SOME BIOLOGICAL PROPERTIES OF DIMETHISTERONE "SECROSTERON" A NEW ORALLY ACTIVE PROGESTATIONAL AGENT

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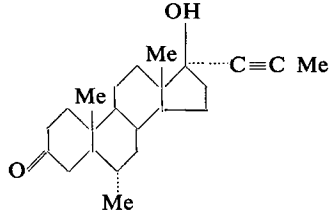
Dimethisterone, 6α:21-dimethylethisterone possesses well-marked oral progestational properties with 11·6 times the activity of ethisterone in the Clauberg assay. It has an oral LD50 in mice of 7·65 g./kg., no apparent anabolic, androgenic properties and no significant effect on sodium, potassium or water excretion in saline loaded rats.

THE preparation and progestational activity of a number of alkylated ethisterones have previously been reported[1] from our laboratories. Dimethisterone, the most active of the series, was submitted for clinical evaluation and its progestational superiority confirmed by Jackson (unpublished) and Matthew[2]. This paper describes its acute toxicity, progestational, anabolic, androgenic and oestrogenic properties and its effect on sodium potassium and water excretion in saline loaded rats.

Dimethisterone is an odourless and tasteless white crystalline compound with a molecular weight of 340·4. It is almost insoluble in water, slightly soluble in acetone and chloroform and soluble in ethanol. Its preparation and properties are described by Barton, Burns, Cooley, Ellis and Petrow[3]. It melts at about 102°, is dextrorotatory with a specific rotation in chloroform +12° and has E(1 per cent, 1 cm.) in *iso*propanol 450 at 240 mμ.

It has the structural formula



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METHODS

Acute Oral Toxicity

Male albino mice, weighing approximately 20 g. each, were fasted overnight. Three groups of 10 animals were given a suspension of dimethisterone, by stomach tube, in the following aqueous suspending medium.

Sodium carboxymethylcellulose ..	1·2 g.
Tween 80	1·5 g.
Methyl-*p*-hydroxybenzoate	0·06 g.
Propyl-*p*-hydroxybenzoate	0·03 g.
Distilled water to 100 ml.	

All volumes were adjusted to 0·5 ml./20 g. body weight. The LD50 was estimated, using Karber's[4] formula from the seven days mortalities.

Progestational Activity

McPhail's[5] modification of the Clauberg test was used. Thirty immature rabbits weighing between 800 and 1,200 g. were sensitised with three doses of 5 μg./kg. of oestrone, intramuscularly in 0·2 ml. of 20 per cent ethyl oleate in arachis oil on days one, three and five of the experiment.

Dimethisterone and ethisterone, the latter being used as the reference compound, were administered by stomach tube as suspensions in 5 per cent mucilage of acacia on days seven, eight, nine and ten of the experiment. Twenty-four hours after the last injection the animals were killed, the uteri removed and frozen sections 20 μ thick prepared and stained with haematoxylin and eosin. The progestational response was estimated by measuring the fraction of endometrium occupied by glandular tissue and also by McPhail's grading system. Relative potencies were estimated from the data obtained by the first method.

Anabolic and Androgenic Activity

A modification of Eisenberg and Gordon's[6] method, using testosterone propionate as the reference compound, was used. Both compounds were administered subcutaneously in 20 per cent ethyl oleate in arachis oil, volumes being adjusted to 0·5 ml./100 g. body weight. Thirty rats, castrated 3 weeks previously, with a mean weight of 156 g. were divided into five groups each of six animals. Seven daily injections were given with a 72-hour interval between the third and fourth injections. Three groups were given 4, 16 and 64 mg./kg. of dimethisterone respectively, the fourth group 2 mg./kg. of testosterone propionate and the remaining group, which acted as castrated controls, the vehicle only. Twenty-four hours after the last injection the animals were killed and the mean wet weight of the levator ani muscle and seminal vesicles per 100 g. weight recorded. The mean pre- and post-injection weights were also recorded.

Oestrogenic Activity

The Allen Doisy vaginal cornification method using spayed mice was employed, for reference see Emmens[7]. All compounds were given subcutaneously in 20 per cent ethyl oleate in arachis oil. The ovariectomised mice were primed with oestrone, 0·5 μg./20 g. weight, on two successive days. Three weeks later thirty-five mice were divided into seven groups of five mice. Oestrone, 0·25 μg. and 0·5 μg./20 g. weight, was given subcutaneously in two divided doses to two groups on days

one and two of the experiment; five doses of dimethisterone 2, 4, 8, 16 and 32 μg. respectively were given in a similar manner to the remaining groups. Vaginal smears were examined 36 hours following the last injection and at 12-hourly intervals on days four and five. The responses were treated quantally and a positive scored if any one of the five smears showed nucleated or cornified cells with the absence of leucocytes.

Effect on Salt and Water Excretion in Saline Loaded Rats

Four groups of 16 male albino rats were deprived of food and water overnight. The following morning a 0·9 per cent saline water load, 25 ml./kg. was given by stomach tube to each rat. At the same time dimethisterone, 2, 8 or 32 mg./kg. was administered as a suspension in 5 per cent gum acacia to three of the groups, the fourth acting as a control. The urine was collected and measured over a 24-hour period after administration and recorded as ml./kg. body weight. The Na^+ and K^+ concentrations were estimated by means of a flame photometer and recorded as mEq./kg. weight.

RESULTS

Acute oral toxicity. The acute oral LD50 in mice was 7·65 g./kg. Table I records the mortalities in 7 days after the administration of 4, 6 and 9 g./kg. of dimethisterone.

TABLE I

ORAL TOXICITY OF DIMETHISTERONE IN MALE ALBINO MICE

Compound	Dose g./kg.	Seven day mortalities	LD50 g./kg.
Dimethisterone	4	1/10	7·65
	6	2/10	
	9	6/10	

Progestational activity. Dimethisterone had an oral progestational activity in oestrone primed rabbits 11½ times that of ethisterone. Table II records the mean responses to varying amounts of dimethisterone and ethisterone.

TABLE II

THE MEAN PROGESTATIONAL RESPONSE IN FIVE RABBITS AFTER ORAL ADMINISTRATION OF DIMETHISTERONE AND ETHISTERONE

Compound	Total dose mg./kg.	Response		Relative potency
		McPhail	Per cent glandular tissue	
Dimethisterone	0·3	1·6	53·4	
,, ...	0·6	2·4	59·6	11·5
,, ...	1·2	3·2	73·4	
Ethisterone	2·5	1·2	45·8	
,, ...	5·0	1·8	54·8	1·0
,, ...	10·0	2·5	68·4	

Anabolic and androgenic activity. Testosterone propionate at 2 mg./kg. had well-marked androgenic and anabolic properties. Dimethisterone in amounts up to 64 mg./kg. had no apparent androgenic or anabolic properties, the weights of the seminal vesicles and levator ani muscles of treated and castrated controls being similar. Table III records the results.

TABLE III

THE ANABOLIC AND ANDROGENIC ACTIVITY OF DIMETHISTERONE GIVEN SUBCUTANEOUSLY TO GROUPS OF SIX CASTRATED RATS

Compound	Dose mg./kg./day	Body weight g.		Wet weight mg. seminal vesicles	Wet weight mg. levator ani
		Pre-injection	Post-injection		
Dimethisterone	4	157	145	10	22
	16	155	161	9	23
	64	155	166	13	24
Testosterone propionate ..	2	156	163	239	76
Controls		156	167	10	20

Oestrogenic activity. Table IV records the number of ovarectomised mice showing a positive oestrogenic response to various doses of dimethisterone and oestrone. Dimethisterone has no oestrogenic properties in amounts up to 32 μg./20 g.

TABLE IV

THE OESTROGENIC RESPONSES IN OVARIECTOMISED MICE GIVEN DIMETHISTERONE SUBCUTANEOUSLY

Compound	Total dose μg./20 g.	Response
Dimethisterone	2	0/5
	4	0/5
	8	0/5
	16	0/5
	32	0/5
Oestrone	0·25	4/5
	0·50	4/5

Effect on salt and water excretion in saline loaded rats. Table V records the results. At 32 mg./kg. dimethisterone had some diuretic effect. There was no Na^+ retention or undue K^+ excretion in the 24 hours after oral administration.

TABLE V

THE MEAN TWENTY FOUR HOUR RENAL OUTPUT OF URINE, Na^+ AND K^+ IN SALINE LOADED RATS GIVEN DIMETHISTERONE ORALLY

Dimethisterone mg./kg.	Urine output		mEq./kg./24 hr.	
	ml./kg./24 hr.	Relative activity	Na^+	K^+
2	6·9	0·9	2·7	1·5
8	8·7	1·2	3·2	1·6
32	12·0	1·6	3·2	1·9
Controls	7·3	1·0	2·3	1·3

Discussion

Dimethisterone, 6-α:21-dimethylethisterone is a comparatively non-toxic synthetic steroid with an acute oral LD50 in mice of 7·65 g./kg. It has oral progestional activity in oestrone-primed immature rabbits with an activity 11·6 times greater than ethisterone. It has no anabolic or androgenic properties in castrated immature rats in amounts up to 64 mg./kg. and no oestrogenic activity in ovariectomised mice in the Allen Doisy test. Salt and water balance were not markedly affected and it has no antidiuretic properties.

Dimethisterone should be of value in gynaecological conditions where an oral progestational agent is indicated.

References

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