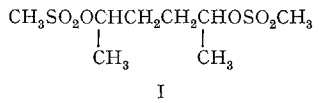
Alkylating Esters VIII. The Action of the Isomers of Dimethylmyleran on Spermatogenesis

With the separation[1] of the *meso*- and (±)- isomers of dimethylmyleran (DMM, **1**) and the establishment of their bimolecular mechanism of alkylation[2], it was necessary to examine the actions of the separate isomers in a number of biological systems. As antifertility



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agents in mice, both isomers inhibit spermatogonial development, the *meso*- isomer being more active on a weight basis though the (±)-isomer is more effective when given at higher doses due to its lower toxicity[2]. When the isomers were examined in male rats, however, they appeared to have only minor activity on spermatogenesis (Table I) contrary to reports[3] that DMM produces an action comparable to that of Myleran[4].

Since previous use has been restricted to the *meso*-isomer it was assumed from the method of synthesis[5] that the DMM used in the fertility tests was an isomeric mixture. Consequently various proportions of the isomers were administered to proven fertile male rats and their fertility assessed. The results (Table I) indicate that at a single i.p. dose level of 4 mg/kg, there is a synergistic effect of the isomers, maximal as a 50% mixture, in inhibiting spermatogonial development. Testicular histology confirms the fertility patterns; since i.p. doses (4 mg/kg) of either the *meso*- or the (±) -isomer results, after 4 weeks, in a diminution of pre-meiotic cells with spermatogenesis continuing at a reduced rate and spermatozoa present in all stages. At the same dose level, the 50% mixture, however, shows a complete loss of pre-meiotic cells with only spermatozoon head and tails present in all tubules. The (±)- isomer is less toxic to rats and administration of higher doses (6 mg/kg) shows that it is more effective at maximum tolerated dose level than the *meso*- isomer (Table I). Both (+)- and (−)-DMM[6] produced similar antifertility responses to the (±)- isomer when administered separately and were equally synergistic as 50% mixtures with the *meso*- isomer[7,8].

Meso- and (±)-DMM have similar types of action on mouse fertility so that a distinct synergistic action cannot be seen. However the 50% mixture of isomers is slightly less toxic than the individual isomers allowing higher doses to be administered which consequently lead to a longer phase of sterility. In the male quail (*Coturnix coturnix japonica*), the lower toxicity of the isomeric mixture is more pronounced. Whereas single i.p. doses of each isomer at the maximum tolerated level (10 mg/kg) produce short periods of complete or sub-fertility comparable to 4× the dose of Myleran[9], twice this level of the mixture can be administered resulting in prolonged, and in some cases permanent, sterility (Table II).

It is known that in the rat *meso*-DMM lowers the blood neutrophil count and that (±)-DMM has very little effect[2]. Mixtures of the isomers do not exhibit any enhanced action on the number of circulating neutrophils but show a decreased effect proportional to the lower amount of the active *meso*- isomer administered.

Even though no explanation can be offered for the unusual action of the DMM isomers on rat spermatogenesis, two things are apparent. First, the initial experiments of the action of DMM on male rat fertility[3]

[1] A. R. JONES, Chem. Commun. *1971*, 1042.
[2] A. R. JONES, Chem.-biol. Interact. *6*, 47 (1973).
[3] H. JACKSON, B. W. FOX and A. W. CRAIG, J. Reprod. Fertil. *2*, 447 (1961).
[4] H. JACKSON, *Antifertility Compounds in the Male and Female* (Thomas, Springfield Illinois, 1966), p. 63.
[5] The usual synthesis of DMM involves the methanesulphonylation of hexane-2,5-diol and recrystallization of the precipitated ester from alcohol. The precipitate is mainly *meso*-DMM but contains, together with mother liquors, an equi-molar amount of (±)-DMM.
[6] The (+)- and (−)-isomers of DMM were synthesised from (+)- and (−)-hexane-2,5-diol. The (±)-diol was resolved through the brucine salt of the bis (monophthallate) ester according to DODSON and NELSON[7]. Optical purity, by comparison to authentic values[8], was 80% for the (+)-and 83% for the (−)-isomers respectively. Treatment for each diol with methanesulphonyl chloride in pyridine gave, respectively, (+)-DMM ((2R, 5R)-2,5-hexanedimethanesulphonate) and (−)-DMM ((2S,5S)-2,5-hexanedimethanesulphonate), both as white prisms from absolute alcohol, m.p. 44–7 °C.
[7] R. M. DODSON and V. C. NELSON, J. org. Chem. *33*, 3966 (1968).
[8] K. SERCK-HANSSEN, S. STALLBERG-STENHAGEN and E. STENHAGEN, Arkiv. kemi. *5*, 203 (1953).
[9] P. JONES and H. JACKSON, J. Reprod. Fertil. *31*, 319 (1972).

Table I. Effects of the isomers of dimethylmyleran on male rat fertility.

No. of animals	% Isomers		Dose (mg/kg)	Average litter size[a] in weeks					
	meso-	(±)-		7	8	9	10	11	12
10	100	0	4	7 (70)	2 (30)	2 (20)	6 (50)	2 (30)	4 (40)
5	90	10	4	2 (20)	3 (20)	0	0	0	0
5	75	25	4	6 (80)	3 (40)	2 (20)	0	0	0
10	50	50	4	2 (20)	0	0	0	0	0
5	50	50	2	9 (100)	11 (100)	2 (40)	7 (80)	3 (20)	0
5	25	75	4	2 (80)	2 (20)	1 (20)	2 (20)	0	0
5	10	90	4	4 (80)	1 (20)	2 (20)	0	2 (20)	0
10	0	100	4	6 (80)	6 (80)	5 (60)	5 (60)	2 (20)	0
5	0	100	6	3 (60)	5 (60)	0	3 (20)	0	0
5[b]	?	?	3.5	5	1	2	0	0	0

The compounds were administered as suspensions in dimethyl sulphoxide arachis oil (1:3) to groups of 5 proven fertile animals and fertility assessed by the serial mating technique[10]. LD_{50} values (single i.p. injections) are *meso*-DMM 5 mg/kg, (±)-DMM 7 mg/kg, 50% mixture 5 mg/kg. [a] Values in parentheses indicate the percentage of treated animals not sterile. Litter sizes of 0 indicate that all treated animals were sterile in that particular week. [b] Values quoted from reference[3].

must have been performed with an isomeric mixture; the present results suggest that it was predominantly *meso*-DMM containing 5–10% of (±)-DMM. Secondly, as the action of DMM on a variety of biological parameters is known to parallel that of much larger doses of Myleran, and as it has been assumed[2] that these studies were carried out with the *meso*-isomer, it could be that the compounds used were similarly 'impure' and contained varying amounts of (±)-DMM. Therefore, it would be interesting to re-investigate these actions of DMM and, if both isomers are active, to see if either a similar type of synergism or a decrease in toxicity[12] occurs. A more detailed histological examination of the effects of the 'pure' isomers and the 50% mixture on the rat testis is at present being investigated.

Table II. Effects of the isomers of dimethylmyleran on the fertility of male quail (*Coturnix coturnix japonica*).

Dose (i.p.)	% of isomers		No. of days
	meso-	(±)-	of sterility
10[a]	0	100	0
10[a]	100	0	2
10	50	50	4
20[a]	50	50	23[b]

Sterility, calculated from day 25 post-administration[9], was assessed as previously described[11]. [a] maximum tolerated doses. [b] of four test birds, two were permanently sterile.

Zusammenfassung. Als Antifertilitätsmittel haben das *meso*-Isomer und die (±)-Isomere von Dimethylmyleran eine synergistische Wirkung auf die Spermatogenese von Ratten. Bei Mäusen und Wachteln hat ein 50%iges Gemisch der Isomere eine niedrigere Toxizität als die einzelnen Isomere, was bei höherer Dosis des Gemisches zu längerdauernder Sterilität führt.

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[10] M. Bock and H. Jackson, Br. J. Pharmac. *12*, 1 (1957).
[11] P. Jones, E. Kominkova and H. Jackson, J. Reprod. Fertil. *29*, 71 (1972).
[12] G. L. Floersheim, Lancet, *1*, 228 (1969).
[13] Acknowledgement. This work was supported by grants from the Ford Foundation and the Medical Research Council.

Mechanism of Action of CDP-Choline in Parkinsonism

CDP-choline (cytidine diphosphate choline), which had been developed as a therapeutic for consciousness disturbance, was found to have an effect in Parkinson's syndrome. A total of 102 patients with parkinsonism were treated with the drug at sixteen medical institutions up to 1971[1,2]. The treatment with CDP-choline yielded effectiveness rate (per cent of cases improved) of 80% approx. The therapeutic effect of the drug in parkinsonism is generally comparable to that of L-DOPA, i.e. prominent effect on bradykinesia, less but significant effect on rigidity and rather modest effect for tremor. Improvement in speech, gait and writing is also conspicuous. The dosage of CDP-choline administration was between 300–500 mg q.d. by the i.v. or i.m. route.

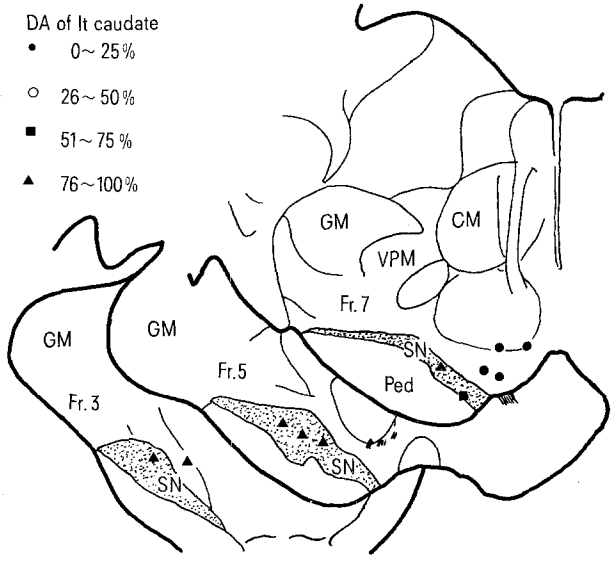

Fig. 1. Interrelation between the site of destruction of substantia nigra and the rate of dopamine diminution. Destruction of the central and caudal regions did not cause significant diminution of dopamine whereas destruction of the rostral region, especially the region medial to it, brings about a marked depletion of dopamine.

CDP-choline is devoid of anticholinergic action and its therapeutic efficacy in consciousness disturbance is attributable to its ability to ameliorate phospholipid metabolism with consequent improvement of deteriorated function of neurons[3–5].

Dopamine in the corpus striatum is originated in the homolateral substantia nigra, and parkinsonism is derived from that dopamine deficiency[6]. The mode of effectiveness of CDP-choline resembles that of L-DOPA, therefore the mechanism of action of CDP-choline in parkinsonism might be related to the activity of the drug to enhance the production of dopamine in the substantia nigra and to improve the deteriorated axonal flow of dopamine from the substantia nigra into the striatum. To clarify this possibility, the following experiments were performed.

[1] S. Manaka, T. Tsuchida, T. Fukushima, H. Sekino, Y. Mayanagi, N. Nakamura and K. Sano, Shinryo *23*, 114 (1970).
[2] S. Manaka, T. Fuchinoue, H. Sekino and K. Sano, Brain Nerve, Tokyo *24*, 1051 (1972).
[3] H. Miyake, I. Hayakawa and K. Takakura, Brain Nerve, Tokyo *16*, 873 (1964).
[4] T. Tsuchida, M. Nagai, T. Hoshimo, S. Kamano and H. Miyake, Brain Nerve, Tokyo *19*, 1041 (1967).
[5] S. Watanabe, S. Kono, K. Mitsunobu, T. Suzuki and S. Otsuki, Brain Nerve, Tokyo *23*, 721 (1971).
[6] L. J. Poirier, P. Singh, R. Boucher, A. Olivier and P. Larochelle, Archs Neurol. Chicago *17*, 601 (1967).