on Sephadex G-50. Detailed procedures have been described (*1*).

The results of these comparisons are shown in Fig. 1. In every case, the elution profiles of glycopeptides from the transformed cells were different from those of the control cells. There was a consistent tendency of material from the transformed cells to elute ahead of material from the control cells. The results were similar for all pairs of cells regardless of the species of origin, that is, hamster (Fig. 1a), mouse (Fig. 1, b and c), or chicken (Fig. 1d). In addition, it appeared to make no difference whether the cells were transformed with DNA-containing (Fig. 1a) or RNA-containing (Fig. 1, b–d) viruses.

Several observations suggest that these differences in the radioactive profiles of material from control and transformed cells reflect an actual difference in the glycoproteins of the cell surfaces. (i) We have found that elution patterns of pronase-digested 'trypsinate" from Sephadex G-50 are similar to the profiles of pronase-digested surface membranes (*1*). (ii) We have previously reported (*1*) that 90 percent of the radioactivity recovered from hamster cells grown for 3 days in the presence of radioactive L-fucose was chromatographically identifiable as fucose. Similar results were obtained by Strauss *et al.* (*7*), who used chick and hamster cells. (iii) Interchanging the isotopes (that is, the use of L-[^{14}C]fucose rather than L-[^{3}H]fucose), increasing the times of trypsinization, or growing the cells for different periods of time or to different cell densities in the presence of radioactive L-fucose did not alter the results. (iv) We have shown (*1*) that pronase digestion as carried out here is complete for material from control and transformed cells. Burge *et al.* (*8*) have found that similar pronase treatment of glycoproteins resulted in digestion of material from viruses grown on either chick or hamster cells. (v) With hamster cells, we have obtained similar results after growing the cells in the presence of radioactive D-glucosamine (*1*) or D-glucose (*9*). (vi) The specific activities of radioactive L-fucose from a pair of control and transformed cells were similar (*1*).

Material from transformed cells was different in the various cell lines. The profiles shown in Fig. 1, a and b, resemble those reported for BHK_{21}/C_{13} cells transformed by the Bryan strains of RSV (*1*). The Balb/3T3 cells transformed by murine sarcoma virus and chick cells transformed by the Schmidt-Ruppin strain of RSV showed less marked, but reproducible, increases in the higher molecular weight glycopeptides.

The material eluting later from the columns (fractions 50 to 75) was found in all cells. The amount of this material was not always the same, even in preparations from the same cell line. Its presence could not be correlated with the presence or absence of glycopeptides in other regions of the profiles.

It will be impossible to know whether the higher molecular weight glycopeptides found in the different virus-transformed cells represent the same molecules until these materials are purified further and characterized chemically. The profiles of surface material from transformed cells shown in Fig. 1 suggest that there may be differences among the glycopeptides from the various transformed cells. The fact that control cells contain small amounts of higher molecular weight glycopeptides that migrate with a large amount of glycopeptides from the transformed cells might suggest that transformation results in a change in the normal glycoproteins or the glycolipids, or both, on the cell surface. Other investigators (*10–12*) have shown that transformation results in the unmasking of regions found in normal cells. The relation of the changes observed here to the unmasking of sites on the cell surface, especially those capable of binding the plant agglutinins, remains to be determined.

CLAYTON A. BUCK*
MARY CATHERINE GLICK
LEONARD WARREN
Department of Therapeutic Research, University of Pennsylvania, Philadelphia 19104

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* Present address: Division of Biology, Kansas State University, Manhattan 66502.

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Latent Meiotic Anomalies Related to an Ancestral Exposure to a Mutagenic Agent

Abstract. *When urethan is administered to cytologically normal F_2 progeny descended from grandsires exposed to ethyl methanesulfonate, meiotic anomalies in the forms of site-specific X-chromosome deletions and bivalent associations are noted in spermatocytes of male Armenian hamsters examined 6 and 8 days after treatment, respectively. These latent anomalies are initiated and retained in a premutated state for two generations after the ancestral exposure to ethyl methanesulfonate, the additional impetus required to complete the mutational processes being supplied by urethan.*

Chemical mutagens, such as nitrogen mustards, ethylenimines, and nitroso compounds induce both complete and fractional (mosaic) mutations in drosophilas (*1*). In several instances where expression of the mutation was delayed until the second and third generations, it was postulated that those mutagens having low energy yields may act by elevating the involved gene locus or a segment of chromosome to a labile, premutated state where it remains until additional energy is available to complete the mutational process (*2*). This report deals with a similar phenomenon encountered in cytologically normal F_2 generation males of the Armenian hamster, *Cricetulus migratorius* ($2n = 22$), whose grandsires were treated with a single dose of ethyl methanesulfonate (EMS). Subsequent treatment of the cytologically normal F_2 generation males with an equivalent dose of urethan (ethyl carbamate), an unrelated and virtually ineffective chromosomal mutagen for mammals (*3*), is interpreted as having provided the additional energy necessary for the latent expressions of two highly specific forms of meiotic chromosomal anomalies.

Table 1. Meiotic anomalies noted on the eighth day of urethan treatment of F_2 males derived from F_1 male progeny. The anomalies are shown as numbers of cells of the 232 cells examined, having fewer (7, 8, 9, or 10) or more (12) bivalents than the normal number (11).

Animal	No. of bivalents						Total No. of cells in diakinesis examined
	7	8	9	10	11	12	
No. 1	1	0	3	49	9	2	64
No. 2	0	0	2	47	2	1	52
No. 3	0	0	0	46	6	2	54
No. 4	1	0	5	52	4	0	62
Totals	2	0	10	194	21	5	232

Details of our experiments are given in Fig. 1. Adult male Armenian hamsters were injected intraperitoneally with a single dose of EMS (100 mg/kg of body weight) in normal saline and bred on alternate days to normal females (days 1 through 11). Following unilateral orchidectomy of 15 F_1 males at 21 to 26 days of age, spermatogonial and meiotic chromosome preparations were examined for chromosomal alterations. Translocations were evident in 2 of the 15 males; the first had two tetravalents, while the other had one hexavalent with an overall incidence of 30 percent translocations among males. The 13 normal (cytologically confirmed) F_1 males and an equivalent number of female littermates were then bred to normal counterparts. Meiotic analyses of the resulting 56 F_2 male progeny derived from the 13 cytologically normal males disclosed that all F_2 males had the normal 11 bivalents (Fig. 2a). Uniform litter size and sex ratios, and failure to detect chromosomal anomalies among the 35 F_2 males derived from F_1 females, indicate that viable (multiple) translocations were restricted to two mutant F_1 males sired on the seventh and eighth days after treatment of sires with EMS (*4*).

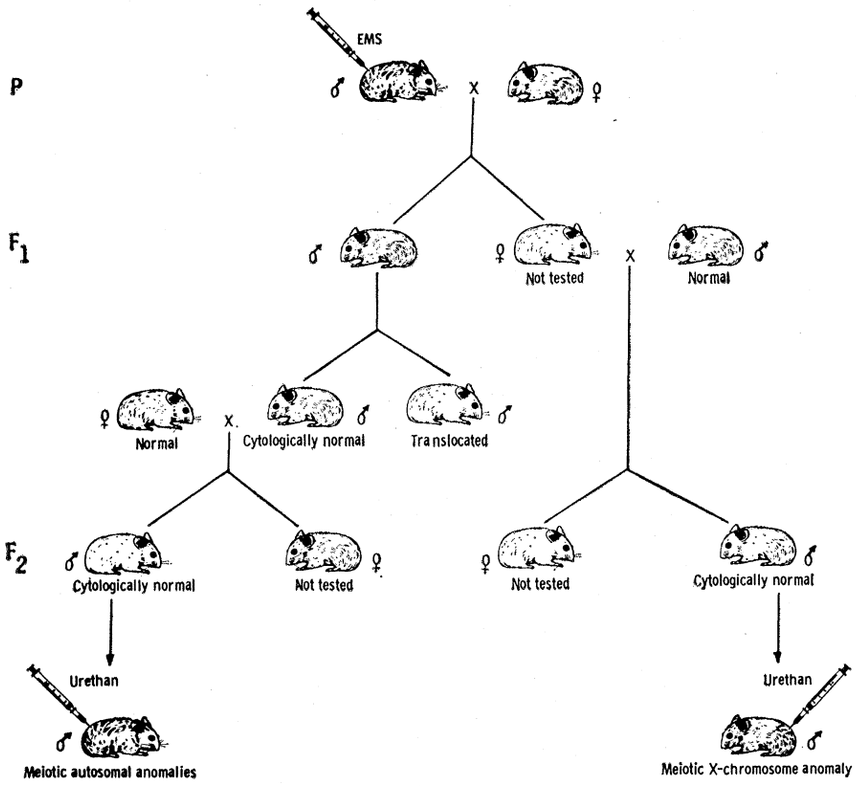

Fig. 1. Scheme of experimental procedures employed. Ethyl methanesulfonate (EMS) was administered to grandsires. F_1 progeny consisted of translocated and nontranslocated males, and females that were not analyzed for meiotic configurations. All F_2 males were cytologically normal. Certain males derived from cytologically normal F_1 animals (left side) exhibited anomalous bivalents following urethan treatment (see text). F_2 males derived from F_1 females (right side) showed mainly X-chromosome deletion following urethan administration (see text).

The 91 unilaterally orchidectomized EMS-background "normal" F_2 progeny were included, along with 20 additional control males, in another trial to determine if urethan is, in fact, inactive throughout the meiotic cycle, as earlier reports employing mice and rats had indicated (*3*). Thus, the three groups of animals (EMS-background F_1 male-derived, EMS-background F_1 female-derived, and normal intact controls) were administered single intraperitoneal doses of urethan (100 mg/kg) (Merck and Co.) in normal saline. Analyses of meiotic bivalents at diakinesis were made daily for a period of 2 weeks. As in earlier reports (*3*), urethan was totally ineffective in the control animals throughout the 14-day period of observation.

However, in sharp contrast to the negative observations on control animals, meiotic cells of particular EMS-background animals treated with urethan exhibited two distinct forms of cytological anomalies, namely, (i) association of bivalents, end-to-end, leading to a reduction in number of paired elements in diakinesis (Figs. 2, b and c), and (ii) site-specific deletions of the X chromosome at the junction of positive and negative heteropycnotic segments of the long arm (Fig. 2d) which in the somatic X chromosome is the junction of late- and early-replicating segments. Furthermore, the former anomaly was restricted to only those F_1 male-derived progeny examined on the eighth day after urethan treatment, while the distinctive X-chromosome breakage was observed in half of the F_1 female-derived progeny examined on the sixth day after urethan treatment. Observations conducted earlier and later than optimal periods for each population of animals were totally negative. Thus, events leading to either bivalent associations or specific X-chromosome lesions had taken place earlier in the reproductive cell cycle, and were detected in spermatocytes which had progressed to diakinesis by the eighth and sixth days, respectively.

Ninety percent of the cells (206/232) in four males examined on the eighth day had reduced numbers of bivalents (Table 1). The association of two or more bivalents at terminal sites (Fig. 2, b and c) resulted in a reduction in the number of bivalent pairs from 11 to 7, 9, and 10. Occasionally, 12 bivalents were noted. Similar preparations viewed from days 1 through 7 and days 9

through 14 failed to disclose bivalent association (Fig. 3), thereby indicating the narrow time span and size of the responding sensitive cell population.

The highly specific medial breakage of the long arm of the X chromosome is, perhaps, the most impressive evidence yet encountered to suggest the possibility for later generations of mammals to express latent, premutated lesions stemming from an ancestral exposure to a weak mutagen. Two of the four males viewed on the sixth day after urethan treatment had the specific break on the X chromosome in approximately half (48/80) of the cells observed in diakinesis (Fig. 2d). Autosomal breaks were, indeed, rare, and spontaneous breakage was virtually nil in control animals. We interpret the susceptible X chromosome to have stemmed from the grandsires treated with EMS. This reasoning is based upon the following observations: (i) X-chromosome lesions were totally absent among the F_1 male-derived progeny which exhibited only associated bivalents, their X chromosome being contributed by a normal female parent; (ii) the control animals were also totally negative; therefore, urethan may be excluded as the principal mutagen; (iii) with 50 percent of the F_1 female-derived male offspring (2/4) displaying the specific X-chromosome lesion, the 1 : 1 (normal : aberrant) ratio fulfills the expected sex chromosome segregation pattern descending from EMS-treated grandsires.

Since the association and reduction in bivalents and X-chromosome breaks were present in spermatocytes at diakinesis only on specific days following urethan treatment, the mutational events had to take place earlier at particular sensitive stages of the reproductive cell cycle. Judging from the time scale for completion of the initial wave of meiosis in the Armenian hamster (*5*), the sensitive period for the induction of bivalent associations is narrowed to late type B spermatogonial transformation into early spermatocytes. The type B spermatogonia containing crusty chromatin in their nuclei are first noted around the 12th day of age, reaching a plateau by the 15th day. Thereafter a sharp decrease is noted with the appearance of early spermatocytes around the 16th day. This reduction in the number of type B spermatogonia along with the appearance of early spermatocytes is referred to as the type B spermatogonial transformation. In the case of the X-chromosome breakage, the sensitive period for this induction is considered to be early spermatocytes, that is, pre-pachytene, when sex bivalents exhibit striking and distinctive patterns of paired (XY) allocycly, particularly along the long arms which are never involved in crossover configurations as displayed by the short arms of the X and Y chromosomes (*5*).

For the moment, a plausible mutational pathway leading to the observed site-specific deletions of the X chromosome is incomprehensible. However, structural features of the breakage site are being clarified. For example, in mitotic cells, the breakage site is the junction between the proximal, late-replicating and distal, early-replicating halves of the long arm of the active X chromosome (*6*). In spermatocytes, this same site is the junction between positively and negatively heteropycnotic segments (*5*). The latter physiological state is peculiar to the X chromosome, autosomal bivalents being relatively isopycnotic throughout meiotic prophase, and the Y chromosome gradually becoming isopycnotic following a pattern which is totally dissimilar to that exhibited by autosomes and homologous segments of the X chromosomes (Fig. 2, a and d). The manner in which the X chromosome may retain a premutable status for at least two generations is, for the moment, unclear.

Neither EMS nor urethan, administered separately or in combination to control animals, resulted in visible mutations during the immediate period (5 to 8 days) of daily examinations of spermatogonial and meiotic cells. Yet, these same agents, administered several generations apart, fostered latent expressions to become evident. Since urethan is postulated as having provided the

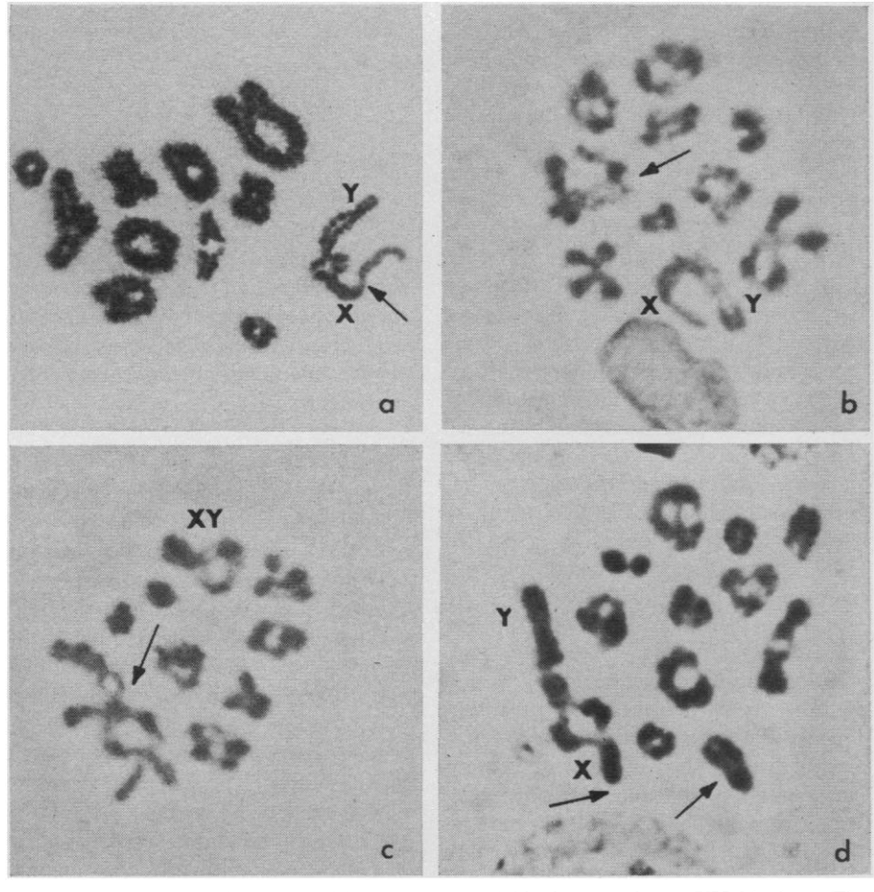

Fig. 2. (a) Normal diakinesis with ten autosomal bivalents and one XY bivalent. The latter exhibits the normal quadriradial configuration indicative of crossing-over medially on the short arms (*5*). Junction of positive and negative heteropycnotic segments of the long arm of the X chromosome is indicated by the arrow (air-dried preparation, lactic-acetic-orcein; × 1400). (b and c) Terminal associations of autosomal bivalents (arrows) noted 8 days after urethan treatment of F_2 males derived from EMS-background F_1 males (acetocarmine squash preparations; × 1400). (d) X-chromosome breaks noted on sixth day after urethan treatment of F_2 males derived from EMS-background F_1 females. Uniformity in size of deletion (right arrow) and length of proximal segment (left arrow) indicates site of breakage to be the junction of early- and late-replicating (positive and negative heteropycnotic) segments of the long arm (× 1900).

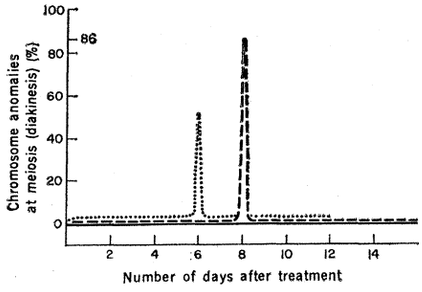

Fig. 3. Frequencies of X-chromosome breakage and association of bivalents in EMS-background F_2 males treated with urethan. Note the sharp increase in X-chromosome breakage (dotted line) and bivalent association (broken line) on the sixth and eighth days, respectively, and the lack of same in control animals (solid line) treated with urethan only. These observations are based on 2326 cells in diakinesis from 105 testes.

additional energy needed to complete the mutational events initiated by EMS in late spermatids-spermatozoa of an earlier generation, it seems reasonable to describe the separate contributions of each agent leading to completion of the mutational process by the term "latent synergism."

As in the earlier findings with drosophilas (*1, 2*), rodents treated with weak mutagens may also express fractional mutations. However, in the case of mammals, delayed expression of fractional mutations in later generations may continue to require exposure to a relatively ineffective and unrelated agent which serves as a source of the energy needed to complete the mutaitonal process. In these trials, latent expressions were witnessed only in particular waves of transitory male germ cells. Thus, direct mutations (dominant lethals and chromosome translocations) induced by EMS tend to occur in late spermatids and epididymal spermatozoa (*7, 8*), whereas latent, incomplete EMS-related mutational states are witnessed only in specific clusters of midspermatocytes of later generations when exposed to urethan. The fact that spermatogenesis in mature males is associated with diurnal waves of meiotic progression means that daily exposures to weak mutagens can be expected to add measurably to the mutational load residing in epididymal sperm.

In the event that fractional mutations appear in future generations of Armenian hamsters when other combinations of agents are substituted, the merits of employing alkylating agents, in particular, for clinical therapy will have to be weighed against plausible overloading of future generations with incomplete forms of mutations. Conceivably, the ever-increasing spectrum and sophistication in the synthesis of new agents can readily serve to provide the required impetus for completion of the mutational process in a later generation. Revelation of highly specific forms of latent mutations in a laboratory rodent, by procedures described herein, is expected to add measurably toward understanding the depth and scope of the many problems related to mammalian mutagenesis.

K. S. LAVAPPA
GEORGE YERGANIAN
Children's Cancer Research Foundation and *Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115*

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Fabry's Disease: Antenatal Detection

Abstract. *A procedure is described for the intrauterine detection, at the 17th week of gestation, of a male fetus afflicted with Fabry's disease. The validity of this determination was substantiated by multiple enzyme and lipid analyses of tissue specimens obtained from the afflicted fetus. Fabry's disease may now be included with other X-linked metabolic deficiency diseases that are amenable to precise genetic counseling, through carrier identification, and the monitoring of ensuing pregnancies.*

Fabry's disease is an inherited metabolic disorder in which the glycosphingolipid galactosylgalactosylglucosyl ceramide (ceramide trihexoside) accumulates in various organs and tissues of the afflicted individuals (*1*). The disease is sex-linked and transmitted on the X chromosome; therefore, only hemizygous males have the severe clinical manifestations of this disease, while the females carry the trait with only mild manifestations. The signs and symptoms of Fabry's disease include severely impaired renal function, a reddish-purple maculopapular rash in the inguinal, scrotal, and umbilical regions, opacities of the cornea, and, in some instances, peripheral neuralgia. The metabolic lesion in this disease is a deficiency of the enzyme which catalyzes the hydrolysis of the terminal molecule of galactose from the accumulating ceramide trihexoside (*2*).

A particularly interesting aspect of this disease is now the subject of considerable controversy. It is believed that the accumulated trihexoside arises from the catabolism of globoside (*N*-acetylgalactosaminylgalactosylgalactosylglucosyl ceramide) from the stroma of senescent red blood cells. The anomeric configuration of the galactosylgalactose linkage in the globoside is considered to be beta (*3*). However, Kint, using artificial substrates, demonstrated a deficiency of α-galactosidase activity in leukocytes obtained from patients with Fabry's disease (*4*). We have fully confirmed Kint's observations with freshly prepared leukocytes from patients with Fabry's disease. This information led us to investigate the possibility that tests with an artificial substrate such as *p*-nitrophenyl-α-D-galactopyranoside might indicate both hemizygous males and carrier females for this condition in assays performed on extracts of skin fibroblasts grown in tissue culture. Fibroblasts from a 4-mm biopsy of skin were grown with minor modifications