XLIV. SOME BIOLOGICAL PROPERTIES OF EQUILIN.

By KÁROLY DAVID AND SAMUEL ELZEVIER DE JONGH.

From the Pharmaco-Therapeutic Laboratory of the University of Amsterdam.

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EQUILIN, an important member of the large group of oestrogenic substances discovered in recent years, is chemically related to menformon (oestrone).

Equilin was originally prepared from the urine of pregnant mares by Girard [1932] and co-workers; the empirical formula ($C_{18}H_{20}O_2$) had 2 hydrogen atoms less than that of oestrone; it had M.P. 238–240° (corr.) as against 255·5–256·5° for oestrone, and $[\alpha]_D^{20}$ was +308° (in dioxan) as against +163°. We obtained very similar figures for our own preparations, viz. M.P. 236–237·5° uncorr. and $[\alpha]_D$ +307° in dioxan.

The biological examination of equilin is justified by the fact that it constitutes the chief impurity even of crystalline commercial oestrone. For comparison of the various biological actions of pure equilin with pure oestrone equal quantities of both have been investigated in comparative assays. By equal quantities we mean not identical weights but quantities which produce the same oestrogenic effect in mice, this animal being the one with which our routine estimations are always performed. The following comparative assays were made: (a) oestrus in the castrated rat, (b) genital growth in the young female rat, (c) opening of the vagina in the young rat, (d) "antimasculine" action in the rat, (e) "paradoxical" effect in the castrated rat (growth of seminal vesicles), (f) nipple growth in the adult male guinea-pig, (g) epithelial metaplasia in the genitalia of the male castrated mouse.

We only determined which of the two substances under consideration would produce the stronger reaction per given number of mouse units. To establish a quantitative ratio in each case would have rendered the investigation too laborious and was not attempted.

We first determined as nearly as possible the exact dose of equilin required to induce oestrus in the castrated mouse, injecting oestrone 3 × 0·1 ml. into one group and equilin into another group of mice and next week performing a cross-test on the same subjects (summary in Table I). The letters *a–g* represent graded types of vaginal smear, progressing in alphabetic sequence towards oestrus, *a–d* being negative, *e–g* positive types [de Jongh *et al.*, 1932]. The oestrone used in our experiments was tested on several hundred animals and found to be equal in oestrogenic potency to the International standard preparation. Accordingly it contains one I.U. in 0·1 γ whether comparison is made in rats or in mice. This

Table I. *Number of animals and stage of cycle achieved or surpassed.*

	≥ a	b	c	d	e	f	g	Positive
I. 0·1 γ oestrone	21	21	21	21	21	18	5	21/21 = 100 %
0·12 γ equilin	21	21	21	17	16	12	6	16/21 = 76 %
	Also 0·12 γ equilin < 0·1 γ oestrone							
II. 0·1 γ oestrone	48	46	45	43	42	33	15	42/48 = 87·5 %
0·15 γ equilin	48	46	44	41	37	36	22	37/48 = 77·1 %
	0·15 γ equilin < 0·1 γ oestrone							
III. 0·1 γ oestrone	24	24	20	20	19	13	6	19/24 = 79 %
0·18 γ equilin	24	24	24	24	23	16	10	23/24 = 96 %
	0·18 γ equilin > 0·1 γ oestrone							

international unit of oestrone will as a rule yield positive smears in 70 % of our animals when given in oil solution (3 injections of 0.1 ml. within 36 hours).

Evidently from Table I the dose of equilin equivalent to 1 international unit in mice is not less than 0.15 γ . A similar effective dose-ratio was obtained when equilin and oestrone were given to mice by mouth (Table I *a*), the actual quantities being 1.5 γ equilin and 1.0 γ oestrone in 3 equal portions of oil solution.

Table I *a*.

	$\geq a$	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	Positive
1.5 γ equilin	23	18	13	12	12	8	3	12/23=52 %
1.0 γ oestrone	23	18	13	13	12	10	3	12/23=52 %

In subsequent experiments oestrone and equilin were therefore given in the ratio of 2 : 3 by weight.

(*a*) *Oestrus in rats.*

1 γ oestrone and 1.5 γ equilin were given in 3 subcutaneous injections; 26 rats were used and cross-tested with the following result:

	$\geq a$	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>
Oestrone	26	21	16	14	8	6	3
Equilin	26	24	23	22	20	20	18

Clearly the action of equilin was stronger than could have been anticipated from its effect on mice, and this was confirmed by a further experiment on 53 rats using half the dose of equilin and the full dose of oestrone.

	$\geq a$	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>
Oestrone	53	46	34	33	28	25	16
Equiline	53	50	41	39	30	27	15

In the rat equilin is four-thirds as oestrogenic per mg. as oestrone, which does not agree with Girard's [1933] finding of only one-seventh.

The I.U. of equilin, when tested in mice, is 0.15 γ , while when tested in rats it is 0.075 γ . From these data we draw the important conclusion that it is not sufficient to describe a preparation as containing a certain number of I.U. without reference to the kind of animals used for the testing. For more intensive discussion of this question see Laqueur [1935].

(*b*) *Growth of genitalia.*

Small doses (0.05 γ oestrone and 0.075 γ equilin) in 0.1 ml. were given twice daily for 14 days (May 29th–June 12th) for fine differentiation of the relative activities. Rats from 3 litters were castrated and equally divided between the

Table II.

Rat	Weight (g.)		Uterus mg.	Vagina mg.	Preputial glands mg.	Substance
	Before	After				
3807	35	55	85	98	17	Equilin
3808	27	47	59	116	16	
3809	34	50	66	145	19	
3810	37	41	59	110	32	
3811	37	63	63	106	24	
3812	31	53	45	97	19	Oestrone
3813	28	67	44	89	22	
3814	39	86	52	94	28	
3815	36	70	48	98	24	
3816	36	83	38	86	45	
3817	26	43	33	73	16	Oil
3818	31	48	24	62	12	
3819	36	76	32	78	25	
3820	35	50	32	61	20	
3821	36	53	30	71	24	

two groups. Increase in body weight was very irregular despite the use of litter-mates.

The increases in weight of the uterus and vagina were greater, though the average body weight was lower, in the equilin group. The weight of the vagina is less significant, because less accurately determinable, than that of the uterus. The action of equilin on the preputial glands is negligible, that of oestrone apparently stronger, largely on account of the exceptional reaction of animal 3816. The confirmatory experiment (June 26th–July 24th) differed from the former only in its greater duration (4 weeks).

Table III.

Rat	Weight (g.)		Uterus mg.	Vagina mg.	Preputial glands mg.	Substance
	Before	After				
4006	44	88	58	98	33	Equilin
4007	52	115	84	110	44	
4008	38	68	58	74	27	
4009	46	100	60	87	27	
4010	36	90	51	58	32	
4011	41	85	47	79	26	Oestrone
4012	42	85	44	86	39	
4013	Died	—	—	—	—	
4014	39	105	51	89	41	
4015	Died	—	—	—	—	
4016	43	75	24	77	22	Oil
4017	47	98	42	85	16 (1!)	
4018	37	68	19	52	15	
4019	44	82	36	68	26	
4020	37	90	17	59	26	

Two animals died in the oestrone group. With the exception of the vagina and to a less extent of the preputial glands, the increase of organ-weight was less than appeared in the former experiment; obviously because, the dose remaining constant throughout both experiments, the tissue hormone concentration became further reduced in the second experiment *pari passu* with greater increase of total body weight. The action of equilin on the uterus is again superior, and this time the preputial glands have also reacted.

The conclusion is that as a stimulus to the growth of secondary sex organs in the female rat equilin is certainly no less powerful than oestrone.

(c) Opening of the vagina.

Immature rats were used at about 3 weeks old. Five injections of 0.1 ml. were given (each containing either 2γ oestrone or 3γ equilin), two on June 5th, two on the 6th, one on the 7th. The weight was approximately the same in all cases (25 g.); observation continued until June 15th.

Table IV.

Rat	Oestrus	Vagina opening	Substance	Rat	Oestrus	Vagina opening	Substance
3879	Dioestrus	9. vi	Equilin	3885	Dioestrus	11. vi	Oestrone
3880	"	9. vi		3886	"	10. vi	
3881	"	8. vi		3887	?	—	
3882	"	9. vi		3888	Dioestrus	11. vi	
3883	"	9. vi		3889	?	—	
3884	?	—		3890	?	—	

Clearly, equilin provoked opening of the vagina more regularly and consistently earlier than oestrone. Subsequent vaginal smears were in all cases negative, in agreement with Girard's observation [1933] that equilin and oestrone in equal doses by weight produce approximately the same effect.

Table VI.

Three groups of five rats were used (from unknown litters) receiving respectively 3.75 γ equilin, 2.5 γ oestrone and control oil.

"Antimasculine" action was clearly evident in all organs weighed and was more pronounced in the equilin group. The "antimasculine" activity of equilin, therefore, is at least as great as that of oestrone.

(e) *Paradoxical effect.*

This is a term which we have applied to the capacity of oestrone and allied substances to stimulate growth in certain parts of the genital tract of the male, especially the castrated male. We have already shown elsewhere [Freud *et al.*, 1933] that, apart from the preputial glands and their stratified epithelium, the reaction principally concerns the organs containing smooth muscle.

We used three groups of young recently castrated rats, some 26 days old (litter A) and some 24 days (litter B). The dose was 5 γ oestrone and 7.5 γ equilin twice daily in the respective groups. Duration of the experiment, 3 weeks.

Table VII.

Rat	Weight (g.)		Litter	Seminal vesicles mg.	Prostate mg.	Preputial glands mg.	Substance
	30. v	22. vi					
3837	27	60	A	54	6	31	Equilin
3838	32	75		37	5	12	
3839	30	55		72	6	21	
3840	31	79	B	54	6	35	
3841	36	80		50	7	15	
3842	32	85	A	52	5	34	Oestrone
3843	34	75		60	7	32	
3844	25	60		62	5	17	
3845	34	73	B	51	6	31	
3846	31	75		59	6	45	
3847	32	85	A	7	5	20	Oil
3848	Died	—		—	—	—	
3849	33	81	B	5	3	39	
3850	35	80		5	5	24	
3851	28	59		4	4	14	

By "prostate" is meant only the easily dissected ventral part of the gland; this grew very little, as expected, whereas the seminal vesicles grew very considerably in both treated groups. The preputial glands were augmented only by oestrone, as in some of the other experiments. The growth of the preputial glands, particularly in the male, is apparently stimulated by equilin less than by oestrone in oestrogenically equivalent unitage.

(f) *Growth of nipples.*

Stimulation of nipple-growth by oestrone and allied substances is most readily seen and measured in the (male) guinea-pig. The present experimental dose (1.5 γ equilin, or 1.0 γ oestrone, twice daily for 3 weeks), based on our former experience, was expected to be just sufficient to cause a distinct reaction, thereby demonstrating fine differences in activity. Table VIII states in millimetres the actual measurements obtained and shows almost mathematically identical activities for the two substances. However, two of the three animals in the equilin group secreted "milk" from the 4th or 5th to the 8th day after the last injections (which is remarkably late), whereas the oestrone group, as would be expected with so low a dosage, showed no secretion.

Equilin, therefore, stimulates the mammary gland at least as strongly as oestrone. The delayed secretion invites speculation concerning possible tissue storage of the hormone, on which point experience is lacking.

Table VIII.

Guinea-pig	Substance	Weight 31. v	Measurement of nipples (mm.) 31. v		Measurement of nipples (mm.) 7. vi			
3852 } 3853 } 3854 }	Equilin	662	2.3-3.4 and 2.0-2.9	2.2-3.0	2.9-4.2 and 3.0-4.4	2.8-4.0		
		540	2.1-2.9		1.9-2.5		2.3-4.5	2.4-3.5
		786	2.5-3.0		2.6-3.3		3.0-3.7	3.1-3.6
3855 } 3856 } 3857 }	Oestrone	608	2.4-2.8	2.4-3.2	2.8-3.6	2.6-3.7		
		536	2.3-3.2	1.9-2.8	2.4-4.0	2.8-4.0		
		784	2.8-3.1	2.5-3.4	2.5-3.8	2.8-4.2		
			14.6		21.6			
3852 } 3853 } 3854 }	Equilin	662	3.7-5.0 and 3.0-5.0	3.7-4.8	3.6-5.0 and 3.7-5.0	3.9-5.0		
		540	3.7-5.1		3.2-4.6		3.6-5.1	3.0-4.8
		786	4.2-4.2		4.2-5.0		4.8-4.8	4.8-5.2
3855 } 3856 } 3857 }	Oestrone	608	4.2-4.6	3.7-4.5	5.0-5.5	4.6-5.0		
		536	3.7-4.5	3.5-4.5	3.7-5.6	3.2-5.0		
		784	3.6-3.8	3.2-4.1	3.5-4.2	3.3-4.6		

(g) *Epithelial metaplasia of the male genital tract.*

This was noted after use of oestrone almost simultaneously by Lacassagne [1933, 1, 2], de Jongh [1933, 1934] and Burrows and Kennaway [1934]. Five prepubertally castrated adult male mice received 0.75 γ equilin subcutaneously twice daily for 3 weeks, while four more received 0.5 γ oestrone twice daily. The genitalia were examined by serial section with the following results.

(1) Stratified epithelium appeared in certain parts of the prostate (Lacassagne, de Jongh) of all mice, somewhat more conspicuously in the equilin group.

(2) The excretory ducts of the seminal vesicles showed similar changes (de Jongh) to a similar extent in both groups.

(3) Connective tissue changes in the ampulla of the vas deferens [Heringa and de Jongh, 1934] were of the same order in all treated animals.

(4) "Paradoxical" thickening of the vesicular smooth muscle (see section (e)) was somewhat weaker, though still distinct, in the equilin group. Similar experiments using rats produced the same order of change in both groups treated. The seminal vesicles were entirely used for serial section and therefore not weighed.

DISCUSSION.

The comparative biological activities of equilin and oestrone have been examined under varying experimental conditions and on various test objects, the minimum dose causing oestrus in mice being adopted as a basis of comparison in preference to a weight basis, for practical reasons.

Equilin is as active as or more active than oestrone in nearly every instance. Two exceptions are the growth of preputial glands (especially in the male) and, in less degree, the "paradoxical" growth of seminal vesicles in the mouse. Neither of these being material to the human subject, our results do not speak against the clinical use of equilin.

The possibility of course remains that human sensitivity may differ from mouse sensitivity in an opposite direction from that of the rat; in which case oestrone would naturally be preferable. The regrettable paucity of quantitative

data concerning the action of oestrone in human subjects renders actual comparison impossible. As matters stand, equilin might even prove a more suitable therapeutic agent than oestrone. On the pharmacological evidence, equilin cannot be regarded as a harmful or even undesirable impurity in commercial oestrogenic preparations, which are standardised in mouse units (though were the comparative basis to be the minimum oestrogenic rat unit, equilin might be expected to show weaker antimasculine and uterine growth-promoting activity).

SUMMARY.

1. Equilin, compared with oestrone in equivalent dosage (calculated from their oestrogenic activities in mice), was more active in producing oestrus in the castrated rat, uterine and to some extent vaginal growth, vaginal opening in the young female rat, inhibition of the testes and male secondary sex organs of the male rat (with the few exceptions mentioned below), stimulation of the mammary gland of the guinea-pig and metaplasia of prostatic epithelium in the mouse.

2. The activities of equilin and oestrone were approximately equal as regards inhibition of preputial glands and seminal vesicles in the entire rat, "paradoxical" stimulation of the growth of seminal vesicles in the young castrated rat or of nipples in the guinea-pig, metaplasia of the vesicular duct epithelium of the mouse and changes in the connective tissue of the vas deferens of the mouse.

3. Oestrone was a more effective stimulus to the growth of preputial glands in the young female and to a greater extent in the male rat; and to the growth of seminal vesicles in the castrated mouse.

4. Equilin, when present in commercial oestrogenic preparations, cannot be regarded as an undesirable contamination.

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