SUMMARY

A study was undertaken in Drosophila of the mutagenic properties of representatives of the various carcinogenic chemical series: alkylating agents, nitroso compounds, hydrocarbons, aromatic amines, and urethan. Mutations arising from direct intramolecular DNA damage, such as point mutations and chromosome breaks leading to viable rearrangements, occurred only with the alkylating agents and the nitroso compounds; urethan and its metabolites were also weakly effective as regards point mutations in mature sperm. A statistical analysis was undertaken for the assessment of whether a rank correlation occurred between the activities of several alkylating agents with respect to the induction of point mutations and pulmonary tumors, but this proved to be insignificant.

All the tested compounds were active in the induction of small chromosome deletions resulting in Minutes, and a suggestive correlation between this function and carcinogenicity seemed to occur both between and within the various chemical series. The induction of these deletions with the hydrocarbons and aromatic amines and, in part at least, even with the alkylating carcinogens, appeared to be indirect; this was probably due to the in situ interference with the reproduction or repair of genic DNA.

INTRODUCTION

The association of cancer with chromosome aneuploidy dates back to Boveri (4), but it was Little and Tyzzer (33) who first recognized the marked similarity between the processes of mutation and malignant change and who demonstrated the role of genes in the initiation, perpetuation, and transplantation of tumors. This led to the formulation of the somatic mutation theory of cancer (1), which has always occupied a central, although controversial, position in fundamental cancer research. An exposition of the development of this theory and an evaluation of its bearing on the cancer problem on the basis of both the genetic and oncological data before 1955 has already been dealt with by Burdette (7). His conclusion was that somatic chromogene point mutations cannot be regarded as a general mechanism of cancer initiation, although they might contribute to this process in special cases. At that time little was known about the role of other classes of mutation in carcinogenesis, and Burdette admitted that his evaluation of the problem was handicapped by the lack of any information regarding the chemical basis of both of the biological phenomena that he was examining.

Recent advances in molecular biology enabled the interpretation of induced mutations in terms of chemical changes in DNA structure, either as a result of direct attack by mutagenic agents (26) or through some indirect interference during its replication (17—24) or repair (44). Considerable information has also accumulated in recent years (especially from 1960 onwards) as to the metabolism of chemical carcinogens and the reactions of their metabolites with cellular macromolecules (39). As a consequence, it has become largely possible to describe the mutation process in terms of chemical reactions and to assess whether these are also likely to occur with carcinogens or their metabolites. The study of the correlation between mutagenesis and carcinogenesis was thus brought to the molecular level and has accordingly been released, in part at least, from the semantic ambiguities of both genetic and oncological terminology.

In this study it will be shown that representatives of all classes of chemical carcinogens were effective in the induction of small chromosome deletions resulting in the Minute phenotype in Drosophila. The induction of these deletions with the more potent carcinogens, such as the hydrocarbons and aromatic amines, was not due to direct damage to the genic DNA, since it was not accompanied by the production of point mutations or chromosome breaks. Their initiation, accordingly, must have been indirect through some interference with any of the cellular metabolic systems (enzymes or their regulators) which are involved in DNA synthesis, replication, or repair. This brings the somatic mutation theory of carcinogenesis in line with the growing body of biochemical evidence that neoplastic initiation does not always seem to be strictly correlated with direct attack on DNA, to the exclusion of other cellular macromolecules, particularly proteins and RNA (29, 36, 37, 39).
MATERIALS AND METHODS

The compounds to be considered in the present study are among the more potent carcinogens of their respective chemical series, since the aim was to determine whether they share some common mutagenic properties. From the chemical point of view they can be classified into the following series: (a) alkylating compounds, (b) nitroso compounds (amine and amide derivatives), (c) polycyclic hydrocarbons, (d) aromatic amines, and (e) urethan and its derivatives. All compounds were tested for mutagenicity on the male germ line of Drosophila melanogaster, either by microinjection into adults or by feeding to larvae. The injection technique necessitated the administration of the compounds in true solution, preferably in water. Accordingly, attempts were made at converting the compounds into water-soluble derivatives. Otherwise they were dissolved in arachis oil or in aqueous dimethylformamide: 1 to 5% was generally sufficient for dissolving the biologically tolerated injection technique necessitated the administration of the compounds in true solution, preferably in water. Accordingly, attempts were made at converting the compounds into water-soluble derivatives. Otherwise they were dissolved in

The mutagenic activity of the various compounds was assayed as regards: (a) the sex-linked recessive lethals by the Muller-5 technique (3), and (b) Minutes and viable X-fragments by the attached-X technique (12, 17). The frequencies of each class of mutation in the various stages of spermatogenesis were determined by the technique of progeny fractionation (or “brooding”). The treated males were repeatedly mated to virgin females (in pair or mass cultures with equal male to female ratio) at set intervals (1 to 3 days), and the mutation frequency in each mating period or “brood” was determined separately. The speed of germ cell sampling with the above technique under standard conditions of culturing and for treated young males (30 ± 5 hours after hatching) was found, in days after injection (14), to be as follows: 0 to 3 for mature sperm; 5 to 9 for spermatids in spermateliosis; 10 to 14 for spermatocytes; and from the 15th day onwards for spermatogonia.

As is the rule with chemical mutagenesis, the genetically active carcinogens induced both complete (across the whole chromosome) and mosaic (subchromosomal) mutations. Both these types can readily be scored as regards the visible (morphologically detectable) mutations in the soma of the F1 progeny from attached-X tests. Accordingly, complete and mosaic mutants were taken into consideration in the assay of visible mutation frequencies: Minutes. On the other hand only the gonadal complete recessive lethals could be measured accurately in the F2 of the Muller-5 technique, and the breeding of further generations would be required even for a partial assessment of the gonadal mosaic lethals; the corresponding mutations in the soma are undetectable. In the present study, only the complete gonadal sex-linked recessive lethal frequencies were assayed. These do not, therefore, represent the full activity of the various compounds as regards the lethal mutations, but they do give a fair indication of the relative activity between compounds for this mutational class.

RESULTS

Mutations in Drosophila may conveniently be classified into 3 levels of primary genetic damage of increasing order of severity: (a) point mutations, (b) gene eliminations, and (c) chromosome breaks leading to major rearrangements. Point mutations are exemplified by the recessive lethals and visibles which are unassociated with any cytologically detectable changes in the salivary gland chromosomes. By analogy to the situation in microorganisms, they are believed to be the result of changes in the order of the bases within a short segment of DNA (26), either through base interchanges (leading to transitions and transversions) or as a consequence of “frame shifts” in triplet reading due to losses or gains of single bases. Gene eliminations are manifested as deletions of one or a few bands in the salivary gland chromosomes which suggest that they involve the loss of whole DNA molecules. These deletions frequently occur among the dominant visible mutations known as Minutes, although they may also be associated with some recessive lethals and visibles. The most drastic genetic effects result from chromosome breaks followed by major rearrangements. Aneucentric rearrangements, such as dicentric bridges and acentric fragments, lead to dominant lethality (11) and could not, therefore, result in permanently viable genetic effects. On the other hand, eucentric rearrangements (12), such as large deletions, inversions, and translocations, do lead to heritable effects either through a mutation at one of the breakage points or through a position effect due to the new genetic sequence after the rearrangement.

Mutagenesis studies in Drosophila have revealed that all of the above 3 types of primary genetic damage could arise spontaneously, and they did occur at low frequencies in the present control experiments (Table 1) with the vehicles used in the administration of the various compounds. The activity of carcinogens in the induction of each class of primary genetic damage will now be examined separately.

Point Mutations. The high efficiency of the alkylating compounds in the induction of point mutations in cellular genetic systems, as exemplified by Drosophila, has already been emphasized (3, 7, 13–16) and need only be mentioned here in brief outline. Representatives of the main alkylating subseries—chloroethylamines, ethyleneimines, epoxides, and sulfonic acid esters—were tested in our laboratory and all proved active to various degrees as regards the induction of point mutations, usually assayed as sex-linked recessive visibles and lethals. The mutation frequency in this respect varied according to the dose and molecular structure of the alkylating compound (10, 15, 16), as well as a function of the biological organizations and physiological state of the genome in the recipient cell (20). The variation in the mutagenic cell stage response among the stages of the testis to the alkylating agents was on the whole quantitative, i.e. a matter of degree. A notable exception, however, is
Somatic Mutation Theory

Table 1
The mean control mutation frequencies in all stages of spermatogenesis occurring after treatment of larvae or adults with the various vehicles used in the administration of the tested compounds

<table>
<thead>
<tr>
<th>Genetic test</th>
<th>Concentration (M)</th>
<th>Treatment</th>
<th>Progeny tested</th>
<th>Mutants</th>
<th>Per 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-recessive lethals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.07</td>
<td>L.F., A.I.</td>
<td>18,234</td>
<td>31</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>0.68</td>
<td>L.F., A.I.</td>
<td>5,408</td>
<td>13</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>DMF (in water)</td>
<td>1.36</td>
<td>A.I.</td>
<td>882</td>
<td>1</td>
<td>0.6 ± 0.6</td>
</tr>
<tr>
<td>Autosomal Minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.07</td>
<td>L.F., A.I.</td>
<td>120,530</td>
<td>193</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>0.68</td>
<td>L.F., A.I.</td>
<td>1,291</td>
<td>2</td>
<td>1.5 ± 1.1</td>
</tr>
<tr>
<td>DMF (in water)</td>
<td>1.36</td>
<td>A.I.</td>
<td>51,271</td>
<td>161</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Viable X-fragments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.07</td>
<td>L.F., A.I.</td>
<td>110,682</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>0.68</td>
<td>L.F., A.I.</td>
<td>1,104</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>DMF (in water)</td>
<td>1.36</td>
<td>A.I.</td>
<td>50,257</td>
<td>1</td>
<td>0.02 ± 0.02</td>
</tr>
</tbody>
</table>

* A.I., adult injection, into the hemocoel at a volume of 0.25 ± 0.05 μl per male; L.F., larval feeding, mixed with the standard culture medium; DMF, dimethylformamide.

S-2-chloroethylcysteine (14), which was highly mutagenic on the spermatocytes and spermatogonia while being virtually inactive on mature sperm. This serves to illustrate the unreliability of negative results in mutagenesis, particularly when based on limited results with a few test systems. The order of mutagenicity of the alkylating agents as regards sex-linked recessive lethals on various sectors of the testis, after larval or adult feeding and with intrahemocoelic injection into adults, can be gathered in Table 2. Another chemical series which showed decisive activity regarding point mutations was the nitroso compounds, both the amine and amide derivatives. The order of their activity in this respect on the testis of adult Drosophila (25, 40) is within the range occurring with the alkylating compounds.

All the tested compounds belonging to the polycyclic hydrocarbons and aromatic amines proved completely inactive in the induction of point mutations, irrespective of whether they were fed to larvae or injected into adults of Drosophila (Table 3). This is in general agreement with the results of previous workers using related compounds on a variety of genetic test systems (7). However, the failure of 7-bromomethylbenz[a]anthracene in inducing point mutations was surprising, in view of its high reactivity in vitro with guanosine and nucleic acids (A. Dipple and P. Brookes, personal communication). This is most probably due to the inactivation of the compound by hydrolysis in vivo, as a result of its fast reaction with water. Another compound which also reacts with DNA but did not produce point mutations is the acetoxy derivative of acetylamino-fluorene. In this instance however the reaction with DNA was shown to be irrelevant to direct mutability, as will be explained later under "Discussion."

The mutagenic activities of urethan and its probable metabolites regarding the sex-linked recessive lethals are shown in Table 4. The carbamate itself, and its N-hydroxy and N, O-diacetyl derivatives, gave indications of weak activity on mature sperm but were ineffective on other stages of spermatogenesis. The mutation rate on the sperm was significantly above the control level with 30 mM of the N, O-diacetyl compound, whereas more than double this concentration was required for a comparable effect with the parent carbamate. This suggests that N-hydroxylation followed by esterification of the hydroxyl-derivative might well be essential steps in the metabolic activation of urethan for attack on the genetic DNA, in a manner akin to that demonstrated for the activation of arylhydroxylamines (36).

The fact that versatile carcinogens, e.g., the hydrocarbons and aromatic amines, were ineffective as regards point mutations clearly indicates that this type of genetic damage was not a crucial prerequisite in carcinogenesis. The next step was to assess whether this damage played a role in tumor initiation with other agents, such as the alkylating compounds, which were highly effective with respect to point mutations and which contained some carcinogenic representatives. This could best be approached through correlation studies of the activities of a given series of compounds with respect to both biological phenomena. A major complication in such a study, however, is that both carcinogenesis and mutagenesis show a certain degree of specificity. Carcinogenic response to a given compound is known to vary between species, among the genetic strains and sexes of the same species, as well as in different organs of the same organism. Specificity plans an even greater role in mutagenesis where response was shown to vary: with the cell...
O. G. Fahmy and Myrtle J. Fahmy

Table 2
Mutagenicity of representatives of the alkylating agents as regards point mutations (sex-linked recessive lethals) in relation to carcinogenicity [pulmonary tumors in strain A mice (42, 43)]

<table>
<thead>
<tr>
<th>Chemical series and compounds</th>
<th>Concentration (mM)</th>
<th>Treatmenta</th>
<th>Route</th>
<th>Mutation frequency of X-lethals per 10^6</th>
<th>Rank of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethyleneimines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tr[(ethylenimino)thiophosphoramide (35)]</td>
<td>0.01</td>
<td>Aq.</td>
<td>A.F.</td>
<td>75 ± 7 st: 46 ± 5</td>
<td>1 5</td>
</tr>
<tr>
<td>2,5-Di(ethylenimino)-3,6-diproxy-1,4-benzoquinone (35)</td>
<td>0.01</td>
<td>Aq.</td>
<td>A.F.</td>
<td>16 ± 4 st: 18 ± 3</td>
<td>2 3</td>
</tr>
<tr>
<td>2,4,6-Tr(ethylenimino)-1,3,5-triazine (Ref. 34 for A.F.)</td>
<td>0.1</td>
<td>Aq.</td>
<td>A.F.</td>
<td>40 ± 11 st: 60 ± 22</td>
<td>3 4</td>
</tr>
<tr>
<td>Chloroethyamines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-NW,Di(2-chloroethyl)aminouracil</td>
<td>2.0</td>
<td>Aq./DMF</td>
<td>A.I.</td>
<td>66 ± 10 st: 66 ± 23</td>
<td>4 1</td>
</tr>
<tr>
<td>5-[3-(Ethyl-2-chloroethyl)amino-6-chloro-2-methoxyacridine; dihydrochloride (8)</td>
<td>2.1</td>
<td>Aq.</td>
<td>A.I.</td>
<td>22 ± 11 st: 60 ± 22</td>
<td>5 13</td>
</tr>
<tr>
<td>p-NW,Di(2-chloroethyl)aminobutyric acid; sodium salt</td>
<td>6.0</td>
<td>Aq.</td>
<td>A.I.</td>
<td>53 ± 12 st: 74 ± 11</td>
<td>7 6</td>
</tr>
<tr>
<td>p-NW,Di(chloroethyl)aminophenyl-L-alanine; sodium salt</td>
<td>12.0</td>
<td>Aq.</td>
<td>A.I.</td>
<td>55 ± 9 st: 61 ± 6</td>
<td>8 2</td>
</tr>
<tr>
<td>2-NW,Di(2-chloroethyl)naphthyamine</td>
<td>2.6</td>
<td>Oil</td>
<td>L.F.</td>
<td>8 ± 3 st: 8 ± 3</td>
<td>10 9</td>
</tr>
<tr>
<td>N,W,Di(2-chloroethyl)amino-N-O-propyleneephosphoric acid ester diamide [Cytoxan (41)]</td>
<td>20.0</td>
<td>Aq.</td>
<td>A.F.</td>
<td>9 ± 3 st: 38 ± 8</td>
<td>11 8</td>
</tr>
<tr>
<td>p-NW,Di(2-chloroethyl)methoxyaniline</td>
<td>40.3</td>
<td>Oil</td>
<td>A.I.</td>
<td>17 ± 6 st: 27 ± 7</td>
<td>13 7</td>
</tr>
<tr>
<td>Epoxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,4-Diepoxybutane</td>
<td>11.6</td>
<td>Aq.</td>
<td>A.I.</td>
<td>117 ± 14 st: 98 ± 11</td>
<td>6 10</td>
</tr>
<tr>
<td>Sulfonic acid esters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Chloroethylmethanesulfonate</td>
<td>10.5</td>
<td>Aq.</td>
<td>A.I.</td>
<td>5 ± 2 st: 49 ± 4</td>
<td>9 12</td>
</tr>
<tr>
<td>1,6-Dimethanesulfonyl-D-mannitol</td>
<td>80.4</td>
<td>Aq.</td>
<td>A.I.</td>
<td>55 ± 10 st: 48 ± 5</td>
<td>12 11</td>
</tr>
</tbody>
</table>

aVehicles: Aq., aqueous solution; Aq./DMF, aqueous solution of dimethylformamide; oil, arachis oil. Routes: A.F., adult feeding; for other abbreviations see the footnote to Table 1.
bsg, spermatogonia; st, spermatids in spermateleosis; st-sg, stages from spermatids to spermatogonia.
cMut., mutagenicity; Car., carcinogenicity.

stage in differentiation, among different chromosome segments, for different genes along the same chromosome, and even between cistrons within the same gene (20). In view of this specificity, correlation studies between the genetic and oncological functions should strictly be based on the response of the same cells within a given test system. This has not so far been achieved because of technical difficulties, and the two biological functions are often tested in phylogenically divergent test organisms. Under such circumstances, no reliance can be based on negative results for either function, since there is always the possibility that the selected test system was nonresponsive to the function examined. However, a safer correlation might be based on wide grades of response for chemically related compounds, especially for the less specific aspects of both carcinogenesis and mutagenesis. Such an analysis can now be undertaken.

Although several alkylating compounds were tested either for carcinogenesis or mutagenesis, only thirteen were studied for both phenomena. Their ranking order for each function is shown in Table 2 (last 2 columns). The carcinogenicity order was based on the dose required to induce one lung tumor per mouse (chiefly, Table 5 in Ref. 43); the highest activity (1st rank) occurred with the uracil mustard at 0.96 μM/kg and the lowest (13th rank) with the propyl-quinacrine mustard which did not produce lung tumors above the control level at 17.5 μM/kg. The mutagenicity order could not be referred strictly to a dose/response relationship, since differences in solubility and toxicity between compounds sometimes necessitated changes in the vehicle and route of administration. Furthermore, the genetic activity for some compounds could only be satisfactorily assayed for the later stages of spermatogenesis because of their sterilizing action on the earlier stages. In spite of these difficulties, however, marked differences were discernible in the activities of the various compounds, which far transcended those encountered with different routes of treatment with the same dose of a given compound. This enabled an unambiguous semiquantitative ranking of the mutagenicity of the tested compounds (ranked 1 to 13, Table 2) on the basis of higher mutation yield within the most responsive recoverable germ cells with lower administered doses. The highest mutagenicity (1st rank) occurred with tri(ethyleneimino)thiophosphoramid e inducing up to 75 mutations per thousand chromosomes with only 0.01 mM administered orally (adult feeding). At the other extreme, the methoxyaniline mustard induced almost one-third of the mutation frequency (27 per thousand) when a concentration some 3 orders of magnitude...
Table 3

Inactivity of carcinogenic representatives or hydrocarbons, aromatic amines, and some of their chemically reactive derivatives in the induction of point mutations (sex-linked recessive lethals)

<table>
<thead>
<tr>
<th>Chemical series and compounds</th>
<th>Concentration (mM)</th>
<th>Treatment</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Route</td>
<td>Stages tested</td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-Methylbenz[a]anthracene-7-carboxylic acid</td>
<td>3.5</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td>Dibenz[a,h]phenazine</td>
<td>1.8</td>
<td>Oil</td>
<td>L.F.</td>
</tr>
<tr>
<td>7-Bromomethylbenz[a]anthracene</td>
<td>0.1</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
<td>18.7</td>
<td>Oil</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td>28.0</td>
<td>Oil</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td>37.3</td>
<td>Oil</td>
<td>A.I.</td>
</tr>
<tr>
<td>Aromatic amines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>0.7</td>
<td>Oil</td>
<td>L.F.</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>Oil</td>
<td>L.F.</td>
</tr>
<tr>
<td>2-Acetylanilinofluorene</td>
<td>1.1</td>
<td>Aq./alc.</td>
<td>L.F.</td>
</tr>
<tr>
<td>$N$-Acetyl-$N$-acetoxy-2-amino fluorene</td>
<td>0.1</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
</tbody>
</table>

$^a$Vehicles: Aq./alc., aqueous alcohol (compound dissolved in ethanol and mixed with culture medium); for other abbreviations see the footnotes to Tables 1 and 2.

$^b$sp, mature sperm; sp—sc, stages from sperm to spermatocytes; sp—st, stages from sperm to early spermatids; other abbreviations as in the footnotes to Table 2.

Table 4

Weak activity of urethan and its probable metabolites in the induction of point mutations (sex-linked recessive lethals) in mature sperm

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (mM)</th>
<th>Treatment</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Route</td>
<td>Stages tested</td>
</tr>
<tr>
<td>Urethan</td>
<td>4.3</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56.2</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>78.7</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N$-Hydroxyurethan</td>
<td>4.3</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N,O$-Diacetyl-$N$-hydroxyurethan</td>
<td>20.0</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean activity on sperm</td>
<td>3302</td>
<td>24</td>
<td>7.3 ± 1.5</td>
</tr>
</tbody>
</table>

$^a$All abbreviations are found in the footnotes to Tables 1 to 3.

higher (40.3 mM) was directly injected around the testes. The rank correlation between carcinogenicity and the induction of point mutations was assessed statistically by Spearman’s coefficient of rank correlation and was found to be insignificant ($p = 0.45, p > 0.1$). It would seem, therefore, that even with the alkylating agents the type of
Table 5

Mutagenicity of representatives of the alkylating agents and nitroso compounds as regards small chromosome deletions (Minutes)

<table>
<thead>
<tr>
<th>Chemical series and compounds</th>
<th>Concentration (mM)</th>
<th>Treatment</th>
<th>Mutation frequency</th>
<th>Stages tested</th>
<th>Minutes per 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylenimines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4,6-Tri(ethyleneimino)-1,3,5-triazine</td>
<td>0.25</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>23 ± 3</td>
<td></td>
</tr>
<tr>
<td>Chloroethyamines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-NN,Di(2-chloroethyl)toluidine</td>
<td>0.1</td>
<td>Oil L.F.</td>
<td>sg</td>
<td>13 ± 3</td>
<td></td>
</tr>
<tr>
<td>2-NN,Di(2-chloroethyl)amino-uracil</td>
<td>1.0</td>
<td>Aq./DMF A.I.</td>
<td>sp-st</td>
<td>9 ± 2</td>
<td></td>
</tr>
<tr>
<td>2-NN,Di(2-chloroethyl)naphthylamine</td>
<td>2.6</td>
<td>Oil L.F.</td>
<td>sg</td>
<td>9 ± 3</td>
<td></td>
</tr>
<tr>
<td>S-2-Chloroethylycisteine</td>
<td>2.7</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>5 ± 1</td>
<td></td>
</tr>
<tr>
<td>p-NN,Di(2-chloroethyl)aminophenyl-D-alanine</td>
<td>10.7</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>14 ± 5</td>
<td></td>
</tr>
<tr>
<td>p-NN,Di(2-chloroethyl)aminophenyl-L-alanine</td>
<td>10.7</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>18 ± 2</td>
<td></td>
</tr>
<tr>
<td>p-NN,Di(2-chloroethyl)methoxyaniline</td>
<td>3.6</td>
<td>Oil A.I.</td>
<td>sp-st</td>
<td>12 ± 3</td>
<td></td>
</tr>
<tr>
<td>p,p'-NN,Di(2-chloroethyl)aminophenoxyphenylalanine</td>
<td>11.9</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>16 ± 7</td>
<td></td>
</tr>
<tr>
<td>Epoxides and oxides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,4-Diepoxybutane</td>
<td>11.6</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>10 ± 2</td>
<td></td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>113.6</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>7 ± 2</td>
<td></td>
</tr>
<tr>
<td>Sulfonic acid esters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl methanesulfonate</td>
<td>4.5</td>
<td>Aq. A.I.</td>
<td>sp-sg</td>
<td>12 ± 2</td>
<td></td>
</tr>
<tr>
<td>Ethyl methanesulfonate</td>
<td>24.2</td>
<td>Aq. A.I.</td>
<td>sp-sg</td>
<td>11 ± 1</td>
<td></td>
</tr>
<tr>
<td>1,6-Dimethanesulfonyl-D-mannitol</td>
<td>80.4</td>
<td>Aq. A.I.</td>
<td>sp-st</td>
<td>6 ± 2</td>
<td></td>
</tr>
<tr>
<td>Nitroso-amides and amines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Ethyl-N-nitrosourethan</td>
<td>5.0</td>
<td>Aq. A.I.</td>
<td>sp-sg</td>
<td>13 ± 2</td>
<td></td>
</tr>
<tr>
<td>N-Diethyl-N-nitrosamine</td>
<td>30.0</td>
<td>Aq. A.I.</td>
<td>sp-sg</td>
<td>8 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

*All abbreviations are found in the footnotes to Tables 1 to 3.*

Direct DNA damage that results in point mutations might not be an essential step in cancer initiation.

Small Chromosome Deletions: Minutes. Representatives of the chemical series that were tested for the induction of point mutations were also examined for their activity regarding the induction of Minutes. These are dominant visible mutations (homozygously and hemizygously lethal) which manifest themselves as various degrees of reduction in the length and thickness of the bristles (most pronounced for the thoracic macrochetes). Those that were localized genetically and cytologically occurred predominantly in the heterochromatic chromosome segments. Furthermore, they were frequently associated with small chromosome deletions, involving a few bands in the salivary gland chromosomes (22, 23, 28). The Minutes described in this study were invariably scored among the male progeny from attached-X tests. They represent, therefore, the viable heterozygous autosomal mutations; those induced in the X-chromosome were eliminated by lethality (Table 5).

The induction of Minutes after biological alkylation was first examined in detail for triethylenemelamine (12). With this compound, Minutes were recovered preferentially from the later stages of spermatogenesis (sperm and spermatids) where their yield increased as the square of the injected molar dose or its biologically effective level as measured in point mutations. Other alkylating agents and nitroso compounds that were subsequently tested in our laboratory also proved active with respect to the same class of mutations, although to various degrees (Table 5). Likewise, the representatives of the hydrocarbons, aromatic amines, and urethan derivatives so far tested also proved decisively active in the Minutes (Table 6).

The yield of Minutes with the various compounds examined in the present study (Tables 5, 6) gave clear indications that this mutational class might be associated with carcinogenesis. Minutes were the only mutations occurring with the hydrocarbons and aromatic amines (Table 6). Furthermore such agents produced Minute frequencies approaching those with some alkylating agents, although they could only be tested at much lower concentrations because of their higher toxicity on the treated organisms (cf. Tables 5, 6). The two nitroso compounds tested induced Minute frequencies within the range occurring with the alkylating compounds (Table 5); the amide derivative being appreciably more effective than the amine, which is compatible with its greater versality in carcinogenesis (37). The rank correlation between the activities in the induction of pulmonary tumors and Minutes was examined for the alkylating compounds in the same way as previously described for point mutations (see "Point Mutations").
Table 6

**Mutagenicity of hydrocarbons, aromatic amines, urethan, and some of their chemically reactive derivatives in the induction of small chromosome deletions (Minutes)**

<table>
<thead>
<tr>
<th>Chemical series and compounds</th>
<th>Concentration (mM)</th>
<th>Treatment</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-Methylbenz[a] anthracene-7-carboxylic acid</td>
<td>3.5 Aq./DMF</td>
<td>A.I.</td>
<td>sp-sg</td>
</tr>
<tr>
<td>Dibenzo[a,h] phenazine</td>
<td>1.8 Oil</td>
<td>L.F.</td>
<td>sg</td>
</tr>
<tr>
<td>Aromatic amines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>0.7 Oil</td>
<td>L.F.</td>
<td>sg</td>
</tr>
<tr>
<td>N-Acetyl-N-acetoxy-2-aminofluorene</td>
<td>0.10 Aq./DMF</td>
<td>A.I.</td>
<td>sp-sg</td>
</tr>
<tr>
<td>Urethan aminoester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N,O-Diacetyl-N-hydroxyurethan</td>
<td>3.5 Aq./DMF</td>
<td>A.I.</td>
<td>sp-sg</td>
</tr>
<tr>
<td></td>
<td>30.0 Aq./DMF</td>
<td>A.I.</td>
<td>sp-sg</td>
</tr>
</tbody>
</table>

*All abbreviations are found in the footnotes to Tables 1 to 3.*

Table 7

**Rank correlation between mutagenicity (Minutes in larval or adult testes, Table 5) and carcinogenicity (pulmonary tumors in strain A mice (42, 43)) for some representatives of the alkylating agents**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (mM)</th>
<th>Minutes per 10³</th>
<th>Rank of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,6-Tri(ethyleneimino)-1,3,5-triazine</td>
<td>0.25</td>
<td>23 ± 3</td>
<td>1 Mut. 3 Car.</td>
</tr>
<tr>
<td>5-NN-Di(2-chloroethyl)aminouracil</td>
<td>1.0</td>
<td>9 ± 2</td>
<td>2 Mut. 1 Car.</td>
</tr>
<tr>
<td>2-NN-Di(2-chloroethyl)naphthylamine</td>
<td>2.6</td>
<td>9 ± 3</td>
<td>3 Mut. 5 Car.</td>
</tr>
<tr>
<td>p-NN-Di(2-chloroethyl)aminophenyl-L-alanine</td>
<td>10.7</td>
<td>16 ± 2</td>
<td>4 Mut. 2 Car.</td>
</tr>
<tr>
<td>p-NN-Di(2-chloroethyl)methoxyaniline</td>
<td>3.6</td>
<td>4 ± 1</td>
<td>5 Mut. 4 Car.</td>
</tr>
<tr>
<td>1,2,3,4-Diepoxybutane</td>
<td>11.6</td>
<td>10 ± 2</td>
<td>6 Mut. 6 Car.</td>
</tr>
<tr>
<td>1,6-Dimethanesulfonyl-L-mannitol</td>
<td>80.4</td>
<td>6 ± 2</td>
<td>7 Mut. 7 Car.</td>
</tr>
</tbody>
</table>

Unfortunately, this analysis could only be undertaken for 7 compounds for which relative activities were determined both for Minutes and pulmonary tumors (Table 7). The Minute frequencies used in the mutagenicity ranking were the highest obtained in any of the testis sectors in the experiments detailed in Table 5. The carcinogenicity ranking has been deduced, as with the point mutations, from the data of Shimkin et al. The coefficient of rank correlation (Spearman's method) between mutagenicity and carcinogenicity (Table 7, last 2 columns) proved significant ($\rho = 0.75, p < 0.05$), although not impressively so. A closer look at the Minute frequencies in Table 7 however indicates that only the 1st, 2nd, and 7th ranks are decisive, whereas compounds occupying the 3rd to 6th ranks might be regarded of roughly equal activity since their mutation yield per unit dose was not significantly different. The 3rd to 6th mutagenicity ranks were therefore substituted by the mean rank (all ranked at 4.5) in the calculation of the coefficient of correlation, but this did not change its value or the level of its significance. This conclusion however must still be regarded as tentative in view of the small number of compounds used in the statistical calculations and the difficulties encountered in their ranking.

Major Chromosome Rearrangements. The induction of chromosome breaks that lead to viable and inviable (dominant lethal) rearrangements after biological alkylation has been quantitatively studied in Drosophila, particularly with triethylenemelamine (10—12). Only those breaks leading to viable chromosome rearrangements are of relevance to carcinogenesis, since these could result in heritable changes through position effects or mutations at the breakage points. Activity as regards the induction of viable X-chromosome fragments was assayed simultaneously with the Minutes in the attached-X experiments with all the tested compounds (Tables 8, 9). Within the examined range of concentrations, all the di- and polyfunctional alkylating compounds (with the exception of diepoxybutane) proved active, while the monofunctional derivatives were ineffective (Table 8). A single X-fragment occurred with each of the nitroso compounds studied, but the overall frequency with the 2 compounds was not significantly above that after monofunctional alkylation (Table 8, bottom rows). All
**Table 8**
The range of activity of the alkylating agents and nitroso compounds as regards the induction of viable X-chromosome fragments (hyperploid \( \text{XX-females} \))

<table>
<thead>
<tr>
<th>Chemical series and compounds</th>
<th>Concentration (mM)</th>
<th>Treatment</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethyleneimines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4,6-Tri(ethyleneimino)-1,3,5-triazine</td>
<td>0.25</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>Chloroethyamines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( p-N,N',Di(2-chloroethyl)toulidine )</td>
<td>0.1</td>
<td>Oil</td>
<td>L.F.</td>
</tr>
<tr>
<td>( 5-N,N',Di(2-chloroethyl)aminouracil )</td>
<td>1.0</td>
<td>Aq./DMF</td>
<td>A.I.</td>
</tr>
<tr>
<td>( 2-N,N',Di(2-chloroethyl)naphthylamine )</td>
<td>2.6</td>
<td>Oil</td>
<td>L.F.</td>
</tr>
<tr>
<td>( S-2-Chloroethylcysteine )</td>
<td>2.7</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>( p-N,N',Di(2-chloroethyl)aminophenyl-D-alanine )</td>
<td>10.7</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>( p-N,N',Di(2-chloroethyl)aminophenyl-L-alanine )</td>
<td>10.7</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>( p-N,N',Di(2-chloroethyl)methoxyaniline )</td>
<td>3.6</td>
<td>Oil</td>
<td>A.I.</td>
</tr>
<tr>
<td>( p/p'-N,N', Di(2-chloroethyl)aminophenoxyphenylalanine )</td>
<td>11.9</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>Epoxides and oxides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2,3,4-Diepoxybutane</td>
<td>11.6</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>113.6</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>Sulfonic acid esters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl methanesulfonate</td>
<td>4.5</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>Ethyl methanesulfonate</td>
<td>24.2</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>1,6-Dimethanesulfonyl-D-mannitol</td>
<td>80.4</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>Nitroso-amides and -amines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N'-ethyl-N-nitrosourethan )</td>
<td>5.0</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
<tr>
<td>( N'-diethyl-N-nitrosamine )</td>
<td>30.0</td>
<td>Aq.</td>
<td>A.I.</td>
</tr>
</tbody>
</table>

Overall frequencies of X-fragments

<table>
<thead>
<tr>
<th></th>
<th>Alkylating compounds: monofunctional</th>
<th>Nitroso compounds:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkylating compounds: monofunctional</td>
<td>per 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Alkylating compounds: difunctional</td>
<td>per 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Alkylating compounds: polyfunctional</td>
<td>21,195</td>
</tr>
<tr>
<td></td>
<td>per 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>per 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>31,173</td>
</tr>
<tr>
<td></td>
<td>per 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>per 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>31,314</td>
</tr>
</tbody>
</table>

<sup>a</sup>All abbreviations are found in the footnotes to Tables 1–3.

The yield of the viable X-chromosome fragments relative to that of the \( \text{Minutes} \) in the same experiments with the different compounds can be gathered from Tables 5 to 9. Some monofunctional alkylating agents and nitroso compounds (such as the cysteine mustard, methyl methanesulfonate, and ethyl nitrosourethan) were of the same order of activity with respect to the \( \text{Minutes} \) as most of the difunctional alkylating compounds (Table 5). This is so in spite of the great differences in the activities of the three chemical series with respect to the induction of chromosome breaks leading to viable rearrangements (Table 8). More significant is the fact that the classical carcinogens, the hydrocarbons, aromatic amines, and diacetylurethan, were ineffective in the induction of viable X-fragments (Table 9) while being decisively active in the \( \text{Minutes} \) (Table 6). Thus it would seem that the induction of chromosome breaks leading to viable rearrangements is neither essential for the production of \( \text{Minutes} \) nor associated with tumor initiation.

**DISCUSSION**

In the light of the present study, chemical carcinogens can be classified into two well-defined groups on the basis of their mutagenicity in cellular genetic systems: (a) gene mutators and (b) gene inactivators. The first group includes the alkylating agents and those compounds which produce alkylating radicals in vivo, such as the nitroso compounds and the lactones. These are characterized by a wide mutational spectrum, inducing point mutations, small chromosome deletions in both the euchromatin and heterochromatin and chromosome breaks leading to major rearrangements. The second group is typified by the classical carcinogens: the hydrocarbons and aromatic amines, and probably also includes urethan and its derivatives. These
agents are inactive as regards point mutations and chromosome breaks but are effective in the induction of small chromosome deletions that result in the Minute phenotype. With both groups of carcinogens, therefore, a common type of genetic damage occurs, namely, small deletions, as is required by the somatic mutation theory.

The mutagenic versatility of the alkylating compounds might reasonably be associated with direct damage to the generic DNA as a result of substitution on the N-7 of guanine moieties and subsequent depurination and main chain scission (31). If this damage is also relevant to tumor initiation, some correlation would be expected to occur between mutagenicity and carcinogenicity after biological alklylation. No such correlation, even at the rank level, was discernible for a series of alkylating compounds with respect to the induction of point mutations on the one hand and pulmonary tumors on the other. Therefore, it is probable that direct DNA damage leading to point mutations might not play a major role in carcinogenesis. Covalent binding to generic DNA has also been reported with various hydrocarbons (6, 27, 29) and aromatic amines or their derivatives (9, 36, 38, 39). The most extensive study, both chemically and biologically, was undertaken with N-acetoxy-N-acetylamino-fluorene. This carcinogen was shown to attack guanosine and guanine residues in DNA at the C-8 position (30, 36, 38) but this reaction, unlike the N-7 alklylation, did not seem to disturb hydrogen bonding in DNA or result in its degradation through depurination and main chain scission (30, 36). No point mutations would, therefore, be expected to ensue from C-8 arylamidation, and this proved to be true in Drosophila.

The exclusion of mutations due to intramolecular DNA damage as the major cause of tumor initiation with most if not all carcinogens, directs attention to the possible role of indirect mutations. Foremost in this connection are the small deletions resulting in the Minute phenotype in Drosophila. These were shown to be induced by chemically nonreactive synthetic polymers, such as polycylic acids, poly bases, and polydimers (17—23). They also occurred with various pyrimidine nucleosides which were not expected to enter DNA and which did not induce point mutations or viable chromosome rearrangements: 5-fluorodeoxyuridine, and 5-azauridine or 5-azacytidine (24). In these instances, therefore, the induction of Minutes was not due to any direct change in the structure of the generic DNA but must have occurred indirectly through interference with its replication, probably as a result of the inhibition of some of the catalyzing enzymes, i.e., kinases, synthetases, or polymerases. The genetic properties of the strongest carcinogens investigated, the hydrocarbons and aromatic amines, were identical with those of the macromolecules and nucleosides. Likewise, they induced Minutes but no point mutations or chromosome breaks, which suggests that they can only initiate indirect mutagenicity. Two molecular mechanisms could be visualized for this process: (a) the carcinogen becomes covalently bound to a chromosomal locus in a manner that physically interferes with its subsequent replication or (b) the compound is physically absorbed within the chromosomal site and subsequently reacts with and inactivates the enzymes involved in its synthesis. The first mechanism could occur with both the hydrocarbons and aromatic amines, since these compounds or their metabolites do react with cellular macromolecules: both nucleic acids and proteins (reviewed in 39). It would seem, however, that the initial target of attack is DNA, since the induced deletions occur predominantly in the heterochromic chromosomal segments which consist largely of condensed DNA. The second suggested mechanism could conceivably occur with some polycyclic hydrocarbons, since these were shown to be solubilized in aqueous solutions of DNA in vitro (5, 32) and

### Table 9

<table>
<thead>
<tr>
<th>Chemical series and compounds</th>
<th>Concentration (mM)</th>
<th>Treatment</th>
<th>Progeny with no hyperploids females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-Methylbenz[a]anthracene-7-carboxylic acid</td>
<td>3.5 Aq./DMF A.I.</td>
<td>sp-sg</td>
<td>3,085</td>
</tr>
<tr>
<td>Dibenzo[a,A] phenazine</td>
<td>1.8 Oil L.F.</td>
<td>sp-sg</td>
<td>1,902</td>
</tr>
<tr>
<td>Aromatic amines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>0.7 Oil L.F.</td>
<td>sp-sg</td>
<td>2,231</td>
</tr>
<tr>
<td>N-Acetyl-N-acetoxy-2-aminofluorene</td>
<td>0.1 Aq./DMF A.I.</td>
<td>sp-sg</td>
<td>8,298</td>
</tr>
<tr>
<td></td>
<td>0.13 Aq./DMF A.I.</td>
<td>sp-sg</td>
<td>34,578</td>
</tr>
<tr>
<td>Urethan aminooesthe</td>
<td>20.0 Aq./DMF A.I.</td>
<td>sp-sg</td>
<td>2,001</td>
</tr>
<tr>
<td></td>
<td>3.0 Aq./DMF A.I.</td>
<td>sp-sg</td>
<td>1,800</td>
</tr>
<tr>
<td>Total nonmutant sperm with all compounds</td>
<td></td>
<td></td>
<td>53,895</td>
</tr>
</tbody>
</table>

### Footnotes

*All abbreviations are found in the footnotes to Tables 1 to 3.*

### Source

Somatic Mutation Theory

Inactivity of representatives of carcinogenic hydrocarbons, aromatic amines, urethan, and some of their chemically reactive derivatives as regards the induction of viable X-chromosome fragments (hyperploid XX-females)
do bind covalently to cellular proteins in vivo via the K-region (2, 45).

From the foregoing discussion, a fundamental modification in our outlook on the somatic mutation theory of carcinogenesis needs to be introduced if it is to comply with the known genetic and biochemical properties of carcinogens. The significant change in neoplastic initiation is the induction of specific small chromosome deletions, analogous to the Minutes in Drosophila. These rarely arise as a result of direct damage to DNA but are mainly due to indirect disturbances in chromosome synthesis (or repair) during replication. The initial target of binding (physical or chemical) between the carcinogen and the genetic material seems to be DNA, but interference with the proper functioning of other cellular macromolecules (proteins and/or RNA) must be involved in the eventual elimination of the attacked locus.

ACKNOWLEDGMENTS

We wish to thank Professor Sir Alexander Haddow, F.R.S., for his interest, and our colleagues of the Chemistry Department for supplying the many compounds used in the present study; their synthesis was undertaken under the guidance of Professor W. C. J. Ross for the alkylating agents, of Drs. P. Sims and A. Dipple for the derivatives of the hydrocarbons, and of Dr. D. R. Nery for the urethan aminoester.

We are also deeply indebted to Professor James A. Miller (McArdle Laboratory, Madison, Wisc.) for supplying the derivatives of aromatic amines and for his advice as to their biochemical mode of action.

REFERENCES

Somatic Mutation Theory


44. Strauss, B. S. DNA Repair Mechanisms and Their Relation to Mutation and Recombination. Current Topics in Microbiology and Immunology. Berlin: Springer-Verlag, in press.

Gene Elimination in Carcinogenesis: Reinterpretation of the Somatic Mutation Theory

O. G. Fahmy and Myrtle J. Fahmy


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/30/1/195

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.