Table 1. Effects of sugars and cations on the dissociation of *Helix pomatia* ovotestes. Numbers of experiments appear in parentheses. Chia, Chiarandini solution.

Dissociation medium	Cells ($\times 10^6$/ml)
Experiment A	
Chia + trypsin (4)	0
Chia + trypsin; no Ca (4)	0.25
Experiment B	
MPS + trypsin (4)	.95
MPS + trypsin; no Ca (4)	.94
Experiment C	
MPS + trypsin (11)	.90
MPS + trypsin + glucose (2)	.94
MPS + trypsin + trehalose (2)	1.04
MPS + trypsin + galactose (2)	1.20
MPS + trypsin + galactose + trehalose (11)	1.70
Experiment D	
MPS + trypsin + galactose + trehalose (4)	1.58
MPS + trypsin + galactose + trehalose; no Ca (4)	1.54
Experiment E	
MPS + trypsin + galactose + trehalose (5)	0.70
Chia + trypsin + galactose + trehalose (5)	1.64
Chia + trypsin + galactose + trehalose; no Ca (5)	.89

major differences between the normal ovotestes and the reaggregates were that in the latter (i) the hemocoel spaces were smaller, (ii) acinar lobes were fewer and smaller, and (iii) fewer cells were found per acinar lobe.

In six experiments any undissociated fragments of tissue were removed by expression of the cells through a 60-mesh stainless steel grid in the base of a 30-ml hypodermic syringe before centrifugation. Sections of the resultant reaggregates yielded results similar to those obtained without use of the grid. Thus the cells are able to form organized tissue during centrifugation.

The reaggregates could be dispersed with a pipette by vigorous aspiration of the solution in which they were bathed. The cells in such suspensions had pseudopodia (Fig. 1A). If the suspension was centrifuged briefly (about 0.5 minute) and a sample was removed and examined without a cover slip, almost all cells showed this activity. There were two kinds of pseudopodia: a broad, slow-moving lobopodium and a thin, flexible, active filopodium. Furthermore, the cells tended to orient on any substrate, such as cotton fibers or sperm tails, with which they came in contact.

The role of the pseudopodia formed by the dissociated molluscan cells is not clear. Trinkaus (*7*) states that both vertebrate fibroblasts and epithelial cells in culture exhibit cytoplasmic extensions at free surfaces. These extensions apparently act as the locomotive organ of the cell and seem to be the means of movement of many cells engaged in morphogenetic cell movements (*7*). The descriptive behavior of these extensions is very similar to that of the pseudopodia of the snail cells; thus it is possible that these pseudopodia function in the rapid reaggregation of the ovotestes cells.

Centrifugation and cellular adhesiveness appear to be the most important factors in rapid reaggregation of the ovotestes cells. The extensive reorganization of the cells in such a short time suggests that adult *H. pomatia* cells retain the ability to (i) recognize a morphogenetic tissue pattern and (ii) maintain cellular adhesiveness after dissociation. Although the reaggregation of dissociated cells has been extensively studied, most research has been concerned with embryonic cells, for only one other group of animals, the sponges, have been reported to have cells showing aggregation behavior in the adult. Sponges can be dissociated both mechanically (*8*) and chemically by removal of divalent cations from the solution (*9*). Moscona (*10*) suggests that, in both sponges and vertebrates, extracellular materials function in the selective attachment and organization of cells. In the case of the sponges, this idea appears to be supported by the fact that cell-free extracts having cell-aggregation activity were obtained from suspensions of sponge cells washed in cold sea water lacking divalent cation (*11*). Such extracts consisted largely of glycoproteins whose activity depended on calcium. Chemical analysis revealed that sponge-cell aggregation depended primarily on the presence of disulfide groups and on protein integrity, although it was stated that these experiments could not entirely rule out a possible role of carbohydrates (*12*).

Most workers (*13*) on reaggregation agree that the regrouping of dissociated cells into coherent multicellular bodies is probably due to the mutual adhesiveness of the cells, and conditions affecting reaggregation have been interpreted as affecting cell adhesion. A study of *Helix* cells similar to that of sponge cells (*11*, *12*) might clarify the reaggregation of dissociated molluscan cells.

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LSD in Mice: Abnormalities in Meiotic Chromosomes

Abstract. *Meiotic chromosomes of six mice injected with high doses of lysergic acid diethylamide (LSD-25) and of six controls were studied. Several breaks, gaps, and unidentifiable fragments were found in the treated but, with a few exceptions, not in the control animals. Secondary constrictions were more numerous in the treated than in the untreated mice. Possible consequences are discussed.*

Lysergic acid diethylamide (LSD-25) has been shown to cause abnormalities in the chromosomes of human leukocytes (*1*). The drug has other teratogenic properties in production of congenital abnormalities and increased rates of abortion in rats and mice (*2*). The possible effect on human embryos has been discussed (*3*). Our preliminary results suggest that LSD can also cause changes of the meiotic chromosomes in germ cells.

Meiotic chromosomes were studied from six healthy male mice injected with LSD-25 (group A); the mice were aged between 6 and 8 weeks. Identical mice were used as controls (group B). The animals were coded, and the investigator did not know the code. But the mice were matched in pairs; one member of each pair received LSD and one did not—in some in-

Table 1. Abnormalties found by differential count in all cells in diakinesis or 1-metaphase. Abbreviations: SECON, secondary constrictions; unid. frag., unidentifiable fragments.

Case (No.)	Day		Cells (No.)	X Chromosomal			Unid. frag.	Autosomal	
	Injected	Killed		Breaks	Gaps	SECON		Breaks	SECON
			Group A, LSD-25, 1 mg/kg						
1a	31 Oct., 1 Nov.	3 Nov.	96	0	0	2	0	0	1
3a	31 Oct.	1 Nov.	129	1	1	0	0	1?	1?
5a	31 Oct., 1 Nov.	6 Nov.	138	0	1	1	0	1	1
6a	31 Oct.	2 Nov.	128	1	0	5	0	2	0
5b	5, 5, 6, 6, 7, 7, 8, 8 Dec.	14 Dec.	50	0	0	1	0	0	0
7b	5, 5, 6, 6, 7, 7, 8, 8 Dec.	18 Dec.	68	0	1	3	0	0	0
			Totals						
			609	2	3	12	0	3 + 1?	2 + 1?
			Group B, saline						
2a		1 Nov.	107	0	0	0	0	0	0
4a		3 Nov.	107	0	1	1	0	0	0
7a		2 Nov.	111	0	0	0	0	0	0
8a		6 Nov.	105	0	0	0	1	0	0
6b	5, 5, 6, 6, 7, 7, 8, 8 Dec.	14 Dec.	58	0	0	0	0	0	0
8b	5, 5, 6, 6, 7, 7, 8, 8 Dec.	18 Dec.	55	0	0	1	0	0	0
			Totals						
			543	0	1	2	1	0	0

stances the second mouse received physiological saline. Each pair were killed and examined together. The doses of LSD were high—1 mg per kilogram of body weight; the numbers of injections and intervals between exposures and examination varied (Table 1).

Preparations of meiotic chromosomes were made by a reported method (*4*). Two of the eight pairs planned were excluded because the preparations were not successful. The distribution of cells in spermatogonial metaphase, diakinesis or 1-metaphase, and 2-metaphase was determined by differential counting of 100 or 200 cells from each mouse. At the same time a detailed analysis was performed on all suitable cells for detection of chromosomal changes. In order to obtain enough cells for detailed study, another 50 or 100 cells (suitable for analysis) in diakinesis or 1-metaphase were examined (except in case 7a), but no differential count was made at this later stage. The results are separated (Tables 1 and 2) because only Table 2 gives a true picture of the percentages of abnormalities found.

An abnormality was scored as a break only if the axis of the two parts of the chromosome was broken (Fig. 1c). Gaps were defined as regions of discontinuity without displacement of the axis (Fig. 1, a, b, and f). Regions of pale-staining material were registered as secondary constrictions (Fig. 1, c and d). Unidentifiable fragments were scored separately (Fig. 1e).

Table 1 shows that 609 cells of a total of 1000 in group A and 543 of a total of 1000 in group B were in diakinesis or 1-metaphase. Analysis was performed furthermore on 400 selected cells in diakinesis or 1-metaphase from group A and 300 from group B. Detailed analysis of these cells showed a difference between groups A and B (Tables 1 and 2). Breaks and gaps in the X chromosome were not found in group B (except in one cell). Secondary constrictions were found in 21 cells in group A, but in only six cells in group B. Unidentifiable fragments, breaks, gaps, and secondary constrictions in the autosomes were not found in group B (except in one cell).

Our results must be considered tentative evidence that high doses of LSD may influence meiotic chromosomes from mice. Although our subjects are few, one should note that the investigator (N.E.S.), on a blind basis, was able to distinguish between the treated and the untreated animal in all pairs. The changes found were gaps, breaks, and fragments and higher numbers of secondary constrictions than were present in the controls. Other abnormalities may have been present but were not recognized. No translocations were seen, but they were not to be expected in view of the short duration of the treatment.

Although the number of abnormalities was small and technical errors could not be excluded, there was difference in number of abnormalities between testes from treated and untreated animals. The results are based on cytological estimation, and the same value cannot be attached to all findings at this point; for this reason and because of the small number of data we consider statistical evaluation premature.

The chromosomal abnormalities were mainly found in diakinesis or 1-metaphase, and here again most changes were located on the X chromosome. Although we cannot explain this fact, it may well be that the same changes are

Table 2. Abnormalities found by detailed analysis in selected cells in diakinesis or 1-metaphase. Abbreviations: SECON, secondary constrictions; unid. frag., unidentifiable fragments.

Case (No.)	Cells analyzed (No.)	X Chromosomal			Unid. frag.	Autosomal	
		Breaks	Gaps	SECON		Breaks	SECON
			Group A				
1a	50	0	1	1	1	1	0
3a	100	0	2	1	3	0	0
5a	50	0	0	2	0	1	0
6a	100	0	1	5	0	1	1
5b	50	0	0	0	0	1?	1
7b	50	2	4	0	1	0	1
			Totals				
	400	2	8	9	5	3 + 1?	3
			Group B				
2a	100	0	0	1	1	0	0
4a	50	0	0	0	0	0	0
7a	0						
8a	50	0	0	0	0	0	0
6b	50	0	0	1	0	0	0
8b	50	0	0	2	0	0	0
			Totals				
	300	0	0	4	1	0	0

as frequent in the autosomes but cannot be recognized with our technique. The XY bivalent is often more clear and distinct than the autosomal bivalents.

The second metaphase also was studied. Because the chromosomes at this stage are spiralized and badly defined, the findings are difficult to interpret. In one instance (case 1a), however, gaps and secondary constrictions were more numerous than in the control mouse.

Assuming that the chromosomal abnormalities seen are due to LSD (as we now believe), one must conclude that the use of LSD may well have serious effects on the size of the litter and the number of congenital malformations in the offspring.

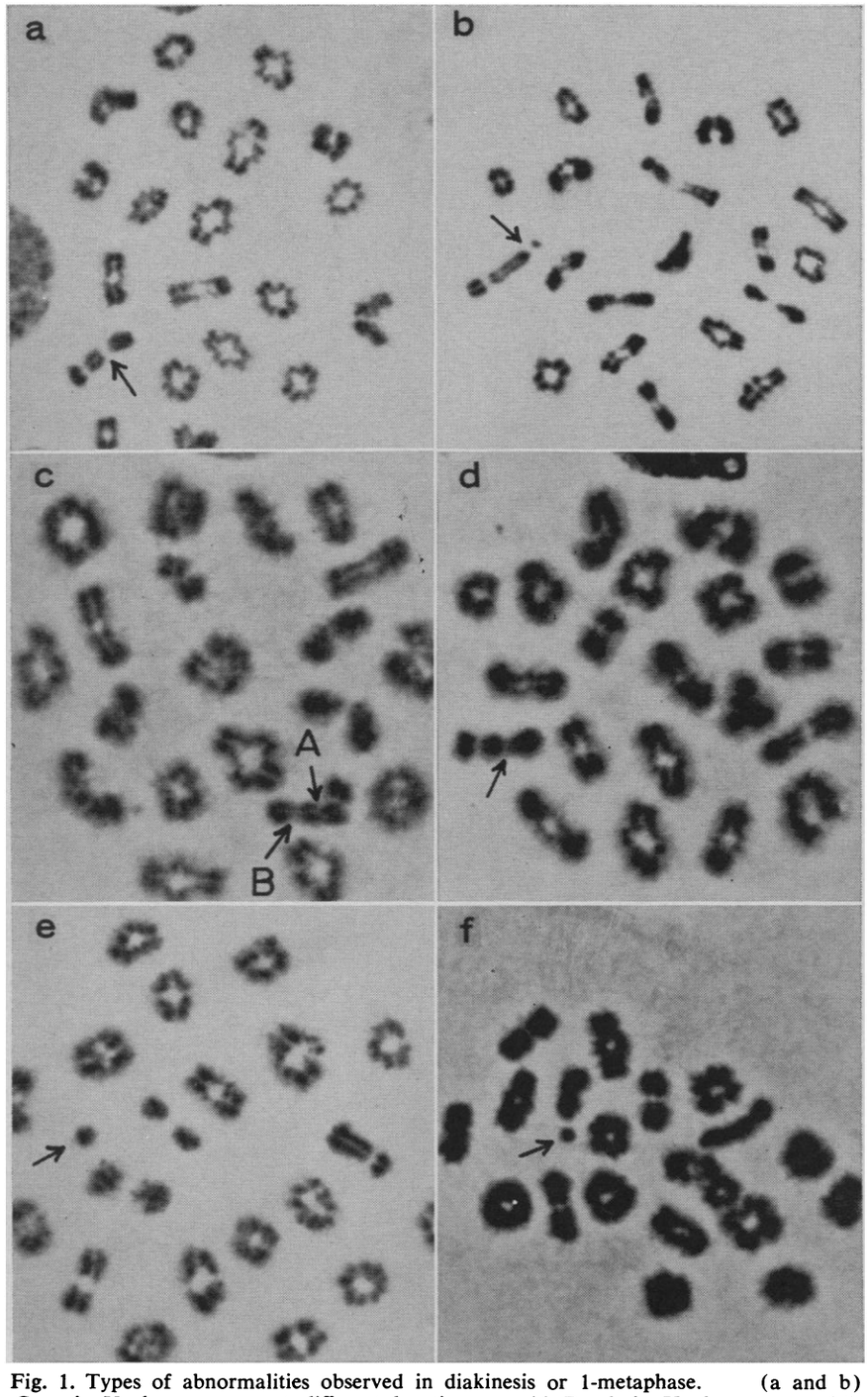

Fig. 1. Types of abnormalities observed in diakinesis or 1-metaphase. (a and b) Gaps in X chromosomes at different locations. (c) Break in X chromosome (*A*); secondary constriction (*B*). (d) Secondary constriction in X chromosome. (e) Unidentifiable fragment. (f) Gap in autosome or fragment.

It is already well known that LSD causes chromosomal changes in lymphocytes of peripheral human blood in vivo (*1*). It has been hypothesized that such changes may cause higher frequencies of leukemia and other neoplastic diseases. If, however, meiotic chromosomes are damaged, the fertility of the gonad may be impaired. It is also well known that chromosome aberrations are present in testes of irradiated mice (*5*). These aberrations, mainly translocations, are hereditary, and the fertility of the carriers is affected (*6*). No translocations would be expected after the very short period of exposure used by us. Translocations may be found in experiments employing a longer interval between exposure and examination, or by breeding experiments with the LSD-treated animals.

Although our experiments with LSD are to the best of our knowledge the first of their kind to be reported, studies of meiotic chromosomes after treatment with Streptonigrin (*7*) and after Cyclophosphamid and Triziquon (*8*) have shown changes comparable to those found after LSD. Investigation of meiotic chromosomes may well be important for clinical pharmacology in the future if the changes seen in mouse also occur in man, and if the changes in the treated animal cause changes in the offspring.

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