THE INFLUENCE OF THYROID FUNCTION ON THE ALLEN-DOISY DOSE RESPONSE LINE OBTAINED BY THE ADMINISTRATION OF OESTRONE AND OESTRADIOL-3,17β IN MICE

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Summary

Hypothyroidism significantly increases the slope of the dose response line obtained by the subcutaneous and intravaginal administration of oestrone and concomitantly decreases the M.E.D. Hyperthyroidism has the reverse effect. Hypothyroidism, however, has no influence on the dose response line obtained by the intravaginal administration of oestradiol-3,17β.

The slope of the dose response line for oestradiol-3,17β is significantly lower than the slope for oestrone.

The significance of these results to an understanding of the mode of action of oestrogens is discussed.

I. Introduction

A considerable literature has now accumulated on the interaction between the hormones of the thyroid gland and the ovary (cf. reviews of Salter (1940, 1950)). Recently Langham and Gustavson (1947) and Barker *et al.* (1950) studied the influence of thyroid function on the Allen-Doisy oestrogen test in rats. They claimed that the thyroid status of the animals influenced their sensitivity to oestrone. As they did not use the method of log dose-probit analysis (or its equivalents), their interpretation of the results is questionable. A superficial examination of the figures of Langham and Gustavson, however, revealed the possibility that the level of thyroid activity could influence the slope of the dose response line. It was decided to investigate the nature of the dose response line in hypo- and hyperthyroid mice, using both the subcutaneous and intravaginal methods of administration.

II. Oestrone

(*a*) *Materials and Methods*

(i) *Preparation of Oestrogen Solutions.*—Nut oil was used as the vehicle for subcutaneous administration and distilled water was used as the vehicle for intravaginal administration. The oily solutions were prepared by the method described by Emmens (1950*b*) and the aqueous solutions by the method described by Biggers (1951*a*). The oestrone employed was a product of Organon Laboratories.

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(ii) *Biological Assay.*—Two colonies (*T*1 and *T*2) of female albino mice bred in the Department were used in this part of the investigation. Colony *T*1 consisted of 240 animals and colony *T*2 of 300 animals. Fourteen days after ovariectomy they were given subcutaneously a priming dose of 1 μg. oestrone in nut oil. Seven days later each colony was randomly divided into two groups; one group was used to determine the dose response line resulting from subcutaneous administration, while the other group was used to determine the dose response line resulting from intravaginal administration. The results are shown in Table 1. The colonies were accepted as satisfactory for use in Allen-Doisy assays.

TABLE 1

TEST SLOPES OBTAINED FOLLOWING THE SUBCUTANEOUS AND INTRAVAGINAL ADMINISTRATION OF OESTRONE TO COLONIES *T*1 AND *T*2

Colony	Route	Slope	Standard Error of Slope
*T*1	Subcutaneous	5·93	0·68
	Intravaginal	1·50	0·25
*T*2	Subcutaneous	5·85	0·97
	Intravaginal	1·79	0·46

Colony *T*1 was used to study the effect of thyroxine and methyl-thiouracil on the oestrone dose response line, while colony *T*2 was used to study the effect of ^{131}I. The plan of each experiment will be described as the results are presented. A standard 2-injection technique was employed for both the subcutaneous and intravaginal tests and the general procedures of randomization and animal management were as described by Biggers (1951*a*).

(*b*) *The Modification of Thyroid Activity*

Three methods have been used to alter the thyroid activity:

(i) The injection of thyroxine;

(ii) The administration of a goitrogen, methyl-thiouracil; and

(iii) The injection of thyroid-lethal doses of radioactive iodine, ^{131}I.

(i) *Thyroxine Administration.*—The normal average thyroid secretion rate in mature female mice has been estimated at 0.40-1.10 μg. DL-thyroxine per day, depending on the strain used (Hurst and Turner 1948). This was estimated from the amount of thyroxine required to maintain normal thyroid weight in animals fed thiouracil. In general it is believed that ovariectomy leads to decreased thyroxine production (see Hurst and Turner 1948).

The following procedure was adopted. Before each test, the colony was randomly divided into two groups, one group being used as a control and the other rendered hyperthyroid. Hyperthyroidism was induced by the subcutaneous injection of 4 μg. DL-thyroxine (B.D.H.) in 0.05 ml. distilled water for four consecutive days. The adequacy of the dose was confirmed by the anoxia

resistance test of Smith, Emmens, and Parkes (1947). The oestrogen test was commenced on the seventh day, and after the conclusion of a test the colony was rested for one week before the next series of thyroxine injections.

(ii) *Methyl-thiouracil Administration.*—Several studies have appeared on the action of goitrogens in mice. Thiouracil has been the most commonly used substance (Dalton, Morris, and Dubnik 1946; Hurst and Turner 1947, 1948; Waldo, personal communication to Hurst and Turner 1948; Gorbman 1947*a*; Dalton *et al.* 1950). Several strains of mice have been studied by these workers, namely Schwing, Rockland, C_3H, yellow A^y Rockland hybrids, A, C57, I, and dba. Thyroid enlargement was noted by all the above workers except Waldo who, studying C57 and dba strains, found little change. Freiesleben, Kjerulf-Jensen, and Schmith (1945) have used methyl-thiouracil and observed only moderate hyperplasia.

4-Methyl-2-thiouracil has been employed in this work; 0.1 per cent. was incorporated in the drinking water and administration of the substance was maintained continually throughout the experiment. Over a period of 6-8 months no deleterious effects have been noted in the colonies, in agreement with the observations of Gorbman (1947*a*). The mice were given methyl-thiouracil for 10-14 days before an assay was commenced. Gorbman (1947*a*) described histological changes in the thyroid indicative of thyroxine deficiency within 7 days from the start of treatment. Also it has been found that methyl-thiouracil effectively increases the thyroid weight in the strain of mice used in these experiments (see Table 2).

TABLE 2

THYROID WEIGHT OF UNTREATED MICE AND MICE HAVING RECEIVED 0.1 PER CENT. METHYL-THIOURACIL IN THE DRINKING WATER OVER A PERIOD OF 3 WEEKS

	Mean Thyroid Weight (mg.)	Standard Error of Mean
Untreated	5·60	0·30
Treated	16·38	1·47

(iii) *^{131}I Administration.*—The first report of the thyroid-lethal properties of ^{131}I was by Hamilton (1942) who showed the effect of 300 μc. ^{131}I per kg. body weight in a dog. The effect of massive doses of ^{131}I in mice has been extensively studied by Gorbman (1947*b*, 1949*a*, 1949*b*, 1950), who has shown that the dose of ^{131}I required to destroy the thyroid depends greatly on the iodine content of the diet. It appears that provided that 4.1 μc. ^{131}I per mg. of thyroid enters the gland the dose will be effective.

Colony *T2* was randomized into two groups, one of 200 and the other of 100 mice. Each animal of the larger group received a single subcutaneous injection of 250 μc. ^{131}I while the animals of the smaller group were kept as controls. The ^{131}I was obtained from the Atomic Research Establishment, Harwell, England. It was received ready for injection, the carrier solution being sodium bisulphite, pH 9.1, which had no obvious irritant action.

(c) *Results*

(i) *Subcutaneous Administration.*—The results obtained in mice with thyroxine and methyl-thiouracil are shown in Table 3. The estimates of slope

TABLE 3

DOSE RESPONSE DATA FOR SUCCESSIVE TESTS OF OESTRONE IN OIL ADMINISTERED SUBCUTANEOUSLY TO UNTREATED MICE, AND MICE RECEIVING THYROXINE OR METHYL-THIOURACIL

Untreated					Treated			
Test No.	Dose (μg.)	No. of Animals per Group	Animals Positive (%)	Mean Slope	Dose (μg.)	No. of Animals per Group	Animals Positive (%)	Mean Slope
					Thyroxine			
1	0·037	19	10·5		0·037	19	15·8	
	0·05	20	35		0·05	20	30	
	0·069	20	65		0·069	20	40	
	0·091	20	75		0·091	20	60	
	0·123	18	83·3		0·123	20	70	
2	0·037	25	0		0·037	25	8	
	0·05	25	32		0·05	25	24	3·34
	0·069	25	40		0·069	25	20	±0·76
	0·091	25	64		0·091	24	50	
3	0·037	19	0		0·037	19	5·3	
	0·05	20	20		0·05	19	15·8	
	0·069	20	5		0·069	20	20	
	0·091	20	50		0·091	20	55	
	0·123	20	95		0·123	20	65	
				5·33 ±0·40	Methyl-thiouracil			
4	0·037	19	5·3		0·037	18	0	
	0·05	19	10·5		0·046	19	10·5	
	0·069	19	36·8		0·058	19	5·3	
	0·091	19	63·2		0·073	19	57·9	
	0·123	19	89·5		0·091	18	83·3	
5	0·037	19	10·5		0·037	16	0	
	0·05	20	30		0·046	19	21·1	
	0·069	18	66·7		0·058	19	31·6	7·34
	0·091	17	52·9		0·073	18	66·7	±0·85
	0·123	17	100		0·091	19	73·7	
6	0·037	18	5·6		0·037	18	0	
	0·05	18	16·7		0·046	18	5·6	
	0·069	18	33·3		0·058	18	27·8	
	0·091	18	66·7		0·073	18	55·6	
	0·123	18	88·9		0·091	18	72·2	

of the individual dose response lines are variable. Table 4 shows that while the *within mean slope* variations can be considered as due to random sampling, there are highly significant differences between the mean slopes obtained with

TABLE 4

PARTITIONING OF χ^2 FOR THE DATA OF TABLE 1

Source of Variation	D.F.	χ^2	P
Parallelism:			
1. Between mean slopes ..	2	18·74	<0·001
2. Within mean slopes ..	9	8·38	0·7-0·5
Heterogeneity	28	40·58	0·1-0·05

the untreated, thyroxine-treated, and methyl-thiouracil-treated groups respectively. The results are illustrated in Figure 1. A comparison of the mean

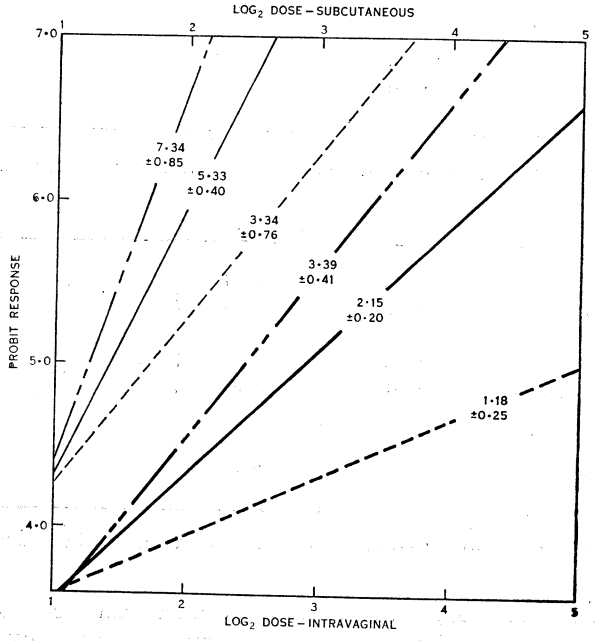


Fig. 1.—Dose response lines calculated from the data of Tables 3 and 9. Thin lines (upper), subcutaneous data. Thick lines (lower), intravaginal data; ——- - -—— hypothyroidism; ———— normal; ------ hyperthyroidism.

slope from the methyl-thiouracil group and that of the untreated group shows them to be significantly different ($\chi^2_{(1)} = 5.00$, $P < 0.05$). Similarly a comparison between the mean slopes obtained with the untreated and thyroxine-treated groups respectively shows a significant difference ($\chi^2_{(1)} = 9.57$, $P < 0.01$).

An analysis of the M.E.D.'s is given in Table 5. The effect of thyroxine is to increase significantly the M.E.D. The effect of methyl-thiouracil appears to be in the reverse direction, i.e. it tends to decrease the M.E.D., but the result just fails to reach conventional significance.

TABLE 5

RATIO OF THE M.E.D.'S AND FIDUCIAL LIMITS OF ERROR ($P = 0.05$) OF THE RATIO FOR THE DATA OF TABLE 2*

Test No.	Treatment	Log_2 M.E.D.	S.E. of Log_2 M.E.D.	Ratio M.E.D. of Untreated to M.E.D. of Treated	Fiducial Limits of Error (P=0·05) of Ratio
1	None	1·262	0·103	0·83	0·65-1·07
	Thyroxine	1·527	0·156		
2	None	1·436	0·106	0·75	0·51-1·11
	Thyroxine	1·857	0·266		
3	None	1·503	0·074	0·82	0·66-1·01
	Thyroxine	1·790	0·136		

Combined data for thyroxine: weighted mean ratio, 0.81; fiducial limits of error ($P = 0.05$), 0.70-0.95.

4	None	1·502	0·090	1·15	0·99-1·33
	Methyl-thiouracil	1·307	0·064		
5	None	1·244	0·123	1·05	0·87-1·25
	Methyl-thiouracil	1·181	0·083		
6	None	1·362	0·088	1·06	0·90-1·25
	Methyl-thiouracil	1·282	0·075		

Combined data for methyl-thiouracil: weighted mean ratio, 1.09, fiducial limits of error ($P = 0.05$), 0.99-1.19.

* In all figures and tables used in assessing results with the subcutaneous method logarithmic transformates, p, of the doses given, X_p, are used, in accordance with the formula $X_p = 2.7 \times 10^{-2} \times 1.35^p$.

The results obtained with Colony *T*2 are shown in Table 6. Table 7 shows that while *within mean slope* variations can be considered as due to random sampling, there is a significant difference between the mean slope for the untreated group and the mean slope of the ^{131}I-treated group.

An analysis of the M.E.D.'s is given in Table 8. The effect of the ^{131}I is to decrease significantly the M.E.D., an effect which was on the borderline of significance with methyl-thiouracil (Table 5).

TABLE 6

DOSE RESPONSE DATA FOR SUCCESSIVE TESTS OF OESTRONE IN OIL ADMINISTERED SUBCUTANEOUSLY TO UNTREATED MICE AND MICE TREATED PREVIOUSLY WITH A THYROID-LETHAL DOSE OF ^{131}I

	Untreated				^{131}I			
Test No.	Dose (μg.)	No. of Animals per Group	Animals Positive (%)	Mean Slope	Dose (μg.)	No. of Animals per Group	Animals Positive (%)	Mean Slope
1	0·037	20	5		0·037	34	5·9	
	0·050	20	10		0·046	34	8·8	
	0·069	28	25		0·058	40	22·5	
	0·091	20	50		0·073	41	65·9	
	0·123	20	85		0·091	34	82·4	
				5·44				7·90
				±0·68				±0·84
2	0·037	20	5		0·037	18	0	
	0·050	20	15		0·046	20	10	
	0·069	27	33·3		0·058	26	23·1	
	0·091	20	60		0·073	20	65	
	0·123	20	90		0·091	20	85	

TABLE 7

PARTITIONING OF χ^2 FOR THE DATA OF TABLE 6

Source of Variation	D.F.	χ^2	P
Parallelism:			
1. Between mean slopes ..	1	5·19	0·05-0·02
2. Within mean slopes ..	2	0·60	0·8-0·7
Heterogeneity	11	6·86	0·9-0·8

TABLE 8

RATIO OF THE M.E.D.'S AND FIDUCIAL LIMITS OF ERROR ($P = 0.05$) OF THE RATIO FOR THE DATA OF TABLE 6

Test No.	Treatment	Log_2 M.E.D.	S.E. of Log_2 M.E.D.	Ratio M.E.D. of Untreated to M.E.D. of Treated	Fiducial Limits of Error (P=0·05) of Ratio
1	None	1·655	0·091	1·38	1·19-1·61
	^{131}I	1·188	0·059		
2	None	1·517	0·088	1·25	1·18-1·44
	^{131}I	1·196	0·059		

Combined data: weighted mean ratio 1.31; fiducial limits of error ($P = 0.05$), 1.18-1.45.

(ii) *Intravaginal Administration.*—The results obtained in mice treated with thyroxine and methyl-thiouracil are shown in Table 9. Table 10 shows that while the *within mean slope* variations can be considered as random fluctuations, there are highly significant differences between the mean slopes obtained with the untreated, thyroxine-treated, and methyl-thiouracil-treated mice respectively.

TABLE 9

DOSE RESPONSE DATA FOR SUCCESSIVE TESTS OF OESTRONE IN DISTILLED WATER ADMINISTERED INTRAVAGINALLY* TO UNTREATED MICE, AND MICE RECEIVING THYROXINE OR METHYL-THIOURACIL

Untreated					Treated			
Test No.	Dose (μg. $\times$ 10^{-3})	No. of Animals per Group	Animals Positive (%)	Mean Slope	Dose (μg. $\times$ 10^{-3})	No. of Animals per Group	Animals Positive (%)	Mean Slope
					Thyroxine			
1	0·1	20	5		0·1	20	5	
	0·2	16	12·5		0·2	19	15·8	
	0·4	19	36·8		0·4	20	25	
	0·8	20	65		0·8	18	27·8	
	1·6	20	85		1·6	20	45	1·18 ±0·25
2	0·1	18	11·1		0·1	20	5	
	0·2	20	20		0·2	19	15·8	
	0·4	18	50		0·4	20	30	
	0·8	19	68·4		0·8	20	35	
	1·6	18	88·9	2·15 ±0·20	1·6	19	52·6	
					Methyl-thiouracil			
3	0·1	18	11·1		0·1	19	10·5	
	0·2	18	27·8		0·18	18	27·8	
	0·4	17	41·2		0·32	17	52·9	
	0·8	18	66·7		0·57	19	89·5	
	1·6	16	87·5		1·00	18	94·4	3·40 ±0·41
4	0·1	18	11·1		0·1	16	0	
	0·2	15	26·7		0·18	19	15·8	
	0·4	18	55·6		0·32	19	52·6	
	0·8	15	80		0·57	19	89·5	
	1·6	19	94·7		1·00	18	94·4	

* In all figures and tables used in assessing results with the intravaginal method logarithmic transformates, p, of the doses given, X_p, are used, in accordance with the formula $X_p = 5.0 \times 10^{-5} \times 2^p$.

The results are illustrated graphically in Figure 2. A comparison of the mean slope from the methyl-thiouracil group and that of the untreated group shows them to be significantly different ($\chi^2_{(1)} = 7.29$, $P < 0.01$). Similarly a compari-

son between the mean slopes obtained with the untreated and the thyroxine-treated groups respectively also shows a significant difference ($\chi^2_{(1)} = 9.10$, $P < 0.01$). An analysis of the M.E.D.'s is given in Table 11. The effect of thyroxine is to increase significantly the M.E.D. while the effect of methyl-thiouracil is to decrease it significantly.

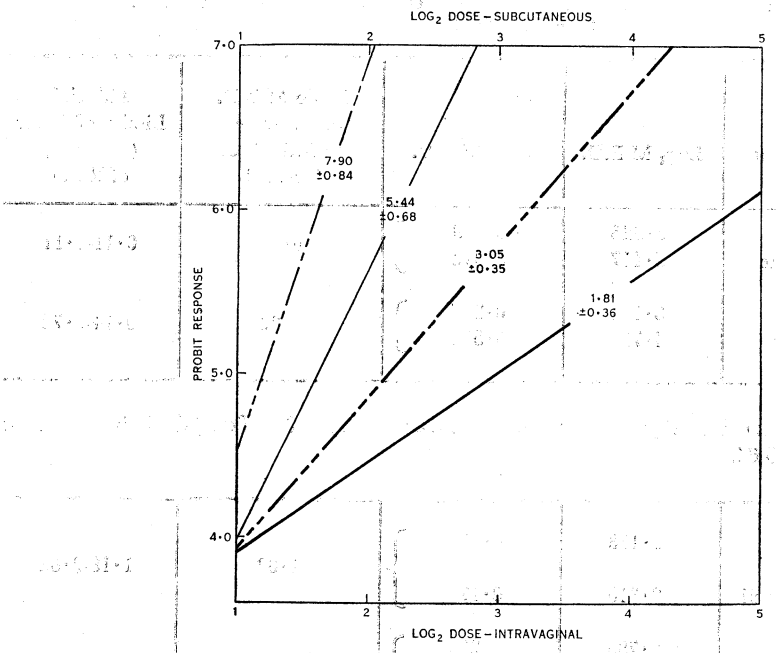


Fig. 2.—Dose response lines calculated from the data of Tables 6 and 12. Key as for Figure 1.

The results obtained in the ^{131}I mice are given in Table 12. They are analysed in Tables 13 and 14 and fully confirm the results obtained with methyl-thiouracil.

TABLE 10

PARTITIONING OF χ^2 FOR THE DATA OF TABLE 9

Source of Variation	D.F.	χ^2	P
Parallelism:			
1. Between mean slopes ..	2	22·66	<0·001
2. Within mean slopes ..	5	1·76	0·9-0·8
Heterogeneity	23	4·68	>0·99

All slopes obtained in hypothyroid animals with oestrone over the experimental period have been combined and compared with the weighted mean slope for oestrone in normal animals (Biggers 1953). The results are shown in Table

15. Ignoring the slight heterogeneity in the distilled water group, a highly significant difference is seen between the two groups ($t = 6.16$, D.F. 50, $P < 0.001$). Thus the higher slope in hypothyroid animals with oestrone is confirmed.

TABLE 11

RATIO OF THE M.E.D.'S AND FIDUCIAL LIMITS OF ERROR ($P = 0.05$) OF THE RATIO FOR THE DATA OF TABLE 9

Test No.	Treatment	Log_2 M.E.D.	S.E. of Log_2 M.E.D.	Ratio M.E.D. of Untreated to M.E.D. of Treated	Fiducial Limits of Error (P=0·05) of Ratio
1	None	3·925	0·219	0·36	0·11-1·11
	Thyroxine	5·417	0·812		
2	None	3·134	0·237	0·32	0·14-0·72
	Thyroxine	4·782	0·552		

Combined data for thyroxine: weighted mean ratio, 0.33; fiducial limits of error ($P = 0.05$), 0.17-0.64.

3	None	3·178	0·269	1·82	1·18-2·82
	Methyl-thiouracil	2·310	0·175		
4	None	2·785	0·225	1·19	0·83-1·71
	Methyl-thiouracil	2·538	0·144		

Combined data for methyl-thiouracil: weighted mean ratio, 1.41; fiducial limits of error ($P = 0.05$), 1.07-1.87.

III. OESTRADIOL-3,17β

As a result of the findings with oestrone it was decided to determine the relative potencies of other oestrogens in hypothyroid mice. Oestradiol-3,17β was selected for the first examination.

(*a*) *Materials and Methods*

Colony *T*3, consisting of 260 ovariectomized albino mice, was prepared for this work. Hypothyroidism was induced by the administration of methyl-thiouracil in the drinking water as before and all other methods were identical with those described for the oestrone experiments. The oestradiol-3,17β was a product of Organon Laboratories.

(*b*) *Results*

The design of the assays and the results obtained are shown in Table 16. Table 17 shows that very little difference exists *within mean slopes* while a highly significant difference exists *between mean slopes.* Thus no estimation of the relative potency can be made. However, Table 18 shows that the M.E.D. for oestradiol-3,17β is significantly lower than the M.E.D. for oestrone (see Fig. 3).

TABLE 12

DOSE RESPONSE DATA FOR SUCCESSIVE TESTS OF OESTRONE IN DISTILLED WATER ADMINISTERED INTRAVAGINALLY TO UNTREATED MICE AND MICE TREATED PREVIOUSLY WITH A THYROID-LETHAL DOSE OF ^{131}I

Untreated					^{131}I			
Test No.	Dose ($\mu g. \times 10^{-3}$)	No. of Animals per Group	Animals Positive (%)	Mean Slope	Dose ($\mu g. \times 10^{-3}$)	No. of Animals per Group	Animals Positive (%)	Mean Slope
1	0·1	21	9·5		0·10	30	10·0	
	0·2	20	25		0·16	33	36·4	
	0·4	26	46·2		0·26	40	55·0	
	0·8	21	66·7		0·41	39	74·4	
	1·6	19	94·7		0·66	32	90·6	
				1·81 ±0·36				3·05 ±0·35
2	0·1	19	21·1		0·10	18	16·7	
	0·2	21	38·1		0·16	19	42·1	
	0·4	26	50		0·26	25	64·0	
	0·8	15	73·3		0·41	18	88·9	
	1·6	20	85·0		0·66	17	94·1	

TABLE 13

PARTITIONING OF χ^2 FOR THE DATA OF TABLE 12

Source of Variation	D.F.	χ^2	*P*
Parallelism:			
1. Between mean slopes ..	1	7·92	0·01-0·001
2. Within mean slopes ..	2	1·88	0·5-0·3
Heterogeneity	12	2·39	>0·99

The results show that the mean slope for oestradiol-3,17β is significantly lower than that of oestrone. This result could mean that the thyroid status of the animal has no effect on the dose response line for oestradiol-3,17β; this

possibility was examined in the ^{131}I-treated colony. Table 19 shows the design of the assay and the results obtained. Tables 20 and 21 show that hypothyroidism has no influence on the dose response line obtained with oestradiol-3,17β.

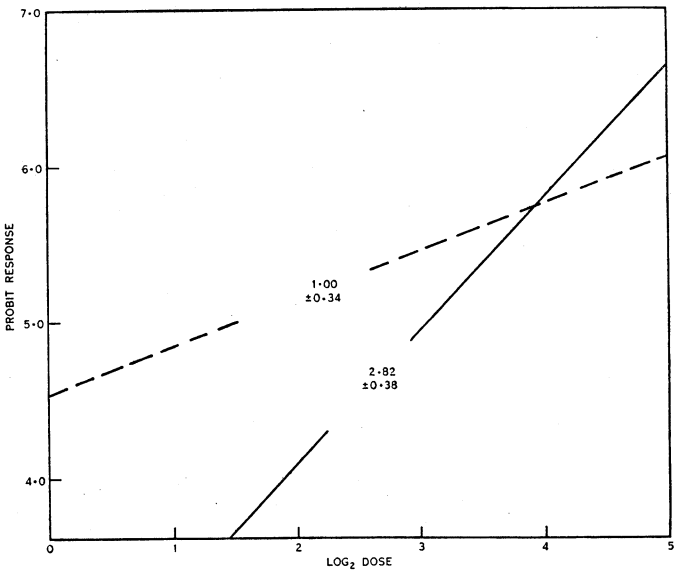


Fig. 3.—Dose response lines calculated from the data of Table 16.
------ Oestradiol-3,17β; ———— oestrone.

TABLE 14

RATIO OF THE M.E.D.'S AND FIDUCIAL LIMITS OF ERROR ($P = 0.05$) OF THE RATIO FOR THE DATA OF TABLE 12

Test No.	Treatment	Log_2 M.E.D.	S.E. of Log_2 M.E.D.	Ratio M.E.D. of Untreated to M.E.D. of Treated	Fiducial Limits of Error (P=0·05) of Ratio
1	None	3·084	0·211	2·25	1·62-3·13
	^{131}I	1·910	0·120		
2	None	2·786	0·294	2·25	1·46-3·46
	^{131}I	1·618	0·156		

Combined data: weighted mean ratio, 2.25; fiducial limits of error ($P = 0.05$), 1.73-2.94.

IV. DISCUSSION

The significant variations in the slope found between the oestrone dose response lines of normal, hypothyroid, and hyperthyroid mice are reflections of the significant differences in variability of threshold doses of oestrone required

to produce cornification. Similarly differences in slope between the oestrone and oestradiol-3,17β dose response lines are reflections of the significant difference in variation of the threshold doses of the two steroids. The method of probit

TABLE 15

A COMPARISON OF THE WEIGHTED MEAN OF THE SLOPES OBTAINED WITH NORMAL ANIMALS (50 PER CENT. AQUEOUS GLYCEROL AND DISTILLED WATER GROUPS) AND HYPOTHYROID ANIMALS

	Normal Group	Hypothyroid Group
Weighted mean slope	2·08	3·07
V_b (theoretical)	0·00543	0·0487
V_b (observed)	0·00913	0·0220
	$F=1·68$, $n_1=44$	$F=2·22$, $n_1=\infty$
	$n_2=\infty$	$n_2=6$
	$0·01<P<0·05$	$P>0·05$

TABLE 16

DOSE RESPONSE DATA FOR SUCCESSIVE TESTS OF OESTRONE AND OESTRADIOL-3,17β IN DISTILLED WATER ADMINISTERED INTRAVAGINALLY TO MICE RECEIVING METHYL-THIOURACIL

	Oestrone				Oestradiol-3,17β			
Test No.	Dose (μg.$\times 10^{-3}$)	No. of Animals per Group	Animals Positive (%)	Mean Slope	Dose (μg.$\times 10^{-3}$)	No. of Animals per Group	Animals Positive (%)	Mean Slope
1	0·28	20	10		0·17	20	40	
	0·5	20	50		0·3	19	57·9	
	0·9	20	65		0·54	20	55	
2	0·28	25	40		0·28	35	65·7	
	0·5	25	64	2·82	0·5	25	80	1·00
	0·9	25	88	±0·38	0·9	25	84	±0·34
3	0·1	25	4		0·1	24	58·3	
	0·2	25	24		0·2	25	68	
	0·4	25	52		0·4	25	88	
	0·8	25	80		0·8	24	83·3	

analysis is derived from considerations of the integrated normal distribution curve* (see Finney 1947, 1949). By changing the theoretical probit values

* There are no *a priori* reasons for the assumption of a normal distribution of threshold doses of oestrogen, e.g. Biggers (1951*b*) has shown that other transformations describe the data equally well.

from the linear regression lines back to percentages the theoretical integrated normal curves are obtained. Differentiation of these curves gives the theoretical normal distribution of threshold doses. Figure 4 shows the theoretical distributions calculated from the intravaginal data for the thiouracil and thyroxine experiments and Figure 5 for the oestrone and oestradiol-3,17β experiments.

TABLE 17

PARTITIONING OF χ^2 FOR THE DATA OF TABLE 16

Source of Variation	D.F.	χ^2	P
Parallelism:			
1. Between mean slopes ..	1	12·90	<0·001
2. Within mean slopes ..	4	0·34	0·99-0·98
Heterogeneity	8	7·61	0·5-0·3

TABLE 18

RATIO OF THE M.E.D.'S AND FIDUCIAL LIMITS OF ERROR ($P = 0.05$) OF THE RATIO FOR THE DATA OF TABLE 16

Test No.	Treatment	Log_2 M.E.D.	S.E. of Log_2 M.E.D.	Ratio of M.E.D. of Oestrone to M.E.D. of Oestradiol-3,17β	Fiducial Limits of Error (P=0·05) of Ratio
1	Oestrone	3·500	0·198	2·19	0·77-6·23
	Oestradiol-3,17β	2·365	0·742		
2	Oestrone	2·675	0·220	2·84	0·56-14·3
	Oestradiol-3,17β	1·145	1·170		
3	Oestrone	2·946	0·182	6·63	1·37-32·1
	Oestradiol-3,17β	0·217	1·008		

Combined data: weighted mean ratio, 3.02; fiducial limits of error ($P = 0.05$), 1.40-6.50.

The demonstration of a difference between the dose response lines of oestrone and oestradiol-3,17β in intravaginal tests indicates a fundamental distinction between the *local* actions of these steroids on the vagina. This seems to be the first demonstration of a qualitative difference in the action of two natural oestrogenic steroids and of considerable importance in the problem of molecular configuration and activity. The results obtained by the subcutaneous administration of oestrone merely confirm the action of thyroxine on the dose response line shown by the intravaginal technique.

It seems that oestrone is in some way modified in the vagina whereas oestradiol-3,17β is not, and that the modification of oestrone is under the control of thyroid activity. There are several reports in the literature on the increase in oestrogenic activity observed when oestrone is incubated with

TABLE 19

DOSE RESPONSE DATA FOR A TEST OF OESTRADIOL-3,17β IN DISTILLED WATER ADMINISTERED INTRAVAGINALLY TO UNTREATED MICE AND MICE TREATED PREVIOUSLY WITH A THYROID-LETHAL DOSE OF ^{131}I

Untreated				I^{131}		
Dose (μg. $\times 10^{-3}$)	No. of Animals per Group	Animals Positive (%)	Mean Slope	No. of Animals per Group	Animals Positive (%)	Mean Slope
0·05	20	40	1·40 $\pm$0·45	20	45	0·92 $\pm$0·43
0·1	20	40		18	50	
0·2	20	75		20	50	
0·4	20	80		20	80	

TABLE 20

PARTITIONING OF χ^2 FOR THE DATA OF TABLE 19

Source of Variation	D.F.	χ^2	P
Parallelism	1	0·61	0·5-0·3
Heterogeneity	4	3·85	0·5-0·3

TABLE 21

THE RATIO OF THE M.E.D.'S AND THE FIDUCIAL LIMITS OF ERROR ($P = 0.05$) OF THE RATIO FOR THE DATA OF TABLE 19

Treatment	Log_2 M.E.D.	S.E. of Log_2 M.E.D.	Ratio M.E.D. of Untreated to M.E.D. of Treated	Fiducial Limits of Error (P=0·05) of Ratio
Untreated ..	1·914	0·384	1·01	0·39-2·64
I^{131}	1·893	0·592		

various tissues. Mamoli (1938) demonstrated the conversion of oestrone esters to oestradiol-3,17β by fermenting yeast. Heller (1940) showed that oestrone activity was greatly enhanced by incubation with minced uterus and to a lesser extent by heart, lung, spleen, and kidney; liver, however, inactivated the hormone unless cyanide was added, following which increased activity was also

observed. Also Miwa, Ito, and Hayazu (1940) demonstrated the enhancement of activity obtained with heart muscle. Recently the effect of incubating oestrone with blood has been examined. Two groups of workers have examined the problem and have obtained opposite results. Werthessen, Baker, and Borci (1948) found that blood inactivated oestrone whereas Bischoff, Katherman, and Yee (1951) have observed increased activity. The latter group have found that blood has no influence on oestradiol-3,17β and oestriol. The evaluation of the bioassay and extraction procedures used by these two groups is difficult if not impossible. However, there is good reason to suppose that oestrone is in some way modified in various tissues of the body, and this idea is further supported by the recent observations of Claringbold (unpublished data) that cyanide significantly increases the intravaginal activity of oestrone but has no effect on the intravaginal activity of oestradiol-3,17β.

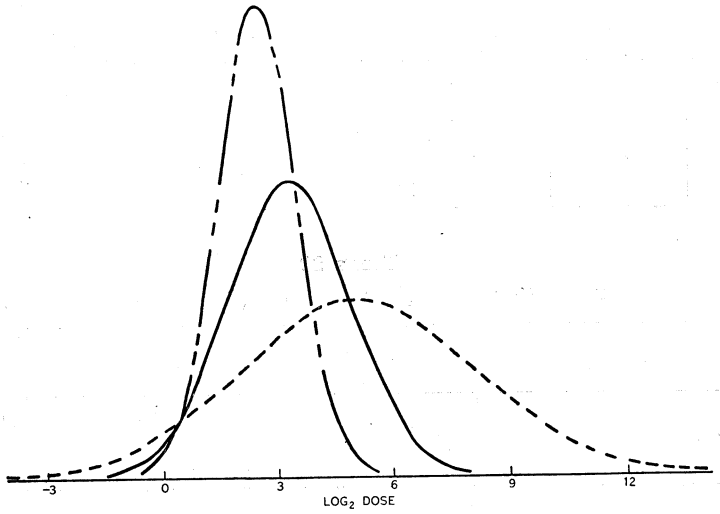
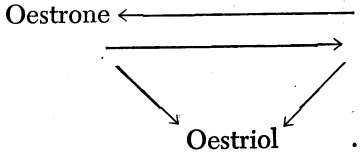


Fig. 4.—Theoretical normal distributions of threshold doses of oestrogen required to produce cornification calculated from the intravaginal dose response lines shown in Figure 1. —— - - —— Hypothyroidism; ———— normal; - - - - - hyperthyroidism.

The various metabolic interconversions of oestrogens have been discussed by Heard (1949). The known interconversions of oestrone, oestradiol-3,17β, and oestriol can be indicated as follows, whence it is seen that oestriol has not so far been shown to change to either of the others:



Bischoff, Katherman, and Yee (1951) have some evidence that in blood at least some of the oestrone is converted to oestradiol-3,17β. It is possible that following absorption into the vaginal cells a similar process may occur. The

depression of slope and the increased M.E.D. observed with oestrone when thyroxine is also administered may be due to interference with this process. The slope and M.E.D. with oestradiol-3,17β are unaffected; furthermore, this steroid is more active than oestrone. These facts may indicate that oestrone acts on the vagina only following conversion into oestradiol-3,17β, but this can only be resolved by further work.

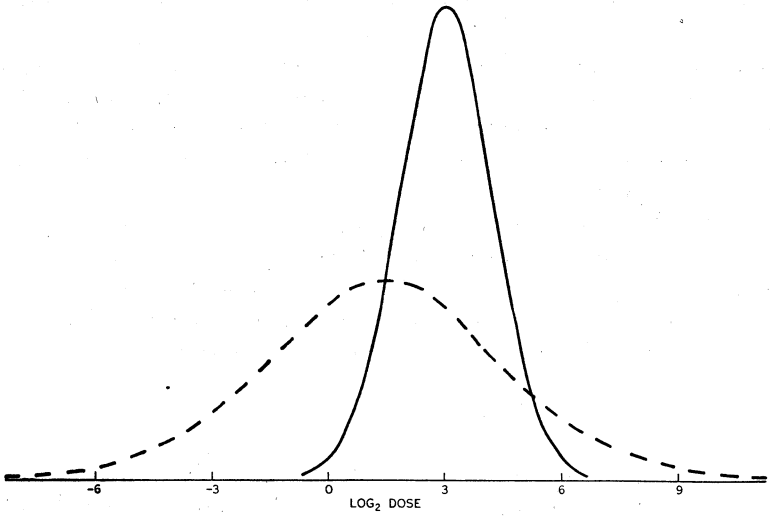


Fig. 5.—Theoretical normal distributions of the threshold doses of oestrogen required to produce cornification calculated from the dose response lines of Figure 3. – – – – – – Oestradiol-3,17β; ———— oestrone.

The findings reported in this paper raise once more the problem of the relationship between the structure and function of oestrogens. The multiplicity of oestrogenic substances has interested people for many years but no theory has been put forward as to what makes a substance oestrogenic. The problem became of special interest following the discovery of synthetic oestrogens (see Dodds *et al.* 1939). For many years attempts have been made to find some steric configuration common to oestrogenic substances and a recent symposium on this subject (Gordon 1950) illustrates the lack of success in this respect. The failure has in part been due to the use of inaccurate and poorly understood bioassay procedures. The discovery of *pro-oestrogens*, as against *true oestrogens* (Emmens 1941, 1942), demonstrated an important group of oestrogenic substances possessing a structure which, in the body, is capable of transformation into a true oestrogen. True oestrogens, only, are capable of acting locally in small concentrations on vaginal cells and stimulating a characteristic biological response. It seems of paramount importance, therefore, that any oestrogenic substance be initially classified into either a pro-oestrogen or a true oestrogen.

As yet the relative activities of all true oestrogens administered intravaginally have not been determined. Those that have been determined indicate that the relative potencies are very similar (see Emmens 1950*b*), and on this basis it

might be supposed that some steric molecular configuration is common to all true oestrogens. Two groups of observations suggest that this is not so:

(1) The finding by all workers using the intravaginal technique that oestradiol-3,17β is the most active compound in 50 per cent. aqueous glycerol (Muhlbock 1940; Emmens 1950*a*), in 1 per cent. egg albumin (Biggers 1951*a*), and in distilled water, as used in the present thiouracil experiments; and

(2) The differential action of thiouracil and cyanide on the oestrone and oestradiol-3,17β dose response lines.

With these results in mind it is tempting to suggest that the property common to all true oestrogens is a molecular structure which can be modified by local metabolic processes in the vaginal cells to give a compound which can initiate the biological response. A large amount of work remains to be done to ascertain the truth of this hypothesis.

The results obtained with thyroxine, methyl-thiouracil, and ^{131}I are interesting from the point of view of thyroid physiology. They provide an example of a peripheral interaction of the ovarian and thyroid hormones. The results emphasize that it is important to specify the steroid hormone being studied and that it is hazardous to generalize from the results obtained with one oestrogen.

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