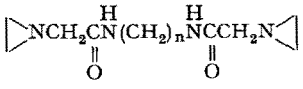
Effect of Alkylating Agents Derived from Diamines on Fertility of the Male Mouse

Alkylating agents have been used quite widely as anticancer drugs, and some have shown promise as insect chemosterilants[1]. Studies in this laboratory have been concerned with both types of activities of alkylating agents derived from certain diamines[2–7]. The effects of some of these alkylating agents on reproduction of the Japanese quail[8] were also evaluated. JACKSON[9] has recently reviewed the field of antifertility compounds including those alkylating agents that are aziridine derivatives which have antispermatogenic activity. It was pointed out that such biological activity varied depending on the carrier-moiety. The selection of alkylating agents on the basis of biological effects or sites of action is important with regard to their utility as anticancer agents, insect chemosterilants or rodent control agents. Thus, we have studied a series of *bis*-aziridineacetyl derivatives of certain diamines (see Figure) for their effects on fertility of male Swiss-Webster random-bred mice (30–40 g).

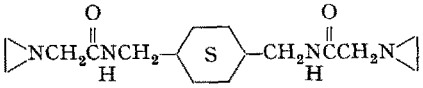
Many investigations on drugs affecting reproduction in the male have been primarily concerned with histological evidence of testicular damage, rather than the actual fertility potential of treated males compared to untreated ones. Therefore, we evaluated our compounds by employing the serial mating technique used by JACKSON[10]. Each compound at the indicated dose was injected i.p. into each of a group of 4 male mice daily for 5 days. On day 7, each treated male mouse was paired with a fertile female which was replaced each week for a total of 12 weeks. The resultant week by week fertility data are summarized in the Table.

The octamethylene derivative (I) differed from the heptamethylene derivative (II) by 1 methylene group.

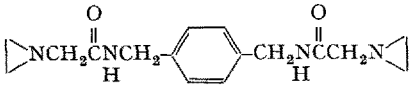


(I) n = 8, N,N′-*Bis*(aziridine acetyl)-1,8-octamethylenediamine

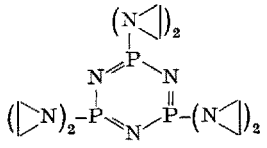
(II) n = 7, N,N′-*Bis*(aziridineacetyl)-1,7-heptamethylenediamine



(III) N,N′-*Bis*(aziridineacetyl)-1,4-cyclohexyldimethylenediamine



(IV) N,N′-*Bis*(aziridineacetyl)-1,4-xylylenediamine



(V) Apholate

Aziridine chemosterilants.

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Aziridine derivatives and male mice fertility

Compound	Dose (mg/kg i.p., × 5)	Effective chemosterilant dose in houseflies (% in diet)	Average weekly litter size 1	2	3	4	5	6	7	8	9	10	11	12
I	20	1	0	3	0	0	0	7	5	8	5	3	5	2
II	20	1[b]	0	0	0	0	0	7	5	10	5	6	8	11
III	20	1	2	0	0	0	0	0	0	0	7	6	3	12
	10		1	2	0	1	1	3	12	7	10	8	9	9
IV	5	0.1	3	0	0	2	0	1	3	5	8	10	8	10
V	1[a]	0.1	0	3	6	15	10	–	–	–	–	–	–	–
Control	Saline		6	9	7	10	10	7	11	4	8	5	8	6

Treated cell types represented at mating: week 1, epididymal spermatozoa; weeks 2–3, spermatids; weeks 4–5, spermatocytes; weeks 6–12, spermatogonia stem cells. [a] Slightly effective at 10 mg/kg but toxic at 20 mg/kg. [b] Only slightly effective at 1% level.

At the same dosage both produced sterility by interference with the spermatozoa, spermatids and spermatocytes. On the other hand, at 1% concentration in the diet, compound I completely inhibited egg-hatch in the housefly while compound II was only slightly effective[3]. Compound I also inhibited egg production in the Japanese quail[8] at 250 ppm in the diet and had a therapeutic index of 15 against Ehrlich ascites carcinoma in mice[5]. The other 2 compounds tested, III and IV, differed in the degree of unsaturation in the carbon ring. Compound III inhibited reproduction during nearly the whole spermatogenic cycle (~42 days) and also spermatogenial proliferation at 5 daily doses of 20 mg/kg while at 10 mg/kg, the spermatogenic cycle was partly affected. Compound IV caused subfertility at 5 mg/kg and was toxic to mice at 10 mg/kg in the drug treatment schedule. Against houseflies compound IV was a more effective chemosterilant (0.1% in diet) than III[4] while the latter was much more potent in its antifertility activity against mice at a higher but non-lethal dosage. The acute LD_{50} values to male mice of I and III were 1070 mg/kg and 71 mg/kg orally respectively, and 88 mg/kg and 45 mg/kg i.p. respectively[8]. Apholate was an effective chemosterilant against houseflies and was either lethal to mice at 20 mg/kg or practically non-effective at lower dosages. Thus, variations in the carrier-moiety, whether drastic or slight, could result in subtle differences in biological activity and more studies are needed to clarify structure-activity relationships.

Recent interest in non-steroidal antifertility agents and the contemplated use of some of them as rodent control agents reflect a more sophisticated rationale for pest control[11]. Presently under laboratory investigations toward such an end are many classes of compounds, including alkylating agents. Compared to other alkylating agents, compounds I, II and III possessed antispermatogenic activity similar to *N*-carbamoylaziridine, ethylene-1,2-dimethanesulfonate (EDS) and *iso*-propylmethanesulfonate but were more effective than, for example, Myleran[10]. Compounds I, II and III or their derivatives may have promise as antifertility agents for mammals and especially as rodent control agents[12].

Zusammenfassung. Nachweis, dass einige alkylierende Substanzen, die von verschiedenen Diaminen erhalten wurden, die Spermatogenese männlicher Swiss-Webster-Mäuse hemmen. Eine davon verursachte achtwöchige Sterilität und scheint zur Kontrolle von Nagetieren besonders geeignet.

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Life Sciences Research Area, Stanford Research Institute, Menlo Park (California, USA), 13 May 1968.

[11] World Health Organization. WHO/Vector Control, *217* (1966).

[12] This work was supported by Stanford Research Institute's Research and Development Program.

Toxicity of Psychotropic Drugs in *Drosophila melanogaster*

We wish to report on the toxicity of the psychotropic drugs, chlorpromazine and tranylcypromine, to growth and development of the common fruit fly, *Drosophila melanogaster*. The flies were reared, mated and housed on standard cornmeal medium[1,2] in a walk-in, constant temperature room (24 °C). Sterilized eggs were incubated in media which contained 0.001, 0.01 or 0.1% of either chlorpromazine or tranylcypromine. As the animals eclosed they were counted and sexed. Observations were made on the mating behavior of 10 pairs of flies using both normal and drug treated males and females. Adults employed for mating observations were aged 4 days to assure sexual maturity.

The ratio of males to females obtained from untreated flies was 1.11 with a standard deviation of 0.06 as determined from 4 experiments. The ratio of males to females in the treated group at the 0.1% level were as follows: chlorpromazine, 0:82; tranylcypromine, 0:76. Tranylcypromine had no effect on the rate of eclosion or number of viable flies obtained. However, inclusion of 0.01 or 0.1% of chlorpromazine in the medium delayed eclosion and reduced the number of viable flies obtained. At the 0.1% level the flies began eclosing 3 days later than untreated flies and the yield of flies was only 52% of the untreated control.

During mating of individual virgin flies it was noted whether or not the males performed the following portions of the mating ritual: vibrating, circling, licking, probing and mounting[3]. The drug treated flies exhibited more instances of activity than untreated flies except in the performance of wing vibration. The only exception was when chlorpromazine treated males were mated with chlorpromazine treated females in which case a decrease in probing activity was observed.

All drug treated flies were able to produce normal F_2 generation flies.

Zusammenfassung. Toxische Wirkungen von Chlorpromazin und von Tranylcypromin auf *Drosophila melanogaster* werden beschrieben.

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