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THE EFFECT OF ALKYLATING AGENTS ON MALE RAT FERTILITY

BY

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The effects of tumour inhibitory doses of tretamine (triethylenemelamine), busulphan, and melphalan on the fertility of male rats have been examined. The aromatic nitrogen mustard, melphalan, was inactive, but busulphan has a highly selective action on spermatogenesis which contrasts strikingly with that of tretamine. The main action of tretamine was exerted upon spermatocytes or spermatids, but, with increasing dose, the effects spread to involve a wide range of spermatogenic cells including mature sperm, so that infertility could be induced very rapidly. Busulphan, however, interfered with the development of spermatogonia for several weeks, although other germinal cells were unaffected and continued to develop into mature spermatozoa. This accounted for the continuation of normal fertility for 7 weeks after a dose, before sterility suddenly developed. The antifertility activity of tretamine could be simulated by a variety of other ethyleneimino compounds, potency being greatest in trifunctional and least in monofunctional compounds. The latter were, however, very destructive to the seminiferous epithelium with increasing dose. In the rat, there appeared to be no definite relationship between the ability of alkylating substances to interfere with the activity of normal and pathological proliferating tissues, as represented by the germinal epithelium, haematopoietic, and tumour tissue. Although carcinogenicity was a biological property of alkylating agents, other chemical types of carcinogen did not interfere with fertility.

In small doses, tretamine (triethylenemelamine) produces selective effects on spermatogenesis in rats, as revealed by fertility studies (Bock and Jackson, 1957). It appears that the drug can interfere with various stages of this complex process so that the sperm produced, although capable of reaching and penetrating ova, are incapable of promoting further development. Tretamine is well known for its cytotoxic effects due, it is thought, to some specific action on dividing cells. Because of its similarity to radiation, tretamine is classified as a radiomimetic drug. The present work describes the comparative actions on male rat fertility of tretamine, busulphan (Myleran), and melphalan as representative of three chemical types of alkylating radiomimetic substances. In addition the activities of a number of tri-, di-, and monofunctional ethyleneimino compounds have been examined.

METHODS AND MATERIALS

The animals used were an inbred strain of Wistar rat of American origin, maintained on a standard diet provided by the Scottish N.E. Agricultural Society. The method of investigating the effect of a drug on male rat fertility has been described previously (Bock and Jackson, 1957). Briefly, it consisted of pairing treated male rats with females of established fertility, and replacing the latter at intervals of 1 week for as long as considered necessary. The date of insemination was determined by examination of a vaginal smear each morning. Because of the number of females required in each experiment, the testing of individual substances has been limited to one or two concentrations related, where possible, to existing information concerning relevant biological activity, such as tumour inhibition, carcinogenicity or interference with the haemopoietic system. Otherwise, the total dose used was close to the maximum tolerated. The majority of substances were administered daily by the intraperitoneal route for 5 days and pairing commenced the first day of the next week, namely day 8 from the first dose. This permitted at least 72 hr. for surplus drug to be eliminated. During the third week after mating, the females were inspected for pregnancy in case litters should be destroyed at birth. The administration of short courses of treatment, rather than single doses, made it less likely that antifertility activity would be missed.

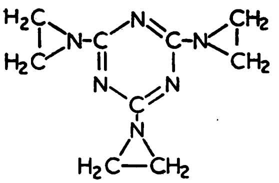
Since ethyleneimino compounds have the reputation of instability, a careful check was maintained on these substances. Only with diethyleneurea was there any reason to suspect deterioration of the pure compound with time. As this was manifest by enhanced toxicity, the compound was freshly prepared before use. All the compounds were kept in tightly stoppered containers at $-20°$ and allowed to warm up to room temperature before opening to avoid condensation of water on to the contents.

Solutions for injection were prepared immediately before use, mainly in water, although arachis oil had to be used in some instances. Nitrofurantoin (Furadantin) was injected as a suspension in 0.5% sodium carboxymethylcellulose solution, whilst nitrofurazone (Furacin) was administered to male rats in the diet as a paste. This latter procedure necessitated an adaptation of the males and females to feeding separately during the day only, with introduction of the female to the male each evening.

Generally speaking, the doses used did not depress the sexual activity of the males so that the great majority of females were inseminated during the series of pairings. The average litter size was calculated by dividing the total viable offspring by the number of females known to have been inseminated. There was always a close correspondence between visible pregnancies and the production of litters, a reassurance that the drugs caused no increased tendency for litter destruction due to abnormal offspring.

Results

The formulae of the important substances referred to in Tables I, II and III are shown in Fig. 1. The terms tri-, di-, and mono-functional simply refer to the number of chemically reactive groups (alkylating groups) present in the molecule.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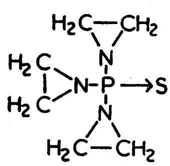
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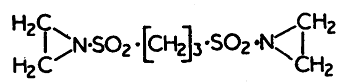
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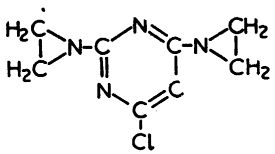
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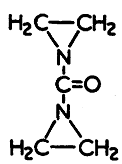
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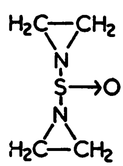
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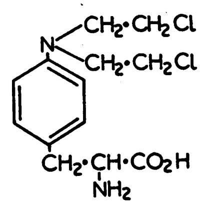
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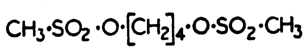
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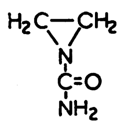
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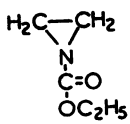
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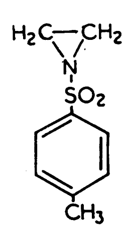
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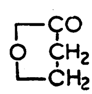
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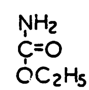
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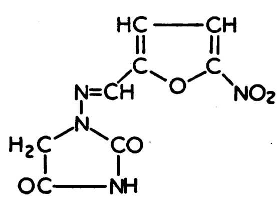
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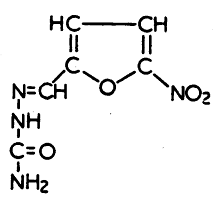
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Fig. 1

Tri- and Di-functional Ethyleneimino Compounds

The results with these compounds are recorded in Table I.

Tretamine (I).—After five daily doses of 0.2 mg./kg., the animals were sterile until the sixth week after the first dose. Between the 37th and 46th day fertility returned, the average litter size being normal by the 8th week. The effect of one dose (0.2 mg./kg.) was infertility 4 weeks later (Bock and Jackson, 1957).

Thiotepa (triethylenethiophosphoramide) (II).—Five daily doses of 0.4 mg./kg. produced a similar effect to that of tretamine except that sterility was not quite complete in week 2. Inseminations from the 37th day onwards resulted in normal size litters.

1,3-Di(ethylenesulphamoyl)propane (III).—1,3-Di(ethylenesulphamoyl)propane (see Paterson and Kunkler, 1954) in five doses of 1.8 mg./kg. induced sterility in most males for four weeks after treatment with return to normal fertility by the 6th week.

6-Chloro-2,4-diethyleneiminopyrimidine (IV).—The quantity of this compound used was well below that required to inhibit the growth of the Walker tumour (Hendry, Homer, Rose, and Walpole, 1951). After a total dose of 1 mg./kg. over 5 days, the animals remained practically sterile for 3 weeks with return to normal fertility by the 6th week. The pattern of events was similar to that following treatment with tretamine and thiotepa.

Diethyleneurea (V).—10 mg./kg. over 5 days only produced a lower average litter size during weeks 2 to 5. The dosage used, which was close to that causing lethal effects, also effectively inhibited the growth of the Walker tumour.

TABLE I

EFFECT OF TRI- AND DI-FUNCTIONAL ALKYLATING SUBSTANCES ON THE FERTILITY OF MALE RATS

Each treated male was paired in consecutive weeks with a different female. Since males and females were of established fertility, insemination should result in litter production. The number of litters produced each week compared with the number of females known to have been inseminated is shown as a ratio in the upper line of each experiment (under mating periods) and the average litter size, representing the number of offspring/female inseminated, shown by the numerals in the lower line. Unless otherwise stated, all doses were given by the intraperitoneal route. Under treatment aq denotes aqueous solution. An asterisk denotes that doses were separated by an interval of 12 days. D denotes all rats dead by week 5.

Drug	Treatment	Mating Periods (in Weeks from First Dose)											
		1	2	3	4	5	6	7	8	9	10	11	12
I Tretamine	1 × 0·2 mg./kg., aq.	6/9 4·0	7/8 2·5	8/9 3·0	0/9 0·0	9/9 7·1	8/9 7·0						
	5 × 0·2 ,, ,, Experiment (1)	— —	0/5 0	0/5 0	0/5 0	0/5 0	2/5 1·6	5/5 6·2	4/5 7·8				
	5 × 0·2 mg./kg., aq. Experiment (2)	— —	0/6 0	0/6 0	0/6 0	0/6 0	2/6 1·3	5/6 5·3	5/6 8·7				
II Thiotepa	5 × 0·4 mg./kg., aq.	— —	3/4 2·7	0/4 0	0/3 0	0/4 0	3/5 6·0	5/5 8·2	5/5 11	4/4 11			
	5 × 0·4 ,, ,,	— —	— —	— —	0/5 0	3/5 0·8	5/5 9·5	4/4 10	5/5 5·6	5/5 9·6			
III 1,3-Di(ethylenesulphamoyl)propane	5 × 1·8 ,, in oil	— —	1/5 0·2	1/5 0·2	2/5 0·6	2/5 1·8	4/4 7·5						
IV 6-Chloro-2,4-diethyleneiminopyrimidine	5 × 0·2 ,, aq.	— —	0/4 0	2/6 1·5	0/8 0	3/5 2·4	7/7 8·1						
V Diethyleneurea ..	5 × 2 ,, ,,	— —	3/4 5·0	3/4 2·4	4/5 4·4	4/5 6·8	3/3 7·7	4/4 7·0					
VI Diethyleneimino-sulphoxide	5 × 2·5 ,, ,,	— —	7/7 5·1	7/7 5·0	4/6 1·3	1/5 0·8	5/7 8·2	7/7 9·8					
VII Melphalan	5 × 0 8 ,, in oil	— —	4/5 6·8	5/5 11	4/5 8·0	5/5 7·8	5/5 10	5/5 8·6					
VIII Busulphan	1 × 10 ,, ,,	— —	5/5 6·8	5/5 8·8	5/5 11	4/4 8·6	5/5 9·4	5/5 6·6					
	1 × 10 ,, ,,	— —	— —	— —	5/5 9·8	5/5 8·4	5/5 11	5/5 11	1/5 2·0	1/5 2·4	0/4 0	0/5 0	0/5 0
	*2 × 10 ,, ,,	— —	— —	3/4 5·7	4/4 7·2	3/4 9·0	3/4 8·0	2/3 7·3	0/3 0	0/2 0	0/2 0		
	4 × 50 ,, ,, p.o.	— —	5/5 5·6	3/3 8·2	1/1 11	}D							

Diethyleneiminosulphoxide (VI).—Five daily doses of 2.5 mg./kg. produced results rather similar to those following treatment with monoethyleneurea (IX, five doses of 5 mg./kg.), except that minimal fertility occurred in the 5th instead of the 4th week. This dose approached the maximum tolerated and did not inhibit the growth of the Walker tumour.

Monofunctional Derivatives of Ethyleneimine

The results with these compounds are given in Table II.

Monoethyleneurea (IX).—Although 1 mg./kg. daily for 6 weeks had no significant effect on fertility, 5 daily doses of 5 mg./kg. produced similar changes to 1 dose of tretamine (Table I). There was a rapid decline in fertility, with sterility in the 4th week from inseminations between 22 and 26 days from the first dose. Normal fertility returned sharply in the 5th week. In contrast to the experiment with a single dose of tretamine, the 5-day course of treatment with monoethyleneurea necessitated postponement of matings until the second week of the experiment. Nevertheless infertility still occurred in all animals in the 4th week from the first dose, as after one dose of tretamine. The effect of this treatment was much more marked in hemi-castrated animals, and in these resembled the action of the short course of tretamine (5 doses of 0.2 mg./kg.). The average litter size from a corresponding group of untreated hemi-castrated males remained normal.

A higher dose rate of monoethyleneurea (5 daily doses of 10 mg./kg.) obliterated fertility for at least 8 weeks. Copulations became nearly aspermic after 7 weeks. Three animals ultimately recovered the ability to father litters of normal size. Histological examination of the testes after treatment were consistent with these findings (Fig. 2).

N-*Ethoxycarbonylethyleneimine* (X). — This ethyleneimino derivative of urethane (ethyl ethylenecarbamate) (5 daily doses of 8 mg./kg.) readily produced complete destruction of the seminiferous epithelium without apparently interfering with sexual behaviour. The animals survived for 12 months, when the experiment was terminated. None had recovered their fertility by this time, a finding consistent with the histological appearance of the testes examined 12 months after treatment. These organs showed gross damage and the epididymis remained quite empty. Inseminations from the 7th week onwards were aspermic (Table II). A smaller dose rate (5 daily doses of 4 mg./kg.) was much less effective, although producing minimal fertility in the 4th week followed by normal fertility from the 5th week. These results demonstrate a very steep dose/response effect with both monofunctional compounds IX and X.

N-*Toluene*-p-*sulphonylethyleneimine* (XI).—With a maximum tolerated dose no sterility occurred during a 3-week period following treatment.

Other Alkylating Agents

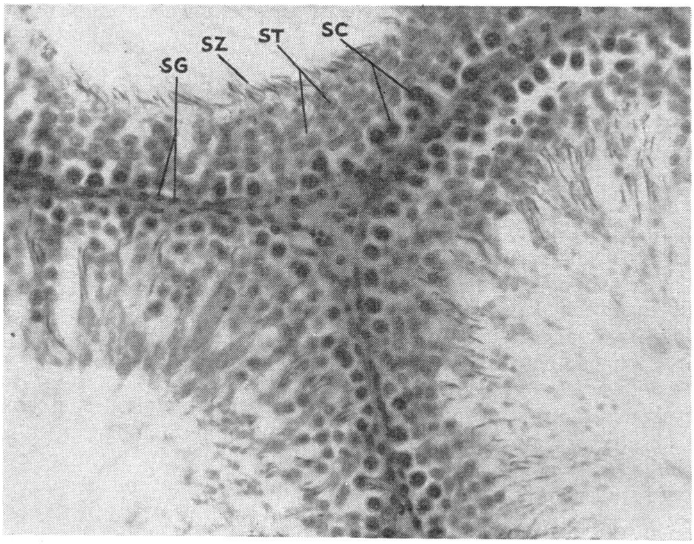
Melphalan (VII).—This difunctional nitrogen mustard derivative of L-phenylalanine had no

TABLE II

MONOFUNCTIONAL ALKYLATING SUBSTANCES AND MALE RAT FERTILITY

Each treated male was paired during consecutive weeks with a different female. Since all animals were of established fertility, litter production could be expected from each pairing. See Table I for explanation of ratio and numerals under mating periods. All doses were given by the intraperitoneal route. An asterisk indicates hemi-castrated rats.

Drug	Treatment	Mating Periods (in Weeks from First Dose)											
		2	3	4	5	6	7	8	9	10	11	12	13
IX Monoethyleneurea ..	5×5 mg./kg., aq.	5/5 6·8	5/5 3·2	0/5 0	5/5 7·0	4/4 9·4	5/5 11						
	*5×5 ,, ,,	5/6 4·7	0/5 0	0/6 0	0/6 0	5/6 6·5	6/6 8·0	4/5 8·0	5/6 7·4				
	5×10 ,, ,,	0/5 0	0/5 0	0/5 0	0/4 0	0/5 0	0/5 0	1/4 0·8	— —	— —	0/5 0	— —	1/5 2·4
	1 ,, ,, daily	5/5 12	5/5 8·6	5/5 7·4	4/4 6·2	5/5 7·8							
X *N*-Ethoxycarbonyl-ethyleneimine	5×4 ,, ,,	4/5 4·8	3/3 4·2	2/4 1·8	3/4 6·7	3/3 9·7	4/4 9·2						
	5×8 ,, in oil	1/4 0·5	0/4 0	0/4 0	0/4 0	0/3 0	0/1 0	Aspermic inseminations No further litters					
XI *N*-Toluene-*p*-sulphonyl-ethyleneimine	5×25 ,, ,,	5/5 11	5/5 8·2	4/5 9·7									



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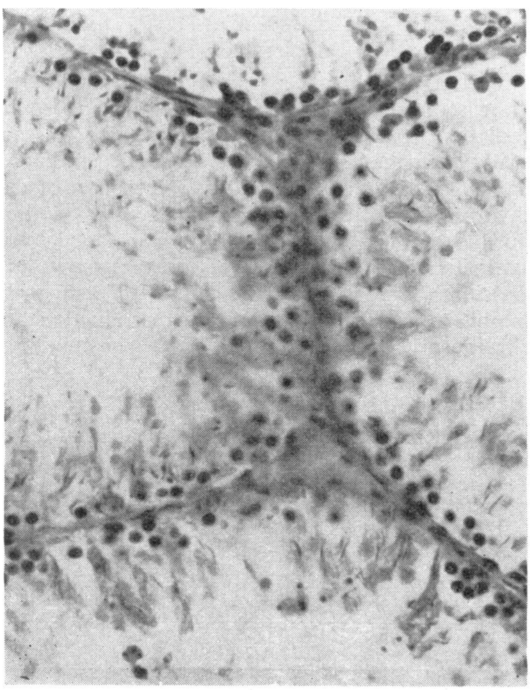

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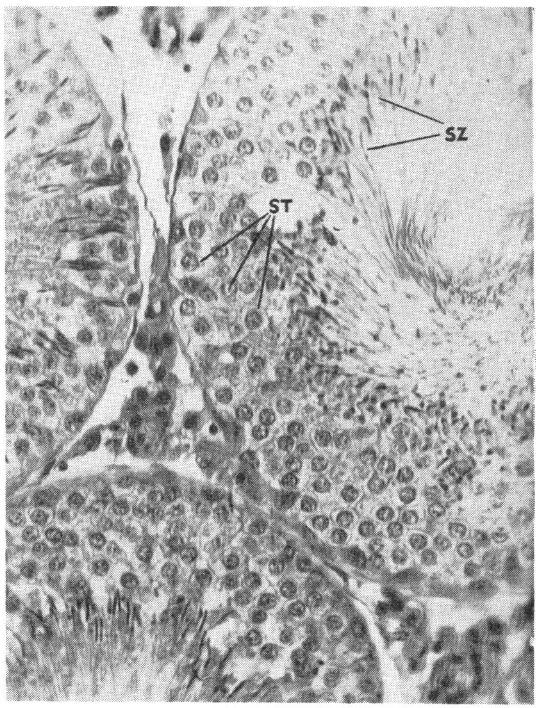

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FIG. 2.—Comparative effects at 30 days of ethyleneurea and busulphan on the testis. (*a*) Normal histological appearance of germinal epithelium after 5 daily doses of ethyleneurea (5 mg./kg., intraperitoneally). (*b*) Destructive effects of 5 daily doses of ethyleneurea (10 mg./kg., intraperitoneally). (*c*) Germinal epithelium after 1 dose of busulphan (10 mg./kg., intraperitoneally). Spermatogonia and spermatocytes are no longer present. The cells are spermatids which are transforming into spermatozoa. **SG**, spermatogonia; **SC**, spermatocytes; **ST**, spermatids; **SZ**, spermatozoa.

effect on fertility (Table I). The course of treatment given was well in excess of that required to inhibit experimental tumour growth (Bergel and Stock, 1953).

Busulphan (VIII).—A single dose (10 mg./kg.) did not interfere with fertility for 7 weeks (Table I). In this preliminary experiment, the possible later development of infertility was not suspected. In a second experiment at this dose, a delay of 3 weeks after the injection was permitted before commencing the series of pairings, and infertility appeared suddenly during week 8 in four of five animals. The remaining animal produced normal litters for 2 weeks longer before becoming abruptly sterile in the 10th week. A second dose of 10 mg./kg. 12 days after the first dose did not hasten the onset of sterility or cause subfertility, but merely enhanced the delayed lethal effects of the drug. Two of three survivors from the 2-dose experiment ultimately recovered fertility. It has again been established that delay in mating does not significantly modify the time of onset of sterility. The latter was associated with oligospermic or aspermic inseminations, indicating a sudden fall in sperm output. Even large and lethal doses such as 4 daily doses of 50 mg./kg. did not interfere with fertility up to the time of the death of the animals. It was also found that one dose of 4 mg./kg. had no effect on fertility over a period of 3 months.

β-Propiolactone (XII).—This monofunctional compound is an alkylating agent which is an effective mutagen and carcinogen (Walpole, Roberts, Rose, Hendry, and Homer, 1954). There was no evidence of antifertility action during the 6 weeks following treatment with a maximum tolerated dose (Table III).

Miscellaneous Compounds

These results are summarized in Table III.

Dibenzanthracene (XIII).—This carcinogen had no effect on fertility at a dose level which subsequently produced hepatic tumours in 4 of 5 animals.

Urethane (XIV).—This substance is a mitotic poison used in the treatment of chronic leukaemia. The dose used in the present experiments (5 daily doses of 250 mg./kg.) produced hypnosis but was not the maximum tolerated quantity; it failed to depress fertility.

Nitrofurane Derivatives (XV and XVI).—These were included because they are known to interfere with spermatocytic transformations in the testes (Nelson and Steinberger, 1953; Steinberger and Nelson, 1957). Neither compound had any significant effect on fertility during the 7 week period of the experiment.

The inactivity of compounds XII to XVI suggests that interference with spermatogenesis and fertility does not readily occur, and emphasizes that the changes in fertility after alkylating agents are not simply manifestations of general toxicity.

Discussion

Various types of alkylating compounds are known whose effects resemble those produced by radiation, so that certain normal cell systems are easily damaged by them. Whereas radiation damage is inflicted at or close to the onset of cell division, alkylating chemicals appear to act earlier, during the " resting " phase, when in fact intense synthetic activity is in progress preparatory to cell division. The result may be cell death, temporary inhibition of mitosis or the production of chromosome damage which may be manifest

TABLE III

MISCELLANEOUS COMPOUNDS AND MALE RAT FERTILITY

Each treated male was paired during consecutive weeks with a different female. Since all animals were of established fertility, litter production could be expected from each pairing. See Table I for explanation of ratio and numerals under mating period. All doses by the intraperitoneal route.

Drug	Treatment	Mating Periods (in Weeks from First Dose)					
		2	3	4	5	6	7
XII β-Propiolactone ..	5 × 20 mg.kg., in oil	4/4 5·7	5/5 8·2	5/5 9·4	5/5 7·6	3/4 7·7	
XIII Dibenzanthracene ..	4 × 20 ,, ,, ,,	4/4 6·5	3/4 4·2	4/4 9·5	3/3 7·7	5/5 9·4	
XIV Urethane	5 × 250 ,, aq.	5/5 10	5/5 9·2	5/5 8·4	5/5 8·8	4/5 6·8	
XV Nitrofurantoin ..	5 × 50 ,, in oil	3/3 6·3	5/5 8·2	5/5 10	4/4 8·0	5/5 9·4	5/5 8·0
XVI Nitrofurazone	0·4 mg./kg., of diet	2/2 7·5	2/2 9·5	4/4 7·5	5/6 5·7	5/5 9·6	6/6 8·3

later in the form of mutagenic or carcinogenic change. In animals, exposure to radiation or radiomimetic compounds is followed by the rapid production of leucopenia, due to damage to bone marrow and lymphoid tissue; later there may be thrombocytopenia and anaemia. These phenomena have been extensively studied, but the nature of the fundamental mechanisms involved remains unsolved.

The antifertility effects of radiomimetic chemicals have not been examined in any detail. Thus Goldeck and Hagenah (1951) described the effects of an aliphatic nitrogen mustard on spermatogenesis and fertility in the laboratory rat. Most males were sterile after injection of the maximum tolerated dose (1 mg./kg., intravenously) owing to inhibition of spermatogenesis and testicular atrophy. In some animals regeneration occurred and potency returned after 2 to 4 months. Landing, Goldin and Noe (1949) had earlier described the testicular lesions in mice following intraperitoneal injection of a number of aliphatic nitrogen mustards. They found " acute and chronic degenerative changes similar to those following metabolic deficiency, irradiation and many other chemical agents." Recovery of spermatogenic function was observed 3 to 4 weeks after non-fatal single or repeated doses. Bollag (1953) reported that busulphan, another type of alkylating radiomimetic agent, produced destructive effects on the rat testis; his results will be referred to later. Apart from the obvious question of the relation between chemical structure and ability to interfere with fertility, the major problem is to correlate changes in fertility with damage to particular types of spermatogenic cell. Our previous work (Bock and Jackson, 1957) with the ethyleneimino compound tretamine has shown that well-defined effects on fertility can be revealed by a systematic mating procedure. A brief account of more recent work with this substance, busulphan and radiation has also appeared (Craig, Fox, and Jackson, 1958).

Comparative Effects of Tretamine, Melphalan, and Busulphan

These three compounds are used in the chemotherapy of malignant disease, and represent three chemical types of alkylating agent—ethyleneimines, nitrogen mustards and sulphonyloxyalkanes respectively. The relevant features of the action of tretamine are as follows: (*a*) Sterility from inseminations occurring on 22 to 26 days from treatment, followed by recovery of normal fertility in the 5th week. (*b*) With increasing dose (Table I), a spread of drug action to involve later and earlier stages of spermatogenesis, and even to render mature sperm in the epididymis infertile. Recovery of fertility occurred 5 to 6 weeks from treatment. (*c*) The absence of noticeable oligospermia in these experiments and the retention of sperm motility and their ability to reach and penetrate ova. The onset of tretamine-induced sterility can be rapid, and the effect of this drug is cumulative so that very small doses can produce and maintain an infertility which is reversible. The 4th week sterility is probably due to interference with spermatocytes or damage to early spermatids (Craig, Fox, and Jackson, 1958; Bateman and Jackson, 1957) and not to spermatogonial damage as was suggested earlier (Bock and Jackson, 1957). Its selective nature is emphasized by the recovery of normal fertility a few days later. Similar results have since been obtained with tretamine in mice (Cattanach and Edwards, 1958) although the time relationships of the sterile periods are different from the rat.

The destructive effects of aliphatic nitrogen mustards on the germinal epithelium have already been mentioned, and in view of this the failure of the aromatic nitrogen mustard, melphalan, to impair fertility was unexpected. This shows, however, that the sensitive germinal cells in the testis may remain unharmed although marked suppression of proliferating cell systems occurs elsewhere. On the other hand, the action of busulphan has proved to be quite remarkable. According to Bollag (1953), two doses of this drug (11 mg./kg., intraperitoneally), separated by an interval of 14 days, caused extensive damage with complete destruction of the germinal epithelium in male rats examined over a period of 150 days. After one dose, the histology of the testis was said to remain normal over an interval of 30 days from the dose. In our experiments with this substance, fertility remained normal until the 8th week when sterility rapidly developed with associated oligo- or a-spermia. This clearly indicated a blocking or destructive action of the drug on an early stage of spermatogenesis. Our examination of the histology of the testis after one dose of busulphan showed that the drug interfered with spermatogenesis in a highly specific manner. The development of new spermatogonia was prevented for several weeks, although the germinal cells present at the time of treatment continued to develop into mature sperm over a period of about 45 days. In this way, the seminiferous epithelium became systematically depleted of spermatogonia, spermatocytes, spermatids and finally spermatozoa in this sequence (Fig. 2). These results account for the continuation of normal fertility after treatment until the supply of spermatozoa is exhausted. Then follows a period of complete sterility until repopulation of the testis tubules has occurred, already apparent by the presence of numerous new spermatogonia at 45 days, and new sperm have developed and traversed the epididymis.

It is curious that increased doses of busulphan, a nucleotoxic alkylating tumour inhibitor (Haddow and Timmis, 1953), do not result in a spread of effect to adjacent spermatogenic cells, the spermatocytes, which are actively proliferating. This specificity is in striking contrast to the actions of tretamine and other ethyleneimines, and is inconsistent with a general nucleotoxic action. The progressive disappearance of germinal elements is reminiscent of that following irradiation with 500 r (Shaver, 1953 ; Steinberger and Nelson, 1957), which is due to the rapid destruction of spermatogonia (Oakberg, 1955, 1957). A suitable dose of radiation (300 r, whole body) produces a fertility pattern indistinguishable from that of busulphan (Craig, Fox, and Jackson, 1959).

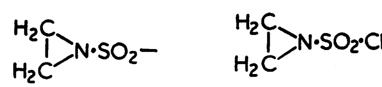
Tretamine, melphalan and busulphan inhibit the growth of a standard test tumour, the Walker carcinoma. Tretamine produces an effect on the peripheral blood which closely resembles that of both aliphatic and aromatic nitrogen mustards, namely an initial lymphocytopenia and neutropenia, followed by a marked neutrophilia (Elson, 1955, 1957). Busulphan, on the other hand, induces a selective and severe neutropenia which persists for a long time compared with the transient mustard effects. Radiation produces a general leucopenia which may be closely imitated by combined treatment with a nitrogen mustard and busulphan (Elson, 1955). It is difficult to reconcile the effects of these 3 types of alkylating radiomimetic chemicals with a common focus of attack on cellular processes.

Ethyleneimino Compounds and Fertility

The activity of tretamine has naturally led to studies of other ethyleneimino compounds. The pharmacological activity of these substances is, in general, related to the number of ethyleneimino groups present as well as to the nature of the molecule which carries them. The results show that qualitatively similar effects on rat fertility can be produced by tri-, di- and mono-functional compounds ; their potency diminishes in this order. It is strange that spermatogonia, the most radiosensitive cells of the germinal epithelium, should be relatively insusceptible to radiomimetic compounds like tretamine, thiotepa, and 1,3-di(ethylenesulphamoyl)propane whereas later phases of spermatogenesis are very sensitive to interference by these substances.

The importance of the carrier moiety of the molecule bearing the ethyleneimino groups is seen by reference to the difunctional compounds (Table I). Diethyleneurea was ineffective even at a near lethal dose. Diethyleneiminosulphoxide caused subfertility and a short period of sterility in the fifth week after treatment but only at a dose close to the maximum tolerated quantity, whilst 6-chloro-2,4-diethyleneiminopyrimidine was highly effective at low dosage.

It was not anticipated that the monofunctional ethyleneimines would have any effect on fertility. However, both monoethyleneurea and the monofunctional urethane derivative, *N*-ethoxycarbonylethyleneimine, at intermediate dose levels caused transient sterility in the 4th week after treatment. Destruction of the germinal epithelium occurred with higher dosage with no recovery of fertility in a period of 12 months following treatment with the urethane derivative ; animals were sterile for at least 8 weeks after treatment with monoethyleneurea. Repeated doses of the latter compound (1 mg./kg. daily) failed to produce infertility whereas small daily doses of tretamine (0.05 mg./kg. daily) were cumulative (Bock and Jackson, 1957). The other monofunctional ethyleneimino compound examined, *N*-toluene-*p*-sulphonylethyleneimine, was ineffective in spite of the chemical reactivity of the alkylating group. This failure was not due to the ethyleneimino group being in a substituted sulphamoyl group



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(XVII) since *N*-methanesulphonylethyleneimine (XVIII) and 1,3-di(ethylenesulphamoyl)propane (III) were active and contain the same grouping. Comparative studies of the effects of these compounds at similar dosages are being made on the peripheral blood count of rats. The male reproductive system appears to be more susceptible to damage by ethyleneimino compounds than haemopoietic tissues. For example, monoethyleneurea induced changes in fertility at doses causing no significant alteration in the peripheral blood count. Even destruction of the germinal epithelium by this substance was associated with no more than a brief period of leucopenia.

It is interesting to compare antifertility effects with antitumour activity. With the exception of diethyleneiminosulphoxide, all the di- and trifunctional ethyleneimino compounds examined inhibited the growth of the Walker tumour, but none of the monofunctional compounds discussed were active in this respect. Diethyleneurea, which had no antifertility effect, was a tumour inhibitor. This evidence of a dissociation of antifertility activity, haematological effects and tumour inhibition suggests that it may be possible to develop compounds with greater specificity for different proliferating cell systems.

Antifertility Activity and Carcinogenicity

A variety of monofunctional ethyleneimino compounds have been shown to be carcinogenic, but of the ethyleneimines used in the present work only tretamine is a known carcinogen in the rat, although not in aqueous solution (Walpole *et al.*, 1954; Bock and Jackson, 1957). Busulphan has induced tumours in rats (Koller, 1953) but not in mice (Shimkin, 1954; Roe and Salaman, 1955; Roe, 1957). The possibility that carcinogenicity and antifertility activity might be associated has been tested with other known carcinogens such as β-propiolactone, dibenzanthracene and urethane. None of these produced changes in fertility, so that there appears to be no consistent connection between carcinogenicity and antifertility activity.

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