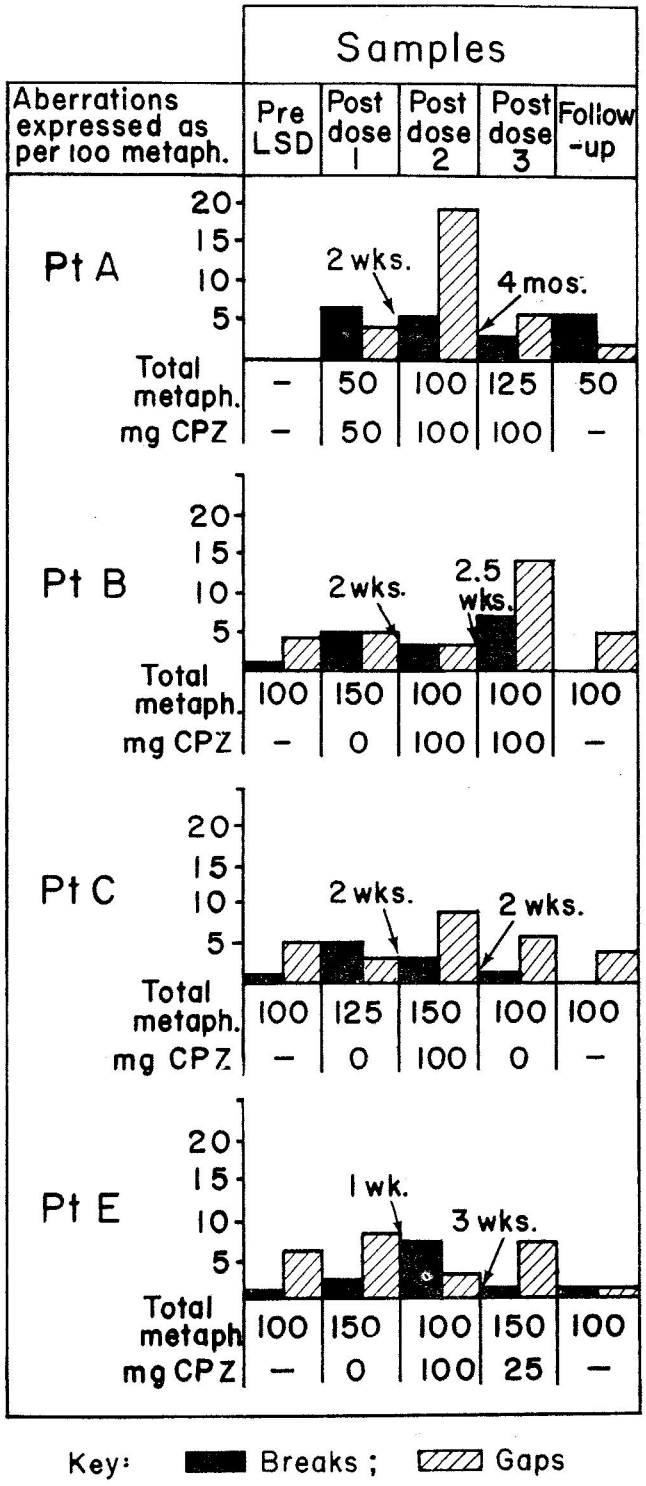
All visible structural abnormalities were scored. The categories observed included unstable rearrangements (acentric fragments, dicentrics, and multiradials), stable rearrangements (deletions), chromatid and isochromatid breaks (defined as involving axial displacement of broken ends), and gaps (nonstaining regions without such displacement). Rings, translocations, and inversions (except for a familial inversion present in all cells of control B) were not observed.

Acentrics and deletions were each scored as representing one break, dicentrics and quadriradials were each scored as two breaks, and triradials were scored as three breaks. An exception was made where an acentric and dicentric were present in the same metaphase; this event was observed only once (patient B, after dose 2), and was scored as two breaks.

Isochromatid breaks and gaps were considered to reflect aberrations which existed prior to deoxyribonucleic acid synthesis, and for this reason each was scored as one aberration rather than as two.

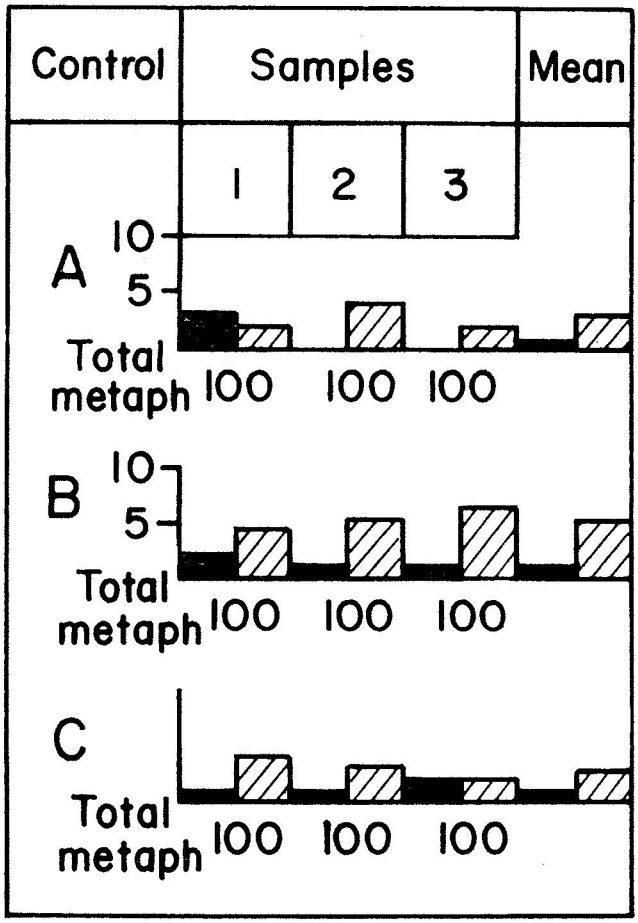
Results

Chromosome aberrations are summarized according to patient, sample, and type of aberration in the Table. In addition, total breaks and total gaps are plotted for each sample from patients (Fig 1) and from controls (Fig 2). It should be noted that these values are individual aberrations, *not* aberrant cells; the number of aberrant cells is somewhat lower because of the occasional occurrence of multiple aberrations in one cell.



1. Chromosome breaks and gaps in leukocytes cultured from patients treated with LSD 25. "Post" indicates studies on samples taken one hour to two weeks after LSD 25; "follow-up", interval of 25 days to four months after third (final) dose; CPZ, chlorpromazine premedication; intervals between doses (*arrows*); breaks (*solid bars*); gaps (*bars with diagonals*).

A number of the features of the data from our patients are noteworthy. In patients B, C, and E



2. Chromosome breaks and gaps in three blood samples from each of three controls. One-week interval elapsed between samples 1 and 2, two-week interval between 2 and 3. Incidence per 100 metaphases (*height of bars*); breaks (*solid bars*); gaps (*bars with diagonals*).

(Fig 1) there was an increase, compared with the findings before LSD 25 was administered, either in breaks or in gaps, or in both, immediately following the second and third doses, and the combined values for all samples after LSD 25 therapy, except those of the follow-up data, were greater than the values before LSD 25 therapy in all three cases. Dicentrics and multiradials were found only following administration of LSD 25 (Table). The other type of unstable aberration, the acentric fragment, was also found in the controls, although in a lower frequency than in the patients' cultures after LSD 25 therapy. Acentric fragments have been reported in control cultures of other experimental studies of human chromosomes.[6,7] Fluctuation in frequencies of breaks and gaps was present among consecutive samples from controls A, B, and C. However, the mean values from the three controls compared well with one another and with the values before LSD 25 therapy from three of the patients. Finally, it is to be noted (Table and Fig 1) that in the three patients for whom pre-LSD 25 values were determined, there was, in the follow-up studies, a return in each case to these earlier levels. The comparatively high follow-up values found in the remaining case (patient A) cannot, of course, be discussed in terms of values before LSD 25 therapy but may be explained by the possibility, mentioned earlier, of his having had occupational exposure to radiation.

Comment

Our data indicate that pharmacologically pure LSD 25 may produce transitory increases in chromosome abnormalities, but that these are no longer evident one month after administration of the final dose of the compound. The transistory nature of these induced changes suggests the existence of a repair or elimination mechanism, or both, operating to reduce the number of aberrations. The phenomenon cannot be explained entirely on the basis of elimination of aberrations during mitosis, since evidence from numerous studies shows that many lymphocytes remain undivided for periods up to several years. Furthermore, a decrease occurs in stable chromosome aberrations as well as in the unstable ones. This supports a concept of repair of chromosomal damage in human lymphocytes such as that recently urged by Nusbacher et al.[8]

The results from our study are complicated to some extent by the fact that CPZ, an agent believed to cause chromosome damage,[9] was used in premedication. However, no premedication was given patients B, C, and E before the first dose of LSD 25, and in all three instances, an elevation in breaks or gaps ensued. These elevations are presumably due solely to LSD 25. In other samples there is no clear correlation between the frequency of breaks and gaps and the amount of CPZ administered. If there is, in fact, any CPZ effect on the chromosomes of our patients, it would be expected to lead to an overestimate of the effect of LSD 25, already presumably maximized by intravenous rather than oral administration of the latter compound.

Comparison of data from different studies concerning the effect of LSD on chromosomes is at best difficult. Some comment, however, seems warranted.

The original publication by Cohen et al[1] established that LSD 25 administered in vitro caused chromosome damage to leukocytes from normal individuals. In the same communication there appeared results from the case of one patient who was extensively treated with LSD 25 and studied eight months afterward. These results suggested that there might also be in vivo effects. Jarvik and Kato[10] also found that LSD 25 increased chromosome breakage in cultured human leukocytes, as did ergonovine (Ergotrate) maleate, aspirin, and streptonigrin; the significance of such results in terms of possible in vivo effects remains to be clarified. It may be relevant in this regard that Ostertag and Haake[11] have shown a 1% caffeine solution both to cause increased chromosome breakage in human leukocytes in vitro and to be mutagenic in *Drosophila*.

In a second communication, Cohen et al[9] describe

studies in a series of 18 LSD users, in all of whom there were elevated frequencies of chromosome damage, the mean frequency being more than three times the mean from a control group of 14 individuals. Similar findings from users have been reported by Irwin and Egozcue,[12] and Egozcue et al.[13] However, Loughman et al,[14] while observing eight individuals from a similar population, found no differences from controls, and Sparkes et al[15] found no significant chromosome damage in four users. Such differences in results from studies of LSD users could occur in a number of ways. Differences in the techniques employed in different laboratories are undoubtedly responsible for variability in control levels. The "LSD" being used by users is from illicit sources; it may thus contain LSD in variable amounts, or none at all, as well as active contaminants. Users' accounts of frequency of drug use may be suspect; their accounts of dosage are certainly so. Users commonly take other drugs, sometimes in great variety and in combination, and the possibility of synergistic effect has not been thoroughly investigated. Finally, it is impossible to assess the conceivably complicating effects of the low nutritional states and high infectious-disease rates which have been reported in "hippie" communities (eg, Smith and Rose[16]).

Patients who have been administered LSD 25 have been studied in several laboratories. Bender and Siva-Sankar[17] report the cases of seven children who received 100μg to 150μg twice daily for periods of weeks or months. No difference from controls was observed, but chromosome studies were not done until 20 to 48 months after the last dose, by which time according to our results effects of the drug would not be apparent. Nielsen et al[18] also report the cases of five patients treated with LSD 25. Compared with control subjects, an increase in gaps, breaks, and frequency of hyperdiploid cells was present. Doses and relationship between treatment and time of study were not given. Sparkes et al[15] found no significant differences between control subjects and four patients given LSD 25, although quadriradials were found only after exposure to the compound. However, these patients were studied 1, 2, 3, and 60 months after their last dose.

Relatively little work has been done on effects of LSD 25 on the chromosomes of experimental animals, or on meiotic chromosomes of any species. Skakkebaek et al[19] have recently reported abnormalities in the meiotic chromosomes of male mice treated with LSD 25.

In addition to the matter of induction of gross chromosome abnormalities, two other issues have been raised in connection with the use of LSD: carcinogenesis and teratogenesis.

It has been pointed out that the types of chromosome aberration observed in high frequency in LSD users are similar to those found in Bloom's syndrome, Fanconi's syndrome, and ataxia-telangiectasia. Patients with these diseases suffer a high incidence of leukemia and other malignancies. Similar effects on chromosomes are caused by known carcinogens, including radiation. On the basis of these facts it has been suggested[9] that malignant disease may be more likely to develop in individuals exposed to LSD. In the absence of appropriate retrospective data concerning cancer patients, and data concerning carcinogenic effects of LSD in experimental animals, the suggestion remains highly conjectural.

Evidence of any teratogenic effect of LSD in humans is sparse. Cohen et al[9] have observed chromosome damage in two children exposed to LSD in utero; Sato and Pergament[20] failed to find the same effect in one such child; and Zellweger et al[21] have described a child with a malformed leg born to a woman who had taken LSD during her pregnancy. Alexander et al[22] have claimed that LSD 25 is teratogenic in rats, results not confirmed by Warkany and Takacs.[23] The latter authors have declined to extrapolate their findings to other species. Lysergic acid diethylamide has been found to be teratogenic in mice[24] and hamsters[25] but not in the New Zealand white rabbit.[26] Once more, the burden of proof concerning teratogenic effects of LSD in humans remains with the proponents of this suggestion.

In conclusion, we feel that our data and those of

Chromosome Aberrations, According to Time of Sampling, in Patients and Controls

Patient	Sample	Aberration Types*						Total Breaks	Total Gaps	Abnormal Metaphases/ Total Metaphases
		A	Di	T	Q	De	OB			
	Before LSD	...	...	...	...	...	...	...	...	...
A	After LSD 1	1	1	0	0	0	0	3	2	4/50
WK120232	2	3	0	0	0	1	1	5	19	18/100
(522H)	3	3	0	0	0	0	0	3	6	9/100
	Follow-up	0	0	1	0	0	0	3	1	2/50
	Before LSD	0	0	0	0	0	1	1	4	5/100
B	After LSD 1	0	1	0	0	0	5	7	8	13/150
PH070738	2	1	1	0	0	0	0	3	3	5/100
(523H)	3	0	0	0	0	0	7	7	14	17/100
	Follow-up	0	0	0	0	0	0	0	5	5/100
	Before LSD	1	0	0	0	0	0	1	5	6/100
C	After LSD 1	2	0	0	1	0	1	6	4	8/125
LWO40532	2	3	0	0	0	0	2	5	13	18/150
(528H)	3	1	0	0	0	0	0	1	6	5/100
	Follow-up	0	0	0	0	0	0	0	4	4/100
	Before LSD	0	0	0	0	0	1	1	5	7/100
E	After LSD 1	0	0	0	0	1	2	3	12	12/150
AL180738	2	0	2	0	0	0	3	7	4	9/100
(550H)	3	0	0	0	0	0	1	4	10	13/150
	Follow-up	0	0	0	0	0	1	1	1	2/100
Control A	1	2	0	0	0	0	1	3	2	5/100
JMO21138	2	0	0	0	0	0	0	0	4	4/100
(499H)	3	0	0	0	0	0	0	0	2	2/100
Control B	1	2	0	0	0	0	0	2	4	6/100
GB240739	2	0	0	0	0	0	1	1	5	6/100
(544H)	3	0	0	0	0	0	1	1	6	7/100
Control C	1	0	0	0	0	0	1	1	4	5/100
WL110332	2	0	0	0	0	0	1	1	3	3/100
(545H)	3	0	0	0	0	0	2	2	2	4/100

*A indicates acentric; Di, dicentric; T, triradial; Q, quadriradial; De, deletion (other than centric); OB, other breaks.

others do not appear to give any stronger contraindication concerning the continued use of LSD 25 in experimental therapy than do those risks established for other therapeutic and diagnostic procedures; in some instances LSD 25 would seem far more innocuous. This statement has, of course, no bearing on the possible consequences of illicit consumption of substances represented as LSD.

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Generic and Trade Names of Drugs

Chlorpromazine—*Thorazine.*
Streptonigrin—*Nigrin.*
Ergonovine maleate—*Ergotrate Maleate.*

References

1. Cohen, M.M.; Marinello, M.J., and Back, N.: Chromosomal Damage in Human Leukocytes Induced by Lysergic Acid Diethylamide, *Science* **155:**1417-1419 (March 17) 1967.

2. Eggert, D.C., and Shagass, C.: Clinical Prediction of Insightful Response to a Single Large Dose of LSD, *Psychopharmacologia* **9:**340-346, 1966.

3. Shagass, C., and Bittle, R.M.: Therapeutic Effects of LSD: A Follow-Up Study, *J Nerv Ment Dis* **144:**471-478 (June) 1967.

4. "Chicago Conference: Standardization in Human Cytogenetics," *Birth Defects: Original Article Series*, New York: The National Foundation—March of Dimes, December 1966, vol 2.

5. Hungerford, D.A.: Leukocytes Cultured From Small Inocula of Whole Blood and the Preparation of Metaphase Chromosomes by Treatment With Hypotonic KCl, *Stain Techn* **40:**333-338 (Nov) 1965.

6. Evans, H.J.; Court-Brown, W.M.; and McLaine, A.S. (eds.): *Human Radiation Cytogenetics*, Amsterdam: North-Holland. 1967.

7. Jacobs, P.A.; Brunton, M.; and Court-Brown, W.M.: Cytogenetic Studies in Leucocytes on the General Population: Subjects of Ages 65 Years and More, *Ann Hum Genet* **27:**353-362 (June) 1964.

8. Nusbacher, J.; Hirschhorn, K.; and Cooper, L.Z.: Chromosomal Abnormalities in Congenital Rubella, *New Eng J Med* **276:**1409-1413 (June 22) 1967.

9. Cohen, M.M.; Hirschhorn, K.; and Frosch, W.A.: In Vivo and in Vitro Chromosomal Damage Induced by LSD-25, *New Eng J Med* **277:**1043-1049 (Nov 16) 1967.

10. Jarvik, L.F., and Kato, T.: Is Lysergide a Teratogen? *Lancet* **1:**250 (Feb 3) 1968.

11. Ostertag, W., and Haake, J.: The Mutagenicity in *Drosophila melanogaster* of Caffeine and of Other Compounds Which Produce Chromosome Breakage in Human Cells in Culture, *Z Vererbungsl* **98:**299-308 (Dec) 1966.

12. Irwin, S., and Egozcue, J.: Chromosomal Abnormalities in Leukocytes From LSD-25 Users, *Science* **157:**313-314 (July 21) 1967.

13. Egozcue, J.; Irwin, S.; and Maruffo, C.A.: Chromosomal Damage in LSD Users, *JAMA* **204:**214-218 (April 15) 1968.

14. Loughman, W.D.; Sargent, T.W.; and Israelstam, D.M.: Leukocytes of Humans Exposed to Lysergic Acid Diethylamide: Lack of Chromosomal Damage, *Science* **158:**508-510 (Oct 27) 1967.

15. Sparkes, R.S.; Melnyk, J.; and Bozzetti, L.P.: Chromosomal Effect in Vivo of Exposure to Lysergic Acid Diethylamide, *Science* **160:**1343-1345 (June 21) 1968.

16. Smith, D.E., and Rose, A.J.: Observations in the Haight-Ashbury Medical Clinic of San Francisco: Health Problems in a "Hippie" Subculture, *Clin Pediat* **7:**313-316 (June) 1968.

17. Bender, L., Siva-Sankar, D.V.: Chromosome Damage Not Found in Leukocytes of Children Treated With LSD 25, *Science* **159:**749 (Feb 16) 1968.

18. Nielsen, J.; Friedrich, U.; and Tsuboi, T.: Chromosome Abnormalities and Psychotropic Drugs, *Nature* **218:**488-489 (May 4) 1968.

19. Skakkebaek, N.E.; Philip, J.; and Rafaelsen, O.J.: LSD in Mice: Abnormalities in Meiotic Chromosomes, *Science* **160:**1246-1248 (June 14) 1968.

20. Sato, H., and Pergament, E.: Is Lysergide a Teratogen? *Lancet* **1:**639-640 (March 23) 1968.

21. Zellweger, H.; McDonald, J.S.; and Abbo, G.: Is Lysergic-Acid Diethylamide a Teratogen? *Lancet* **2:**1066-1068 (Nov 18) 1967.

22. Alexander, G.J., et al: LSD: Injection Early in Pregnancy Produces Abnormalities in Offspring of Rats, *Science* **157:**459-461 (July 28) 1967.

23. Warkany, J., and Takacs, E.: Lysergic Acid Diethylamide (LSD): No Teratogenicity in Rats, *Science* **159:**731-732 (Feb 16) 1968.

24. Auerbach, R., and Rugowski, J.A.: Lysergic Acid Diethylamide: Effect on Embryos, *Science* **157:**1325-1326 (Sept 15) 1967.

25. Geber, W.F.: Congenital Malformations Induced by Mescaline, Lysergic Acid Diethylamide, and Bromolysergic Acid in the Hamster, *Science* **158:**265-267 (Oct 13) 1967.

26. Fabro, S., and Sieber, S.M.: Is Lysergide a Teratogen? *Lancet* **1:**639 (March 23) 1968.

ROMOS and SOmOR.—. . . chromosomes could also vary in other ways than by changes within the genes, by ways therefore no longer characteristic of each gene. There are gains and losses and inversions of whole segments, whole groups of genes.

It is as though the word *chromosome* were to be changed by breakage and rearrangement of letters to give *chromomosome,* or *chrosome,* or *chrosomome* or *chromoso.* This kind of change, going on at one level, while simple gene mutations such as make *cHromosome* or *chromosome,* are going on at another level, give a more complicated basis to variation. Two chromosomes from opposite parents when they meet at meiosis have often been found to be of this relationship:—

CHROMOSOME
CHSOmOROMe

Two such chromosomes tie themselves in a knot when they pair. If crossing-over takes place between them, in the middle of the knot, two new chromosomes are formed which usually fail to live, thus:—

CHROMmOSHC
EMOSOOROMe

Or, if crossing-over does not take place, then the gene-blocks ROMOS and SOmOR will be held together as unbreakable units in heredity, as new *super-genes.*

All these conditions were soon to be demonstrated directly from the study of the chromosomes at meiosis in plants and animals and in the nucleus of the salivary glands of *Drosophila* itself.—Darlington, C.D.: *Genetics and Man,* New York: Macmillan Co., 1964, pp 117-118.