Table I. Effects of estrogens and progesterone upon HIOMT activity in the pineal glands of castrated female rats

	Controls	Estradiol benzoate	Estradiol benzoate + progesterone	Progesterone
Estradiol benzoate	a)	b)	c)	d)
(μg/day)	0	20	20	0
Progesterone (μg/day)	0	0	200	200
Animals Number	4	4	4	4
Weight (g)	167±9.2	174.8±8.3	175.8±2.8	183.8±12.4
Pineal gland Weight (μg)	1287± 55	1012± 50	950± 87	1300± 104
HIOMT (activity)	43.6±3.0	55.8±4.9	28.8±3.0	2.0± 0.3

Probabilities (employing Student's *t*-test)

	a) vs b)	a) vs c)	a) vs d)	b) vs d)	c) vs d)
Animal wt.	n.s.	n.s.	n.s.	n.s.	n.s.
Pineal wt.	<0.01	<0.02	n.s.	n.s.	<0.05
HIOMT activity (μμmol/mg)	<0.05	<0.02	<0.001	<0.01	<0.001

Table II. Effects of progesterone upon HIOMT activity in the pineal glands of castrated female rats

Dose	a)	b)	c)
(μg/day)	0	20	200
Animals			
Number	4	7	8
Weight (g)	239± 5	232± 9	234± 8
Pineal			
Weight (μg)	1612±183	1378±85	1487±121
HIOMT activity (μμmol/mg)	42.0± 3.7	33.1±4.0	16.6± 4.7

Probabilities (employing Student's *t*-test)

	a) vs b)	a) vs c)	b) vs c)
Animal wt.	n.s.	n.s.	n.s.
Pineal wt.	n.s.	n.s.	n.s.
HIOMT activity	n.s.	<0.01	<0.02

during the estrous cycle of the rat, which might be mediated by gonadotrophins or by nervous pathways. On the other hand, it is of considerable physiological interest to have found a steroid hormone able to alter markedly the pineal gland functions in the rat.

Zusammenfassung. Da die Aktivität der Pinealdrüse bei weiblichen Ratten mit dem Cyklus wechselt, wurde die Wirkung von Oestradiol und Progesteron auf ihre Aktivität bei kastrierten weiblichen Ratten untersucht. Oestradiol bewirkte Gewichtsabnahme und Funktionssteigerung, während Progesteron die Pinealis-Aktivität herabsetzte.

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R_M Values and Biological Action of Testosterone Esters

In a recent communication to this journal, Biagi, Barbaro and Guerra[1] demonstrated a linear correlation between the R_M values of some testoster oneesters, determined by a Boyce-Milborrow technique[2], and the times of maximum biological effect in fowl. Times of maximum effect (BR) were expressed on a molar basis relative to testosterone. Observations in these laboratories[3] reveal that time of maximum effect in rat is not affected significantly when the dose of testoserone ester is increased threefold, indicating that conversion on a molar basis was

[1] G. L. Biagi, A. M. Barbaro and M. C. Guerra, Experientia *27*, 918 (1971).
[2] C. B. C. Boyce and B. V. Milborrow, Nature, Lond. *208*, 537 (1965).
[3] G. T. Richards, unpublished data.

R_M values and biological activity of testosterone esters
Log. relative time of maximum effect

Compound	R_M values	Fowl (DORFMAN)		Rat (DORFMAN)		Rat (JAMES)	
		Observed	Calculated	Observed	Calculated	Observed	Calculated
Testosterone	1.31	0.00	–0.07	0.00	–0.02	0.00	0.01
-17-formate	0.58	0.07	0.11	0.30	0.30	0.15	0.20
-17-acetate	0.46	0.07	0.14	0.30	0.35	0.17	0.23
-17-propionate	0.11	0.18	0.22	0.49	0.51	0.27	0.32
-17-butyrate	–0.09	0.30	0.27	0.60	0.59	0.39	0.38
-17-valerate	–0.26	0.37	0.31	0.72	0.67	0.57	0.42
-17-decanoate	–0.71	0.43	0.42	0.85	0.86	–	–

not necessary. We have therefore correlated BIAGI's results directly with the logarithms of the times of maximum effect in fowl and rat[4] and obtained equations (1) and (2).

	n	r	s	
In fowl				
Log BR = 0.217 + 0.298 R_M	7	0.888	0.081.	(1)
In rat				
Log BR = 0.554 + 0.544 R_M	7	0.913	0.128.	(2)

n signifies the number of results, r correlation coefficient and s standard deviation.

The R_M values of some n-fatty acid esters of testosterone have been measured using the Bush system petroleum ether/methanol/formic acid (90/10/100). Experimental details are described elsewhere[5]. The R_M values, which are shown in the Table, correlate with the biological results, according to equations (3) and (4). Calculated biological results are given in the Table.

	n	r	s	
In fowl				
Log BR = 0.249 — 0.240 R_M	7	0.946	0.059.	(3)
In rat				
Log BR = 0.554 — 0.438 R_M	7	0.993	0.037.	(4)

The coefficients in R_M are negative because in this system the stationary phase is more polar than the moving phase.

The times of maximum effect of the n-fatty acid esters of testosterone generally increase uniformly as the homologous series is ascended, but an exception occurs in moving from formate to acetate, when no difference is observed. This is shown in the Table. Correlations (3) and (4) are therefore particularly encouraging because they are based on results which include those for formate ester and predict its effect reasonably well.

Despite the fact that equations (1) and (2) are disappointing, BIAGI's results correlate very well with biological response if only the acetate to valerate esters are considered, but their predictive value is considerably reduced if the decanoate results are taken into account. The lipid-water distribution coefficients of the n-fatty acid esters of testosterone do not appear to increase in the theoretical logarithmic manner as the homologous series is ascended. Measurements made in these laboratories suggest that the plot of the logarithm of lipid-water distribution coefficient against position in the homologous series, is curved. It is therefore probable that BIAGI's R_M values, which increase linearly from homologue to homologue, correlate logarithmically with the times of maximum effect over only a limited range of the homologous series, where the curve approximates to a straight line, and that the correlation breaks down when an extensive range of the series is examined. The better predictive ability of the results presented here is attributed to the fact that the two chromatographic phases are more representative of lipid-water distribution coefficients than those of the BOYCE-MILBORROW system. This opinion is developed in detail elsewhere[6].

The biological half lives of carbon-14 in rat after i.m. injection of oily solutions of [4-^{14}C] testosterone and its esters, have been published[7], and it was considered of interest to correlate these with both sets of R_M values. Regression analysis yielded equations (5) and (6) and the calculated biological responses which are shown in the Table.

	n	r	s	
BUSH System				
Log BR = 0.354 — 0.265 R_M	6	0.747	0.150.	(5)
BOYCE-MILBORROW-System				
Log BR = 0.351 + 0.651 R_M	5	0.977	0.053.	(6)

Correlation is not as good as for the other two systems and suggests that although the carbon-14 work served the purpose for which it was used, the half lives, because they represent the elimination of both ester and metabolites, are not directly related to time of maximum biological effect.

Zusammenfassung. Eigene Befunde zum Zusammenhang zwischen R_M-Werten und biologischer Wirkung von Testosteron-Estern werden mit denjenigen von BIAGI (Experientia *27*, 918 (1971)) verglichen und diskutiert. Durch Anwendung geeigneter chromatographischer Phasen können allgemeiner gültige und mathematisch ausdrückbare Resultate erhalten werden.

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