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THE MUTAGENIC PROPERTIES OF THE NUCLEOSIDES OF PYRIMIDINE ANALOGUES IN *DROSOPHILA MELANOGASTER*

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Abstract—The mutagenic activity of the 5-halogen derivatives of deoxyuridine (FUdR, BUdR and IUdR), cytosine arabinoside (CA), 6-azauridine (AzUR) and 6-azacytidine (AzCR) were tested on the male germ line of *Drosophila*. All compounds were inactive as regards the induction of point mutations (sex-linked recessive lethals and visibles). They were also virtually ineffective in the induction of viable chromosome rearrangements; the highest yield of X-chromosome fragments occurred with IUdR, but only reached a rate of 1 per 10^4 treated gametes.

The only mutations induced at an appreciable rate by the tested nucleosides were the small chromosome deletions resulting in the *Minute* phenotype. Maximal activity in this respect was of the order of 5 mutations per 10^3 treated sperm and was obtained at an injected concentration of $2{\cdot}0 \times 10^{-2}$ M; further increase in dose did not raise the mutation rate. Mutagenic activity was roughly the same for compounds that could enter DNA (BUdR, IUdR and CA) as with those which were not expected to do so (FUdR, AzUR and AzCR). Activity was higher on cells that could undergo nucleoprotein synthesis and chromosome replication (spermatocytes and spermatogonia) than on stages that were inert in this respect (spermatids and sperm). The damage induced consisted of chromosome deletions involving more than one band of the salivary gland chromosome, suggesting the elimination of several DNA molecules. The chromosome breaks leading to the deletions extended across both chromatids, since the resulting *Minutes* were almost exclusively complete (non-mosaic). The distribution of the *Minutes* among the chromosomes was somewhat more preferential for the IVth chromosome with the tested compounds, except CA.

Consideration was given to the possible genetic hazards to man involved in the chemotherapeutic use of IUdR, AzUR and AzCR. Attention was drawn to the fact that long term genetic hazards were mainly caused by the rise in point-mutations, which persisted for many generations in the human gene pool. Such hazards would not occur with the nucleosides since they were virtually inactive as regards the induction of intragenic changes. Nevertheless, the small chromosome deletions which these agents produced, were expected to result in short term genetic damage in the treated patients and their offspring.

INTRODUCTION

RECENT advances in the understanding of the biosynthesis of nucleic acids, suggested various techniques for the external control of the process, which the pharmacologist utilized in the design of drugs for influencing cellular growth and multiplication. Most successful in this connection was the utilization of various analogues of purines and pyrimidines and their nucleosides. These either inhibit the production of particular deoxyribonucleic acid (DNA) precursors, thus altering or stopping the progress of its

synthesis, or become themselves incorporated into the macromolecule and change its structural and functional characteristics. Biologically, this could either lead to cell death, or its survival with some genetic damage. In the chemotherapy of bacterial, viral and neoplastic diseases, it is desirable to maximize the cytotoxic effect and minimize the genetic hazards. The question now arises, as to how far this has actually been achieved with the structural analogues of pyrimidines.

The non-genetic biological activity of pyrimidine analogues and their nucleosides, mainly pertain to carcinostatic and anti-viral effects. Foremost in this connection are 5-iododeoxyuridine (IUdR) and its cytosine analogue (ICdR), as well as 6-azauridine (AzUR) and 6-azacytidine (AzCR), which were shown to possess tumour inhibitory activity, both in experimental animals and in man.[1, 2] Furthermore, IUdR and ICdR, were shown to have an inhibitory activity against two DNA viruses, *herpes simplex* and *vaccinia*, which suggested that they might be useful in the chemotherapy of potentially lethal viral infections of man,[1] such as smallpox, vaccinial and *herpes simplex* encephalitis, and simian B virus.

The genetic activity of some of the pyrimidine analogues and their nucleosides has, so far, been examined in micro-organisms: mainly bacteriophage and bacteria. When these organisms were cultured under conditions of thymine starvation, but in the presence of 5-bromo, chloro, or iodouracil (BU, ClU, IU) or the corresponding deoxynucleosides (BUdR, ClUdR, IUdR), the thymine in the DNA of the progeny was quantitatively replaced by the administered pyrimidine analogue. During this replacement errors in base incorporation or pairing might occur in the course of DNA synthesis, thus resulting in the appearance of mutations.[3, 4] The activity of some nucleosides on the genetic systems of higher organisms has only been reported in relation to chromosome breakage: 5-fluorodeoxyuridine (FUdR)[5] and deoxyadenosine (AdR)[6] in *Vicia* root tips, and cytosine arabinoside (CA)[7] on human leukocytes in tissue culture. The chromosome breaks induced by these agents, however, fail to rejoin and would be expected to lead to cell death rather than to viable mutations.

The complete lack of information about the mutagenic effects (on the gene level) of the nucleosides in metazoa, prompted the present investigation of the problem on the genetic system of the fruit fly *Drosophila melanogaster*. This system has the great advantage of permitting the detection of all classes of genetic aberrations: point mutations, gene eliminations (small chromosome deletions) and viable chromosome rearrangements. The compounds selected for the investigation included representatives of those which: (a) are incorporated into DNA, such as BUdR, IUdR and CA, (b) fail to enter DNA, but are incorporated into RNA, such as FUdR and (c) cannot enter into nucleic acids, such as AzUR and AzCR. This should enable the determination of whether mutagenicity is a function of DNA incorporation, or whether it is the outcome of metabolic disturbances in DNA synthesis which are common to all the tested compounds. Special attention was given to the mutagenicity of IUdR, because of its potential value in human chemotherapy.

MATERIAL AND TECHNIQUE

The compounds used in this investigation were the 5-halogen derivatives of uracil deoxyriboside: fluoro-, bromo- and iodo-deoxyuridine (FUdR, BUdR and IUdR, respectively), as well as cytosine arabinoside (CA), 6-azauridine (AzUR) and 6-azacytidine (AzCR); all were synthesized in the Department of Pharmacology, Yale

University School of Medicine, and supplied by the kind permission of Professor Arnold D. Welch. For the mutagenicity tests they were dissolved in isotonic saline (0·4% NaCl) at the required concentration and administered by micro-injection into the haemocoel of adult Oregon-K males (30 ± 5 hr after eclosion) so that each fly received an average of 0·25 ± 0·05 μl of solution.

The mutagenicity tests used were: (1) the attached-X technique—with the markers yellow (*y*), vermilion (*v*), forked (*f*)—for the detection of visibles (morphological changes) whether sex-linked recessives or autosomal dominants (especially *Minutes*), as well as the viable chromosome fragments; and (2) the Muller-5 technique for the detection of the sex-linked recessive lethals and visibles. *Minutes* were assayed on the basis of the male progeny only of the attached-X tests, so as to avoid the difficulty of their detection in conjunction with the forked (*f*) phenotype in the females.

Newly arising *Minutes* were classified phenotypically into extremes, $M_{(e)}$; intermediates, $M_{(i)}$ and slights, $M_{(s)}$, according to the degree of expression of the mutation. The criteria used in this classification were the same as those used in comparable experiments with macromolecules.[8] Fertile *Minutes* were crossed to a stock homo zygous for brown (*bw*), ebony (*e*), poliert (*pol*) for the determination of their position on the IInd, IIIrd, or IVth chromosomes respectively. No Ist chromosome *Minutes* could occur among the male progeny of attached-X experiments, since they would be eliminated as hemizygous lethals. The IVth chromosome mutations were further tested for allelic coverage against the recessive markers: cubitus interruptus (*ci*), grooveless (*gvl*), abdomen rotatum (*ar*), bent (*bt*), eyeless (*ey*), sparkling-poliert (spa^{pol}) and shaven (*sv*). These markers are spread along the whole length of the IVth chromosome and permitted the genetical determination of the extent of the induced deletions.

In all experiments the progeny of the treated males was fractionated by repeated matings to an approximately equal number of virgin females at 3 day intervals. The mutation rate for each mating period (or 'brood') was determined separately, so as to permit the determination of the mutagenic response in the different cell stages of the male germ line. Breeding was undertaken in mass cultures, each starting with 10 treated males and an equal number of marker females. Separate scoring records were kept for each culture throughout the various broods, so as to permit the detection of any marked differences in response between males at each injected dose.

RESULTS

1. *Recessive point mutations: visibles and lethals*

The mutation rate with the nucleosides as regards the sex-linked recessive visibles and lethals was determined by both the attached-X and Muller-5 techniques (Table 1). The control rate for the test stock (after injection with isotonic saline) was of the order of 1×10^{-4} for the visibles (with both attached-X and Muller-5) and $1\text{–}2 \times 10^{-3}$ for the lethals (Muller-5). In virtually all the experiments with the nucleosides the rate of point mutations did not rise significantly above the control level, even at the higher tested concentrations. Further increases in dose were impracticable, since the higher concentration used approached the maximal tolerated levels as regards toxicity, and were accompanied with considerable mortality among the treated flies and sterility among the survivors. One of the attached-X experiments with FUdR ($2{\cdot}0 \times 10^{-2}$ M, Table 1) gave a visible rate significantly above the controls, but this was not confirmed in later tests with the same compound. With BUdR, IUdR, AzUR and AzCR, the

TABLE 1. X-CHROMOSOME VIABLE FRAGMENTS, RECESSIVE VISIBLES AND LETHALS INDUCED BY VARIOUS NUCLEOSIDES (MOSAIC MUTANTS ENTERED IN BRACKETS)

		Attached-X						Muller-5				
			Hyperploids		X-recessive visibles				X-rec. visibles		X-rec. lethals	
Compound	Conc. ($\times 10^{-2}$ M)	Females observed	Fragments No.	Fragments Type	Males observed	No.	Mutants per 10^3	Chromosomes tested	No.	Mutants per 10^3	No.	Mutants per 10^3
Control		110682	0	—	120530	16(4)	$0{\cdot}1 \pm 0{\cdot}03$	18234	2	$0{\cdot}1 \pm 0{\cdot}07$	31	$1{\cdot}7 \pm 0{\cdot}31$
FUdR	2·0	31024	1	y^+	33214	12(1)	$0{\cdot}4 \pm 0{\cdot}11$					
	4·1	29236	0	—	30648	2(1)	$0{\cdot}1 \pm 0{\cdot}06$	2592	0	$0{\cdot}0 + 1{\cdot}42$	1	$0{\cdot}4 \pm 0{\cdot}39$
BUdR	1·6	18931	0	—	21737	5	$0{\cdot}2 \pm 0{\cdot}10$					
	3·3	15699	0	—	17586	5	$0{\cdot}3 \pm 0{\cdot}13$	2544	0	$0{\cdot}0 + 1{\cdot}45$	4	$1{\cdot}6 \pm 0{\cdot}79$
IUdR	1·4	37859	3	$3v^+$	41415	7	$0{\cdot}2 \pm 0{\cdot}07$					
	2·8	24043	3(1)	v^+, (v^+f^+), f^+	26128	4	$0{\cdot}2 \pm 0{\cdot}09$	2052	2	$1{\cdot}0 \pm 0{\cdot}70$	3	$1{\cdot}5 \pm 0{\cdot}85$
CA	2·1	15827	1	y^+	16919	2	$0{\cdot}1 \pm 0{\cdot}08$					
	4·1	6664	0	—	6922	0	$0{\cdot}0 + 0{\cdot}53$	1736	1	$0{\cdot}6 \pm 0{\cdot}59$	6	$3{\cdot}5 \pm 1{\cdot}42$
AzUR	2·0	14073	1	y^+	15483	3	$0{\cdot}2 \pm 0{\cdot}11$					
AzCR	2·1	6663	0	—	7385	3	$0{\cdot}4 \pm 0{\cdot}23$					

frequency of visibles was occasionally higher than among the controls, but in no instance did the increase reach decisive statistical significance.

In all experiments the progeny of the injected males was sampled for long periods after treatment (minimum of 5 broods, lasting 15 days) so as to ensure the recovery of sperm exposed to the tested compounds in the earlier stages of spermatogenesis: the spermatocytes and the spermatogonia. It was noticed, however, that the few recovered point mutations were not restricted to any particular brood, indicating that they could arise in all stages of spermatogenesis. Their mechanism of induction, therefore, must be independent of the uptake of the nucleoside into the genic DNA; otherwise they would have been more frequent in the late progeny, during the utilization of sperm derived from treated immature germ cells, which are the only stages capable of nucleoprotein synthesis. Further evidence against the incorporation mechanism of mutagenesis becomes apparent from a consideration of the results with the different nucleosides used in the present study. The only instance of possible activity—as regards point mutations—occurred with FUdR, which is not incorporated into DNA, while its analogues BUdR and IUdR were inactive, although they are known to be incorporated into metazoan DNA.

2. *Viable X-chromosome fragments*

The frequency of hyperploid females in the control and test series with the nucleosides is shown in Table 1. In the control series, none were recovered among a sample of 110,682 tested sperm, which meant that their spontaneous rate could approach $0{\cdot}3 \times 10^{-4}$ (the upper limit of the Poisson expectation for $P = 0{\cdot}025$). This rate was not exceeded with the majority of the tested compounds (Table 1), indicating their virtual inactivity in the production of viable chromosome fragments. In contrast, IUdR showed decisive activity in this respect, producing hyperploid females at the rate of 1 per 10^4 treated sperm.

An idea about the genotype of the hyperploid females induced by the nucleosides can be gathered from the coverage of the 3 markers (*y*, *v*, *f*) used in the attached-X tests. The 3 fragments occurring with FUdR, CA and AzUR, all covered *y*, indicating their inclusion of the free tip of the X-chromosome. This is the most frequent situation with mutagens of semi-random action, like radiation. On the other hand, among the 6 hyperploids with IUdR, 4 showed coverage of *v*, one *v* and *f* (mosaic) and another *f* only. This high frequency of v^+ hyperploids was not encountered with other mutagens, including strong chromosome breakers like radiation and some alkylating compounds. In an attached-X experiment with γ-rays (3300 R), involving the same test markers, none were found to cover only *v*, in a sample of 69 viable fragments, whereas a minimum of 20 (lower limit of the binomial expectation at $P = 0{\cdot}025$) would be expected on the basis of their frequency with IUdR.

The v^+ hyperploids with IUdR were recovered either in different experiments, or in separate cultures of the same experiment, indicating that they were independent mutational events. Of the 4 instances, 2 occurred in the Ist brood and 1 in each of the IVth and VIth broods, suggesting that they could be induced in both the pre- and post-meiotic germ cells. The v^+f^+ mosaic, showed coverage of both markers on the right half of the head only (v^+ right eye); the rest of the fly was *y*, *v*—left eye, *f*, showing that it was devoid of the extra-fragment. All the F_1 hyperploid females were

bred for an F_2 against wild-type males, but they were either sterile or reverted to the marker phenotype. The simplest genetical interpretation of the recurrent IUdR hyperploids, therefore, is that they carried acentric fragments derived from the middle part of the X-chromosome including v^+, hence their loss on breeding. Alternatively, these fragments could have been carried as insertions into the Y-chromosome or the autosomes. Either mechanism must involve at least two breaks—one on each side of v^+—and the recovery of an intercallery segment of the X-chromosome. It is not known, however, whether this is due to the differential induction of the breaks themselves, or is a consequence of variation in their rejoining ability along the chromosome.

3. *Small deletions* (Minutes)

The only mutations induced at an appreciable rate by the nucleosides are the small chromosome deletions resulting in the *Minute* phenotype. At a dose range of 0·5–1·0 per cent (w/v), corresponding to a molarity of $1{\cdot}4$–$4{\cdot}1 \times 10^{-2}$ M for the different agents, they yielded at least double the spontaneous rate in attached-X tests (Tables 2 and 3). The culture series (progeny of 10 males over all broods) in these tests fell clearly into two types recognizable on the basis of the frequency and classes of *Minutes* they contained. These will be referred to as:

i. *Homogeneous*, in which—(a) the mutation rate between series fell within the fiducial limits of the overall mean of the containing experiment—and (b) the various *Minute* phenotypes were represented, and more or less randomly scattered in the successive broods.

ii. *Heterogeneous*, in which—(a) the mutation rate was above the upper limit of the Poisson expectation ($P = 0{\cdot}025$) on the basis of the overall mean of the containing experiment—and (b) the *Minutes* were predominantly of the same phenotype. The majority phenotype was either restricted to one brood of the series and was designated a "mutation bunch", or recurred in most—or all—broods and was referred to as a "mutation run".

The data from the homogeneous culture series only are given in Table 2. Statistical analysis showed that the induced mutation rates with all compounds and at the various dose levels used were significantly higher than among the controls. Replicate experiments at the same dose with each compound gave virtually identical mutation rates, indicating that with these agents there were no serious variations due to secondary factors independent of the treatment, such as male sensitivity and cellular penetration. None of the tested compounds, however, showed an increase in the mutation rate proportional to the injected molar concentration. On dose doubling, the mutation rate remained unchanged with FUdR and BUdR, increased slightly with IUdR and was even depressed with CA, although the change with the latter two compounds was not statistically significant. It can safely be concluded, therefore, that within the dose range here investigated, there was no dose effect in the mutagenicity of the nucleosides as regards the induction of the *Minutes*. The mean mutation rate recovered in the various genetic tests with the different compounds can, therefore, be looked upon as fairly accurate estimates of the maximal mutagenicity that these agents can produce. There were no dramatic differences in the activity of the series of nucleosides investigated; the mutation rates with the cytosine derivatives (CA and AzCR) were somewhat higher than with those of uridine (FUdR, BUdR, IUdR and AzUR), but the difference did not reach statistical significance.

TABLE 2. THE OVERALL AND BROOD *Minute* RATES AMONG THE F_1 MALES FROM ATTACHED-X EXPERIMENTS WITH VARIOUS NUCLEOSIDES (DATA FROM CULTURES WITHOUT RUNS OR BUNCHES OF IDENTICAL MUTANTS; RATES ON LESS THAN 1000 MALES IN SMALL TYPE AND MOSAICS IN BRACKETS)

Compound	Conc. ($\times 10^{-2}$ M)	Males observed	Minute per 10^3 in broods I	II	III	IV	V	VI	VII+	Mutation rate No.	per 10^3
Control		120530	2·0	2·6	1·5	1·2	0·6	1·9	1·1	193(12)	1·6 ± 0·1
FUdR	2·0 (a)	15903	2·1	4·0	4·3	6·9	0·0	3·8	5·8	67(2)	4·2 ± 0·5
	(b)	17311	2·9	2·1	2·8	3·4	4·6	8·1	7·8	71(3)	4·1 ± 0·5
	Total	33214	2·6	3·0	3·4	5·2	2·1	5·9	6·7	138(5)	4·2 ± 0·4
	4·1 (a)	13189	2·5	2·3	6·8	7·7	13·3	11·8	—	60(1)	4·6 ± 0·6
	(b)	17459	4·6	3·4	5·1	4·0	11·4	1·9	0·0	82(1)	4·7 ± 0·5
	Total	30648	3·7	2·8	5·8	5·1	12·1	3·8	0·0	142(2)	4·6 ± 0·4
BUdR	1·6 (a)	12048	2·5	2·7	1·4	3·8	3·0	8·8	—	37	3·1 ± 0·5
	(b)	9689	0·8	0·9	5·1	1·5	8·3	0·0	—	20	2·1 ± 0·5
	Total	21737	1·6	1·7	3·2	3·0	4·6	7·8	—	57	2·6 ± 0·3
	3·3 (a)	7410	4·7	1·8	1·0	4·6	5·2	—	—	21	2·8 ± 0·6
	(b)	10176	0·8	4·4	2·9	3·2	8·7	0·0	—	31	3·0 ± 0·5
	Total	17586	2·3	3·2	2·0	3·7	6·9	0·0	—	52	3·0 ± 0·4
IUdR	1·4 (a)	21093	1·8	1·6	2·0	4·0	2·9	3·7	4·4	58(3)	2·8 ± 0·4
	(b)	20322	1·1	3·3	2·0	3·2	2·6	2·1	4·9	53(4)	2·6 ± 0·4
	Total	41415	1·5	2·4	2·0	3·6	2·8	2·9	4·6	111(7)	2·7 ± 0·3
	2·8 (a)	12766	3·6	1·8	3·1	5·6	7·0	8·6	8·0	44(2)	3·4 ± 0·5
	(b)	13362	2·8	3·6	3·4	8·5	9·1	11·6	6·8	61(3)	4·6 ± 0·6
	Total	26128	3·2	2·7	3·3	7·1	8·3	10·4	7·1	105(5)	4·0 ± 0·4
CA	2·1 (a)	8849	5·7	4·9	5·4	5·6	12·7	6·2	—	51(3)	5·8 ± 0·8
	(b)	8070	6·8	4·7	3·6	7·5	17·7	0·0	—	48	5·9 ± 0·9
	Total	16919	6·2	4·8	4·5	6·6	14·6	3·7	—	99(3)	5·9 ± 0·6
	4·1 (a)	2886	3·7	4·4	3·4	10·5	—	—	—	12	4·2 ± 1·2
	(b)	4036	1·6	4·0	4·9	16·8	9·6	0·0	0·0	15	3·7 ± 1·0
	Total	6922	2·4	4·2	4·0	14·6	9·6	0·0	0·0	27	3·9 ± 0·7
AzUR	2·0	15483	1·6	1·7	4·0	2·4	3·8	2·6	4·0	45	2·9 ± 0·4
AzCR	2·1	7385	1·8	9·1	3·4	6·3	6·3	4·3	0·0	37(1)	5·0 ± 0·8

TABLE 3. THE FREQUENCY AND BROOD DISTRIBUTION OF THE *Minutes* AMONG THE F_1 MALES FROM ATTACHED-X EXPERIMENTS WITH VARIOUS NUCLEOSIDES (DATA FROM CULTURES WITH RUNS (R) OR BUNCHES (B) OF IDENTICAL PHENOTYPES; RECURRING MUTANTS IN ITALICS AND MOSAICS IN BRACKETS)

Compound	Conc. ($\times 10^{-2}$ M)	Series No.	Series R	Series B	Males observed	Minutes in broods I	II	III	IV	V	VI	VII+	Mutation rate* No.	Mutation rate* per 10^3
Control		46	1		3639	1+*12*	1+*3*	1	*1*	0	1+*3*	1+*57*	81(2)	$17{\cdot}5 \pm 11{\cdot}0$
				1	4172	0	3	4	3	1	1+*43*	2	56	
FUdR	2·0	12	1		2905	1+*2*	1+*2*	2+*13*	*2*	*1*	1	2+*15*	42	$14{\cdot}5 \pm 11{\cdot}8$
	4·1	21	2		2090	*2*	*6*	0	*15*	1+*6*	1	—	31	
					1875	0	*9*	*3*	0	*4*	*10*	—	26	$16{\cdot}0 \pm 7{\cdot}7$
				1	1965	3	3	1	4+*25*	1	1	—	38	
BUdR	1·6	12		2	2551	4	0	0	*24*	1	2	—	31	$12{\cdot}9 \pm 6{\cdot}3$
					2094	3	3	0	1+*21*	1	—	—	29	
	3·3	12		1	1311	1	1+*19*	0	3	—	—	—	24	$18{\cdot}3 \pm 14{\cdot}1$
IUdR	2·8	12		1	1259	1	2+*15*	1+*14*	1	0	0	—	34	$27{\cdot}0 \pm 22{\cdot}6$
CA	4·1	17		1	516	2	0	*15*	0	—	—	—	17	$32{\cdot}9 \pm 28{\cdot}6$
AzCR	2·1	4	1		3365	2+*17*	3+*8*	5+*33*	3+*6*	3+*32*	*12*	2+*88*	214(1)	$63{\cdot}6 \pm 56{\cdot}3$

* Standard deviation calculated by Muller's method[9].

The data from the heterogeneous culture series are given separately in Table 3. The recurring mutants (in italics) they contained (whether in runs or bunches) were invariably of the same phenotype (either $M_{(s)}$ or $M_{(t)}$) and behaved similarly as regards fertility. Genetical placing of the fertile recurring mutants showed that they were carried on the same chromosome, and could even have been allelic (involving the same locus). The mutation bunches, and the larger mutant concentrations (> 5 instances) within the runs, were not restricted to any particular broods, although in the treated series they were somewhat more prevalent in the IInd–IVth broods.

For an assessment of the significance of the runs and bunches of identical *Minutes* in the experiments with the nucleosides, it is necessary to compare their frequencies with those occurring in the control tests. As can be seen from Table 3, among 46 series in the control experiments there was an instance of a run and another of a bunch. In comparison, among 90 test series with the different nucleosides there were 4 runs and 6 bunches, neither of which being significantly above the corresponding frequencies among the controls. Clearly, therefore, the occurrence of runs and bunches is quite independent of the treatment. The phenomenon can best be interpreted as due to the spontaneous occurrence of a *Minute*, either in the course of the fly's ontogeny or early in its gametogenesis, which became multiplied by mitoses during the subsequent poliferation cycles. The mutation runs must have been contributed by the males, since these are the only flies used in the successive rematings (broods), among which the recurring mutant was observed. On the other hand, the mutation bunches could have been contributed by either the males or the females used in the genetic test. Their restriction to individual broods might suggest that they were probably contributed mainly by the females, which are supplied fresh for each remating. However, the double bunch in the IUdR experiment must have been supplied by the male, since apparently the same recurring mutant was recovered late in the IInd and early in the IIIrd broods.

It is difficult to determine the number of independent mutations represented by the total *Minutes* recovered in cultures with runs and bunches. The limited phenotypes among the recurring mutants ($M_{(t)}$ or $M_{(s)}$) could easily lead to the inclusion of independent occurrences within bunches or runs of comparable expression. Furthermore the frequency of recurrence of the same mutant would vary with the cell stage affected during embryogenesis, being higher the earlier is the time of the initiation of the mutation. The largest run in the present experiments occurred with AzCR and involved 196 mutants spread over all 7 broods, indicating that the initial mutation could have involved an embryonic cleavage nucleus. It is clear, therefore, that these atypical cultures cannot be used in any accurate analysis of mutagenesis, whether spontaneously or after treatment, and they were accordingly excluded from any further consideration in the present study. It is, nevertheless, interesting to note that the mutation rates in the atypical culture series, when their large errors—calculated by Muller's method[9]—were taken into consideration, did not prove to be significantly different from those for the typical counterparts (compare last columns, Tables 2 and 3).

4. *The role of the cell stage*

The mutagenicity of nucleosides (as regards the *Minutes*) in relation to DNA synthesis and chromosome replication was investigated by a comparison of the

mutation rate induced in the pre- as compared to the post-meiotic stages of spermatogenesis. With the breeding technique used in the present study, the post-meiotic stages (sperm and spermatids) are sampled during the first 9 days after treatment, whereas the meiotic and pre-meiotic stages (spermatocytes and spermatogonia) are used from the 10th day onwards.[10, 11] The post-meiotic stages are inert, as regards *de novo* nucleoprotein synthesis, and their chromosomes do not undergo replication until after fertilization, during embryonic cleavage. On the other hand, the meiotic and pre-meiotic stages are active in nucleoprotein synthesis, since the chromosomes of the spermatocytes undergo at least one replication cycle, and those of spermatogonia undergo several such cycles in the course of their mitotic proliferation. It follows that the relative mutation frequency in the early progeny (first 9 days) as compared to that in later broods (10th day onwards), should give an idea as to the role of DNA synthesis during chromosome replication on the mutation process.

Table 4 demonstrates that the activity of the nucleosides on the post-meiotic stages (sperm and spermatids) is, in general, rather low and in some instances even doubtful. A statistical comparison of the induced and control mutation frequencies in the various genetic tests (0–9 days, Table 4) revealed that the induced rate was significantly higher than the spontaneous level with CA, AzCR, FUdR and the higher concentration of IUdR; but was the same with BUdR, the lower concentration of IUdR and AzUR. In contrast, the same comparison for the meiotic and pre-meiotic stages ($>$ 9 days, Table 4) revealed that the induced rate was invariably and most significantly higher than the control.

Another measure of the role of chromosome replication in the mutagenicity of nucleosides is given by the ratio of the mutation rates in the pre/post-meiotic stages of spermatogenesis (initial ratio, Table 4). This ratio is less than unity for the controls, doubtless because of germinal selection against the *Minutes* in the pre-meiotic stages. Some of the mutant cells among the early stages seemingly fail to complete their differentiation to mature sperm (as is generally the case with hemizygous recessive lethals), thus depressing the spontaneous mutation rate in the later progeny, when the sperm from the early germ cells is utilized. In contrast to the controls, the experimental series invariably showed pre/post-meiotic mutation ratios above unity. When these ratios were corrected for germinal selection (on the basis of the control series) it was found that the mutagenicity of the nucleosides was higher on the pre- as compared to the post-meiotic stages, at least by a factor of 2 (AzUR and AzCR), but often by as much as 3–5 fold (FUdR, BUdR, IUdR and CA).

Minutes, being deletion mutations, are slow in development and of low viability and fertility. They could, therefore, encounter some difficulty in emergence, particularly with poor culture conditions associated with high population density. If this environmental selection is different for the progeny fractions derived from pre- and post-meiotic sperm, this could affect the ratio of their mutation rates. The nucleosides were preferentially cytotoxic on the early germ cells, which meant that the population size would be larger in the early as compared to the late progeny (Table 4). Care was, therefore, taken to avoid culture crowding even in the early progeny, by using two heavily yeasted half-pint bottles per brood (per 10 pairs of parents) and scoring the offspring daily. This resulted in an average number of progeny of about 400 flies per bottle (containing about 65 g of food) in the early fertile broods, which is unlikely to result in adverse selection even against the *Minutes*. In fact, the detailed examination

TABLE 4. THE MUTABILITY RATIOS AS REGARDS *Minutes* IN EARLY GERM CELLS (SPERMATOCYTES AND SPERMATOGONIA; SAMPLED ON THE 10TH AND LATER DAYS) RELATIVE TO THAT IN THE LATE STAGES (MATURE SPERM AND SPERMATIDS; SAMPLED 0–9 DAYS)

Compound	Conc. ($\times 10^{-2}$M)	Progeny sampled: 0–9 days Males observed	0–9 days Minutes No.	0–9 days Minutes per 10^3	> 9 days Males observed	> 9 days Minutes No.	> 9 days Minutes per 10^3	Mutation ratio late/early progeny: initial	Mutation ratio late/early progeny: corrected for control
Control		56928	116(3)	$2{\cdot}0 \pm 0{\cdot}2$	63602	77(9)	$1{\cdot}2 \pm 0{\cdot}1$	$0{\cdot}6 \pm 0{\cdot}1$	$1{\cdot}0 \pm 0{\cdot}2$
FUdR	2·0	18300	54(1)	$3{\cdot}0 \pm 0{\cdot}4$	14914	84(4)	$5{\cdot}6 \pm 0{\cdot}6$	$1{\cdot}9 \pm 0{\cdot}3$	$3{\cdot}2 \pm 0{\cdot}5$
	4·1	23912	94(2)	$3{\cdot}9 \pm 0{\cdot}4$	6736	48	$7{\cdot}1 \pm 1{\cdot}0$	$1{\cdot}8 \pm 0{\cdot}3$	$3{\cdot}0 \pm 0{\cdot}5$
BUdR	1·6	14912	29	$1{\cdot}9 \pm 0{\cdot}4$	6825	28	$4{\cdot}1 \pm 0{\cdot}8$	$2{\cdot}2 \pm 0{\cdot}6$	$3{\cdot}7 \pm 1{\cdot}0$
	3·3	13634	35	$2{\cdot}6 \pm 0{\cdot}4$	3952	17	$4{\cdot}3 \pm 1{\cdot}0$	$1{\cdot}7 \pm 0{\cdot}5$	$2{\cdot}8 \pm 0{\cdot}8$
IUdR	1·4	20737	41(2)	$2{\cdot}0 \pm 0{\cdot}3$	20678	70(5)	$3{\cdot}4 \pm 0{\cdot}4$	$1{\cdot}7 \pm 0{\cdot}3$	$2{\cdot}8 \pm 0{\cdot}5$
	2·8	21140	65(3)	$3{\cdot}1 \pm 0{\cdot}4$	4988	40(2)	$8{\cdot}0 \pm 1{\cdot}4$	$2{\cdot}6 \pm 0{\cdot}6$	$4{\cdot}3 \pm 1{\cdot}0$
CA	2·1	14066	75(3)	$5{\cdot}3 \pm 0{\cdot}6$	2853	24	$8{\cdot}4 \pm 1{\cdot}7$	$1{\cdot}6 \pm 0{\cdot}4$	$2{\cdot}7 \pm 0{\cdot}7$
	4·1	6334	21	$3{\cdot}3 \pm 0{\cdot}7$	588	6	$10{\cdot}2 \pm 4{\cdot}1$	$3{\cdot}1 \pm 1{\cdot}4$	$5{\cdot}2 \pm 2{\cdot}3$
AzUR	2·0	6786	17	$2{\cdot}5 \pm 0{\cdot}6$	8697	28	$3{\cdot}2 \pm 0{\cdot}6$	$1{\cdot}3 \pm 0{\cdot}4$	$2{\cdot}2 \pm 0{\cdot}7$
AzCR	2·1	4691	22	$4{\cdot}7 \pm 1{\cdot}0$	2694	15(1)	$5{\cdot}6 \pm 1{\cdot}4$	$1{\cdot}2 \pm 0{\cdot}4$	$2{\cdot}0 \pm 0{\cdot}7$

of the data for the separate experimental bottles showed no disproportionate correlation between the size of the emerging population and the number of mutants they contained, neither for the pre-, nor for the post-meiotic progeny. The role of environmental selection in disturbing the ratio of the mutation rates in the two compared germ-line sectors, therefore, can be safely ignored. It would thus follow that the higher activity of the nucleosides in the later progeny, is a genuine manifestation of the high mutagenic response of the early germ cells. This can best be interpreted as the result of disturbances produced by the nucleosides during chromosome replication in the course of spermatogonial proliferation. The weak mutagenicity on the post-meiotic stages—when it occurs—might be attributed to comparable effects during cleavage. The administered nucleoside could have been carried passively in the sperm and subsequently interfered with chromosome replication during the first mitosis after fertilization. Evidence for this process was found in recent experiments with homologous DNA,[12] where the paternal and maternal X-chromosomes were distinguishable by genetic markers. Injection of the polymer into the males induced a mutation rate significantly above the control level in some loci of the untreated maternal-X.

The frequency of mosaic *Minutes* (entered in brackets in Table 4) produced by the nucleosides in the pre- as compared to the post-meiotic sperm is of significance to the understanding of the nature of the damage induced across the treated chromosome. On the whole, the frequency of mosaics with the nucleosides was low and of the same order as that among the spontaneous mutants. The proportion of mosaicism in the test series was roughly the same in the pre- and post-meiotic stages. This is so in spite of the fact that the younger germ cells are: (a) more affected by the nucleosides, and (b) often undergo proliferation after damage; both factors favouring complete rather than mosaic mutations. It would thus seem probable that the nucleoside mutations arise as complete damage across the chromosome, and the few mosaics encountered in the test series are of spontaneous origin.

5. *Phenotypes and sites of induction of* Minutes

The three phenotypic classes of *Minutes*, $M_{(e)}$, $M_{(i)}$ and $M_{(s)}$, distinguishable mainly on the basis of the length of the macrochaetes (see technique), were all represented among the mutations induced by the nucleosides. Samples of mutants from these classes were placed genetically as to chromosome against a stock homozygous for brown (*bw*), ebony (*e*), poliert (*pol*). A total of 200 mutants were analysed in this way and were found to show the same correspondence between phenotype and genetic position as was established for the macromolecules.[8] Among 107 $M_{(e)}$ mutations, 105 occurred on the IVth chromosome, and only 2 were on the large autosomes (one on each of IInd and IIIrd). The $M_{(i)}$ and $M_{(s)}$ mutants (93 tested instances), all occurred on the IInd or IIIrd chromosomes.

The $M_{(e)}$ mutations with the nucleosides were phenotypically identical to those induced by macromolecules, and showed the same pattern of allelic coverage against the IVth chromosome recessive visibles. Positive interaction (revealing of the marker's phenotype) invariably occurred in crosses against *ci–pol* inclusive: *ci*, 61; *gvl*, 38; *ar*, 8; *bt*, 23; *ey*, 32; *pol*, 95. Crosses between $M_{(e)}$ and *sv* (as with *ar*) were virtually lethal, but in 12 instances, *Minutes* were recovered and were sv^+, indicating negative interaction (suppression of the marker's phenotype) with this locus. There can be no

doubt, therefore, that the $M_{(e)}$ deletion with the nucleosides is identical to that of macromolecules; genetically it extends from *ci–pol* inclusive, but falls short of *sv*, and cytologically it involves the deletion of the major part of the IVR salivary chromosome—from the centromere to the end of segment 102D.[8] It is, nevertheless feasible that the deleted IVth of the $M_{(e)}$ mutation could secondarily be lost, thus leading to its conversion to the *haplo*-4 genotype.

The establishment that the $M_{(e)}$ mutants with the nucleosides were the result of IVth chromosome damage, permitted the determination of the selectivity for this chromosome as the proportion of these mutants among the induced *Minutes* (Table 5).

TABLE 5. PHENOTYPIC EXPRESSION OF *Minutes* RECOVERED IN THE ATTACHED-X EXPERIMENTS WITH VARIOUS NUCLEOSIDES

Compound	Conc. ($\times 10^{-2}$ M)	*Minutes* No.	*Minutes* per 10^3	Phenotype $M_{(e)}$	Phenotype $M_{(s+t)}$	Percentage $M_{(e)}$ IVth chrom. mutants
Control		181	1·6	63	118	34·8±3·5
FUdR	2·0	133	4·2	69	64	47·3±3·0
	4·1	140	4·6	60	80	
BUdR	1·6	57	2·6	27	30	41·3±4·7
	3·3	52	3·0	18	34	
IUdR	1·4	104	2·7	50	54	46·6±3·5
	2·8	100	4·0	45	55	
CA	2·1	96	5·9	32	64	31·7±4·2
	4·1	27	3·9	7	20	
AzUR	2·0	45	2·9	20	25	44·4±7·4
AzCR	2·1	36	5·0	16	20	44·4±8·3

This proportion was fairly homogeneous and independent of the injected dose for each of the tested compounds. On the other hand, when the relative frequency of $M_{(e)}$ to $M_{(s+t)}$ for the control and the 6 tested compounds were compared in a 2×7 contingency table, a decisive heterogeneity was revealed ($\chi^2_{[6]} = 14{\cdot}3$; $P = 0{\cdot}03$). The largest and only significant contributions to the heterogeneity χ^2 were those of the controls and CA, indicating their deviation from the rest. A comparable statistical test on the proportion of $M_{(e)}$ with FUdR, BUdR, IUdR, AzUR and AzCR showed no heterogeneity ($\chi^2_{[4]} = 1{\cdot}2$; $P = 0{\cdot}9$), all producing a mean $M_{(e)}$ percentage of $45{\cdot}7 \pm 1{\cdot}9$. The corresponding proportion for CA, only reached $31{\cdot}7 \pm 4{\cdot}2$, which was of the same order as that occurring spontaneously and after mutagens of semi-random action, like radiation.[8] The significantly higher frequency of the $M_{(e)}$ mutants ($P < 0{\cdot}01$) with the rest of the tested nucleosides can be looked upon as an indication of their slightly higher selectivity for the IVth chromosome.

6. Genetic hazards of nucleosides in man

The nucleosides which proved of some value in human chemotherapy are IUdR, AzCR and AzUR; all being used in the treatment of leukaemia and allied neoplastic diseases. On the whole massive doses are required: an average daily dose of 80–400 mg/kg body weight; the lowest being used with IUdR, the intermediate with AzCR and the highest with AzUR. The daily amounts are administered into the blood stream usually in 3 fractions, either by intravenous injections at 8-hr intervals or by 2-hr

infusions. Most leukaemic patients require a 5-day therapeutic course, which means a total exposure to the chemical agents ranging from 0·4–2·0 g/kg.

In the design of the mutagenicity tests in *Drosophila*, an attempt was made to simulate the human therapeutic exposure, both as regards dose range and method of administration. The compounds were dissolved at a concentration of 0·5–1·0 per cent (w/v) and administered by micro-injection into the haemocoel (blood circulation) at an approximate volume of 0·3 μl per male—each weighing about 1 mg. The physical dose around the male's testes, therefore, ranged from 1·7–3·4 μg/mg fly, which is equivalent to—or slightly above—the higher levels of human therapeutic exposure (per mg of tissue) to the used nucleosides (compare first two rows, Table 6). Chemical

TABLE 6. THE MUTAGENIC EFFECTS IN *Drosophila* PRODUCED BY THE EQUIVALENT OF THE TOTAL AMOUNTS OF NUCLEOSIDES USED IN CANCER CHEMOTHERAPY, RELATIVE TO THOSE PRODUCED BY CELLULAR MACROMOLECULES

Compound	IUdR	AzCR	AzUR
Dosimetry			
Total therapeutic dose in man [g/Kg (= μg/mg) body weight]	0·4–0·6	0·6–0·8	0·5–2·0
Mutagenicity dose-range in *Drosophila* [μg/mg body weight]		1·7–3·4	
Mutagenic effects in *Drosophila*			
Absolute: deletions per 1000 sperm	3·2	5·0	2·9
Ratio of mutation rates relative to			
Spontaneous	2·0	3·1	1·8
Macromolecules			
Histones (0·5 μg/mg)	0·8	1·2	0·7
Undegraded DNA (1·7 μg/mg)	1·3	2·0	1·2
RNA (3·4 μg/mg)	1·3	2·1	1·2

exposure in the genetical experiments was in a single dose in an open blood circulation (bathing all tissues), whereas the therapeutic exposure was fractionated and was introduced in a closed vascular system. The open circulation would speed up excretion and detoxication and the lack of fractionation would shorten the time of treatment with the active drug. Both these factors would operate against the biological activity of the drugs in the genetical experiments, but would perhaps be compensated for by the higher administered doses. The exposure of the tissues to the nucleosides in the therapeutic treatments and the genetical tests, might accordingly be looked upon as very roughly equivalent.

The mutagenic effects produced in *Drosophila* by the equivalent of the human therapeutic exposure to the nucleosides were compared with those occurring spontaneously and after treatment with natural macromolecules (lower part, Table 6). Only small chromosome deletions (*Minutes*) were induced, at a rate varying from 3–5 per 1000 sperm with the different compounds. This is about 2–3 times the spontaneous rate for the same mutations in the fly, and might accordingly be considered as approximately its "doubling dose". How far this is applicable to man, however, is difficult to assess. Deletion mutations doubtless occur in human populations—as in the case of chronic myeloid leukaemia—but nothing is known about their spontaneous frequency or rate of induction in mammalian tissues with any mutagen. The indirect method[25] used for the extrapolation of the chemical mutagenicity results in *Drosophila* to man (on the basis of the mutagenically equivalent doses of radiation in the two

organisms) was inapplicable in the case of the nucleosides, which—unlike radiation—did not give a proportionate increase in mutability with increase in dose. Nevertheless, the fact that exposure of the fly's testes to the equivalent of the nucleosides' therapeutic dose only doubles the spontaneous deletion rate, must mean that the genetic hazards they might induce in man cannot be very great.

A better appreciation of the genetic hazards of the nucleosides, appears from a comparison of their activity relative to natural macromolecules, which were shown[12–15] to induce the same types of mutations. When solutions of nucleic acids (both DNA and RNA) as well as proteins (basic, acidic and neutral)were injected into the haemocoel of *Drosophila* males, they were exclusively active in the induction of *Minutes*. The doses of histones, DNA and RNA injected, and the ratios of the mutation rates they produced compared to the nucleosides, are shown in Table 6. The range of concentrations used with both chemical series were roughly the same, and so also was the order of their mutagenicity; the ratios between their mutation rates ranged from a minimum of 0·7 for AzUR compared to histones, to a maximum of 2·0 for AzCR compared to DNA or RNA.

DISCUSSION

Mutagenesis studies with DNA base analogues and their nucleosides on micro-organisms (bacteriophage and bacteria) left no doubt that the mechanism involved consisted of base pairing errors during DNA replication.[3, 4] These errors were attributed to the tendency of some substituted base analogues (such as 5-bromouracil, BU; or 2-aminopurine, AP) to undergo ionization, or tautomerism, thus initiating a dual potentiality in their base-pairing properties. The switch in pairing—from the regular to the rare complementary base—could either occur at the moment of incorporation of the mutagenic base into DNA, or at some later stage in the course of its replication. Errors of incorporation must be initiated during the exposure pulse and their realization as mutants in synchronized systems would occur in a single burst. On the other hand, errors of replication could be initiated any time during DNA synthesis, and the resulting mutants would not accordingly be restricted to any particular generation during cell multiplication.

The mode of action of nucleosides on the genetic system of *Drosophila* was found to be completely different from that with micro-organisms. All the tested nucleosides—including the strongest microbial mutagen BUdR—were inactive in the induction of point mutations (sex-linked recessive visibles and lethals) which, in metazoa, are likely to include changes in the order of the bases of the genic DNA, analogous to the transitions and transversions of micro-organisms. This is not surprising in the case of FUdR, AzUR and AzCR which cannot be incorporated into DNA, but is rather unexpected for BUdR and IUdR, which do enter into the DNA of metazoan nuclei.[16–19] IUdR was also found to be readily incorporated into the chromosomes of *Vicia*.[20] This means that the pyrimidine analogues that do enter into the DNA of higher organisms either successfully undertake the functions of the normal bases, or lead to cell death; but do not cause base pairing errors leading to viable mutations. In the present experiments BUdR and IUdR caused a high mortality among the injected males and produced sterility among the survivors, both indicating considerable cell death as a result of the treatment.

Metazoan cell death after treatment with nucleosides could be associated with their

chromosome breaking activity, which was demonstrated cytologically in both plant and animal material. This activity was most marked with FUdR,[5] AdR[6] and CA.[7] However, BUdR and IUdR do not seem to be effective chromosome breakers,[5, 21] although there are some reports indicating that they also might well be weakly active.[22] An important feature of the breaks induced by the nucleosides, is that they tend to remain open rather than rejoin. They would, therefore, be expected to lead to dominant lethals and cell death, instead of chromosome restitution and viable rearrangements. This is confirmed by the present results with the attached-X technique where very few viable X-chromosome fragments (hyperploid females) were recovered with the various tested nucleosides. The highest frequency in this respect occurred with IUdR, but it only reached a rate of about 1 per 10^4 treated sperm. Most interesting is the fact that the majority of the IUdR fragments covered *v*, a situation which was not encountered with comparable experiments with radiation. This is an indication of some selectivity in the induction, or rejoining, of the chromosome breaks occurring with IUdR.

The only viable genetic damage occurring with the nucleosides is the induction of small chromosome deficiencies and deletions resulting in the *Minute* phenotype. The mutation rate in this respect was of roughly the same order for the compounds tested, reaching about 4 mutations per thousand treated sperm at an injected dose range of $1{\cdot}4\text{–}2{\cdot}0 \times 10^{-2}$ M. This rate was not substantially increased on doubling the injected concentration and was independent of whether the nucleoside could enter the genic DNA (BUdR, IUdR, CA) or was unacceptable in this respect (FUdR, AzUR, AzCR). Clearly, therefore, the mechanism of induction of the *Minutes* by the nucleosides, cannot be a function of their incorporation into the genic DNA. The only other biochemical property of these agents, which could lead to mutagenic effects, is their inhibitory action on some enzymes required for the synthesis of DNA precursors.[23] The metabolic disturbances are known to start at the nucleotide level, but this could lead to the inhibition of large stretches of DNA, whose synthesis requires the availability of all four nucleotides. This is compatible with the size of chromosome deletions known to be associated with *Minutes*, which were found to involve more than one band in the salivary gland chromosomes, suggesting the elimination of several DNA molecules. The process of DNA elimination would be expected to involve the new chromosome replica, rather than the original template, a situation which would lead to mosaic mutations. The fact that such mosaicism did not occur with the nucleosides, could only mean that the induced damage in the newly synthesized DNA strands was sufficiently extensive to lead to the "nipping out" of a corresponding part of the old complementary chain, possibly as a result of mechanical strain during anaphase chromosome movements.

Evidence has been adduced that *Minutes* are due to deletions in the heterochromatin[24] and this has further been supported by the recent finding that the macromolecular mutations were restricted to the heterochromatic segments of the chromosome complement.[8] The fact that nucleosides are only active in the induction of *Minutes*, must also mean that they are selective for the heterochromatin. This selectivity, however, is differential for the different heterochromatic segments of the genome, as indicated by the variation in the proportion of IVth chromosome mutations between agents. The IVth *Minutes* constituted about a third of the CA mutations (which is the same as with radiation), as compared to nearly half those induced by

the halogen derivatives of deoxyuridine. This could mean that there is a higher concentration of thymidine in the atypical heterochromatin[24] of the IVth chromosomes, thus making the shortage of thymidylate more damaging to them.

The selective activity of macromolecules and nucleosides for heterochromatin helps in the understanding of a puzzling feature of their mutagenicity. They were active in the induction of *Minutes*, which are deletion mutations, but not effective as regards the sex-linked recessive lethals, although these also are sometimes associated with the same chromosome aberrations. This might suggest that deletions need to involve some loss of euchromatic material (perhaps on the molecular level) in order to be expressed as recessive lethals. Nevertheless, a large heterochromatic deletion (of the size associated with the $M_{(e)}$ mutation) occuring in the X-chromosome would be expected to lead to a sex-linked recessive lethal. However, the frequency of these large X-deletions—on the basis of the induced rate of autosomal *Minutes*—would be too small ($<$ 1 per 1000 chromosomes) to be detectable above the spontaneous variation in experiments of manageable size.

The elucidation of the mutagenic mode of action of the nucleosides in *Drosophila*, enabled some assessment of the possible genetic hazards involved in their use in human chemotherapy. As would be expected, the severity of chemical genetic hazards to man is not only dependent on the number of mutations a given exposure could add to the human gene pool, but also on the persistence and spreading of these mutations beyond the exposed population. The amounts of nucleosides used in the treatment of leukaemia were shown to be mutagenically equivalent to the "doubling dose" in *Drosophila* and could probably contribute to human hazards by nearly the same factor. There was no way, however, of determining this factor quantitatively, because of the lack of a dose effect in the mutagenicity of the nucleosides. This stricture did not occur with the alkylating compounds used in cancer chemotherapy, for which it was possible to assess the human genetic hazards—in terms of "doubling dose"—on the basis of their mutagenicity equivalence compared to radiation.[25] A full course of treatment with "Myleran" or "Melphalan" was estimated to produce 1–2 times the "doubling dose", while that with triethylenemelamine (TEM), was equivalent to 300 times this dose. However, the real genetic danger of the alkylating compounds, as compared to the nucleosides, lies in the persistence—rather than the frequency—of the mutations they induce. A large proportion of these mutations are recessive intra-genic changes, which are expected to persist in human populations for many generations. In contrast, the nucleosides only induce chromosome deletions, some of which might show dominant genetic expression. Gametes carrying the larger of these deletions will probably die either before or after fertilization (gametic or zygotic lethals), thus preventing their entry into the human gene pool. The less drastically affected gametes (with single small deficiencies) might occasionally lead to the production of viable offspring, and hence the introduction of some dominant mutations into the population. The persistence of these mutations, however, is expected to be rather short, and they would soon be eliminated through the death or sterilization of their carriers.

Deletion mutations were induced by natural macromolecules in *Drosophila*, sometimes even at a higher frequency for the same dose than the nucleosides, as with the histones and degraded DNA. A comparable situation might occur in man, but it would be difficult to assess the amounts of natural macromolecules released in the body relative to those of the nucleosides used in therapy. Nevertheless, a substantial

quantity of such molecules must be released in the human body in the course of a lifetime, as a result of the continuous process of cellular degeneration and the multitudes of viral infections. The magnitude of genetic disabilities produced in this "natural" way, could easily approach that resulting from the chemotherapeutic use of nucleosides.

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