Effects of Testosterone Acetate and Propionate and of Estradiol Dipropionate upon the Resistance of the Rat to Evipal Sodium, Nostal, Pernoston and Pentobarbital Sodium*†

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That prolonged administration of male sex hormone from human urine raises the resistance of female and castrated male rats to the barbiturate, evipal, was first shown by Holck, *et al.* (1). Preliminary reports of similar effects by testosterone esters against several barbiturates were subsequently published (2, 3). Antinarcotic effect of testosterone against pentobarbital in the rat has recently been reported by Kinsey (4). Crabtree, Ward and Welch (5), by administering this hormone, raised the resistance of castrated male rats to red squill to the normal level. Störtebecker (6) concluded that estrogenic hormones increased the resistance of rabbits, guinea pigs and mice to depression by ether, magnesium, alcohol, curare and the barbiturate, pernoston. Also, androsterone elevated the rabbit's resistance to ether anesthesia.

Our present studies are intended to establish the necessary doses and durations of administration of various synthetic sex hormones and the limitations of their action, depending on the barbiturate and species of animals.

EXPERIMENTAL

Our albino rats were originally bred from Wistar stock; the hybrids were from our College of Agriculture (Table IV) or bred from rats presented by Professor Carmichael of the University of Alabama (Table V). The diet consisted of Purina Dog Chow. The rat quarters were kept at approximately 25° C. During the barbiturate tests, each rat was kept alone at 27–28° C. and considered recovered when it retracted either hind foot promptly from an extended position.

In the pentobarbital (8097 x 974417, Lilly) experiments, ten sites of injection were used in rotation and the 1% solution was prepared fresh several times daily and kept well protected during the interim between each series of injections.

PART I. INFLUENCE OF VARYING DOSES AND DURATIONS OF ADMINISTRATION OF TESTOSTERONE ACETATE (PERANDREN) UPON THE RESPONSE OF ALBINO RATS TO SINGLE DOSES OF PERNOSTON (SODIUM 2-BUTYLBROMALLYL BARBITURATE) AND TO SODIUM EVIPAL (SODIUM METHYL-CYCLOHEXENYL-*N*-METHYL BARBITURATE) AND UPON THE RESPONSE OF ALBINO MICE TO THE LATTER

The results of six experiments with pernoston are shown in Table I. With a daily dose of 125 γ of the hormone per normal female rat, it was necessary to continue the administration for about two weeks to get a significant difference in recovery time. However, with 500 γ, such a difference was found after one full week of treatment. Although the prolonged hormone medication shortened the female rat recovery time from pernoston, this time never approached the recovery time of the male rats, which is much shorter in the normal male rat. Whether still higher hormone doses could have shortened the female recovery time to the male level we cannot say, but we will return to this problem in Part II. It is also interesting that the percentage of delayed deaths in the hormone-treated females was only 6%, whereas in the normals it was 18%. However, even heavy doses of the hormone failed to completely prevent delayed deaths.

The first experiment in Table II shows that 10-day administration of only 62½ γ of testosterone acetate per rat daily definitely made such treated females recover faster, but did not influence the male rat response to evipal. Three experiments with much higher hormone dosage also failed to change the male response. In an experiment upon mice, the relatively large dose of 125 γ of male hormone per mouse daily for a week failed to influence the recovery time of female mice.

In Table III are shown the results of experiments upon two groups of adult female albino rats in case of which rats of Group 2 were given testosterone acetate before spaying and rats of Group 1 were given this hormone after spaying. With administration of 125 γ per rat daily for about one week, the

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Table I.—Summary of the Results of the Intraperitoneal Administration of 50 Mg./Kg. of Pernoston (Sodium 2-Butyl-Bromallyl Barbiturate, 1% Solution) to 16 Groups Consisting of 12 Albino Rats Each, 3 to 5 Months Old, Which Had Been Given Varying Doses of Testosterone Acetate (Perandren)

The two values under each group in each test represent the average recovery time in hours and the actual number of delayed deaths. The testosterone acetate (perandren) was administered subcutaneously in sesame oil; the final dose was given 3 hrs. before the pernoston; thus, 5 doses indicates that the hormone was allowed to act slightly over 4 full days. The rats were observed over a period of 5 days for delayed deaths. One hormone-treated rat died acutely in Group 2. In case of the two female groups, the critical ratios were calculated in the usual manner from the average recovery times and their standard errors.

No. of Test	Normal Males		Normal Females		Hormone-Treated Females		Critical Ratio	Daily Gammas of Testosterone Acetate per Rat	No. of Doses
1	1.3	0	4.1	4	3.9	0	0.7	125	3
2	1.4	0	4.5	3	4.2	2	1.2	125	5
3	1.2	0	4.1	4	3.2	0	3.1	125	15
4	1.3	0	3.9	0	3.4	1	2.2	500	5
5	1.2	0	4.2	0	3.4	0	2.9	500	8
6	[a]	..	[a]	..	2.7	1	6.0	500	15
Total percentage of delayed deaths				18		6			

[a] The normal rats for Group 3 also served as controls for Group 6.

Table II.—Summary of the Results of the Administration of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate) to 10 Groups Consisting of 10 to 14 Rats Each and to 2 Groups Consisting of 14 and 15 Mice, Respectively, Which Had Been Given Varying Doses of Testosterone Acetate (Perandren)

The rats were from 3 to 6 months old, and the mice weighed from 20 to 28 Gm. each. In Groups 1 and 3, all were albinos; Group 4, albinos with a few hybrids; and Group 2, all hybrids. Values under each group are the average recovery time in hours. Sodium evipal concentrations were such that 0.5 cc. of solution was administered per 100 Gm. of body weight. Testosterone acetate (perandren) was administered as under Table I. One rat died acutely in the hormone-treated group under 4. The critical ratio between the two female groups under 1 was 3.1.

		Average Hours of Recovery					
No. of Test	Sodium Evipal, Mg./Kg.	*A*, Normal Males	*B*, Hormone-Treated Males	*C*, Normal Females	*D*, Hormone-Treated Females	Gammas of Testosterone Acetate per Rat or Mouse	No. of Doses
Rats							
1	80, ip.[a]	0.9	1.0	1.6	1.2	62.5	11, two daily
2	200, subc.[b]	2.8	2.6	...	...	125	8, one daily
3	200, subc.	2.4	2.4	...	...	125	11, one daily
4	200, subc.	2.7	2.8	...	...	500	11, one daily
Albino Mice							
5	180, subc.	...	...	1.8	1.8	125	8, one daily

[a] Intraperitoneal administration.
[b] Subcutaneous administration.

Table III.—Summary of the Results of Subcutaneous Administration of 100 Mg./Kg. of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate, 2% Solution) to Two Groups of 5½-Month-Old Albino Female Rats, 15 in Each Group at the Start

Group 2 was administered testosterone acetate (perandren) as indicated in the dose column before spaying, and Group 1 was treated after spaying. In Group 1, one rat died during the 5-week interval, and a second one during the last interval. In Group 2, one rat died actuely during the eighth test; it had lost much body weight since the previous test. The testosterone acetate (perandren) was administered and the critical ratios calculated as under Table I.

Group No.	Average Hours of Recovery										Gammas of Testosterone Acetate per Rat per Dose	No. of Doses
	1*	1	1	2	1½		1	2	5	2		
1	3.8	3.6	2.9	3.4	3.8	All Spayed	2.6[a]	3.4	3.0[b]	3.3	[a]125	9
											[b]125	8
2	3.6	3.5	2.4[c]	2.4[d]	3.7		3.4	3.2	3.6	3.5	[c]125	8
											[d]500	7
Critical Ratios:			3.7						4.3			

* Time interval in weeks between administrations of evipal.

Table IV.—Summary of the Results of Subcutaneous Administration of 100 Mg./Kg. of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate, 2% Solution) to Five Groups of Hybrid Rats

The hormone was administered subcutaneously in sesame oil (1 mg. of testosterone in 1 cc. of oil). In addition to the daily administration of testosterone propionate during the preceding 2 weeks as indicated, one dose was also given 3 hrs. before the evipal administration.

Group	No. of Rats	Age in Months	Sex	Average Hours of Recovery 17*	21	14	35	69		Daily Mg. of Testosterone per Rat (Given as the Propionate)
1	8	6–7	Male	1.7	1.9	1.8	1.8	...	...	Not treated
2	7	6–7	Female	2.9	1.9[a]	2.3	2.7	...	...	[a] 0.25 during preceding two weeks
3	8	11–12	Male	1.9	1.9	1.6[b]	1.5[c]	1.7	2.0	[b] 0.25 during preceding two weeks [c] Then 0.50 for two weeks
4	14	2	Male	1.0	1.1	1.3	1.3	1.6	1.7	The usual increase as untreated males become mature
5	14	2	Female	1.7	1.9	1.8	1.3[d]	2.5	2.9	[d] 0.25 during preceding two weeks

* Time interval in days between administrations of evipal.

hormone was very efficient in shortening the recovery time from evipal in both the normal and the spayed females. Five hundred gammas daily were given to the normal females for a week following the 125-γ administration; this raised the difference between the hormone-treated and untreated rats. In all cases the improvement induced by the male sex hormone had disappeared two weeks after stopping the hormone administration.

PART II. INFLUENCE OF VARYING DOSES AND DURATIONS OF ADMINISTRATION OF TESTOSTERONE PROPIONATE (PERANDREN) UPON THE RESPONSE OF HYBRID RATS TO SINGLE DOSES OF SODIUM EVIPAL AND OF NOSTAL (ISOPROPYL-BROMALLYL BARBITURATE)

While conducting experiments with testosterone acetate, the more potent propionate became available. We therefore substituted this drug to ascertain and compare its effects. The results of experiments with sodium evipal are presented in Table IV. A large dose of 250 γ of the propionate per rat daily for two weeks made the female rat response equal to that of the male. Also, for the first time, there was an indication of shortening the male response; apparently 500 γ of the propionate were not significantly more effective than 250 γ. These rats were about one year old.

Our earlier experiments (1) with urinary male hormone indicated that when the rat exhibited no sex difference to a barbiturate (barbital), the male hormone did not influence the recovery time. We therefore administered testosterone propionate to both male and female rats before giving nostal, in case of which the rat in previous tests had shown only slight sex difference. The results are shown in Table V. Although, for the first time in our experience, a clear-cut sex difference was apparent, both in the recovery time with nostal and the proportion of delayed mortality cases, the 10-day administration of 1 mg./Kg. daily of testosterone (given as the propionate) did not influence either the acute recovery time or the delayed mortality percentage. Control injections of sesame oil likewise had no effect upon these responses to nostal.

Table V.—Summary of the Results of Intraperitoneal Administration of 50 Mg./Kg. of Nostal (Isopropyl-Bromallyl Barbituric Acid, 1% Solution) Given as the Sodium Salt to Six Groups Consisting of 7 to 8 Hybrid Rats Each, 3 to 3½ Months Old

The testosterone propionate (perandren) was administered subcutaneously in sesame oil, and the final dose was given 3 hrs. before the nostal. The average for the females that were given sesame oil is omitted because one apparently normal rat took about 8 hrs. to recover; the average for the other six was 2.6 hrs. All three groups showed normal gains in body weights during the oil and hormone administration.

Group	Males: Average Hours of Recovery	Males: Delayed Mortality	Females: Average Hours of Recovery	Females: Delayed Mortality	Treatment
1	1.8	3/8	2.6	6/7	None
2	1.8	2/8	...	6/7	Sesame oil, 1 cc./Kg. daily, 11 doses
3	1.8	3/8	2.4	7/8	Testosterone propionate,[a] 1 mg./Kg. daily, 11 doses

[a] Calculated as testosterone.

PART III. EFFECTS OF CASTRATING AND OF VARYING DOSES AND DURATIONS OF ADMINISTRATION OF TESTOSTERONE PROPIONATE (PERANDREN) UPON THE ABILITY OF THE ALBINO RAT TO BUILD UP TOLERANCE TO AND TO DETOXIFY SODIUM PENTOBARBITAL

To elucidate further the increased resistance caused by male sex-hormone administration, we next employed a new technique developed by Holck and Fink (7). The rats are first given a small dose of sodium pentobarbital per Kg. of body weight, and every 90 min. the dose is increased by a small percentage. One can then compare the percentages of rats which develop tolerance and determine quite closely the highest dose each tolerant rat can detoxify in 90 min. By choosing the proper dosage, these authors and also Holck and Mathieson (8) found that nearly all normal adult males develop tolerance, that castration of such males diminishes the number which develop tolerance, that very few

normal adult females develop tolerance, except during pregnancy and the period of lactation, and, finally, that almost all one- or two-month-old rats of either sex develop tolerance, and that within these limits, the younger the rat, the more pentobarbital it can destroy in 90 min. The rats were allowed food and water throughout these tests.

Two male and two female rats, two months old, were given subcutaneous control injections of Ringer's solution; two other males and two other females were given one and one-half times this control dosage. At the end of 82 injections all of the rats were alive, but showed subcutaneous edema. One male receiving the larger saline dose died on the second day after the injections were stopped. Also, one one-month-old male rat was permitted to recover after receiving 80 doses of pentobarbital; it had apparently recovered an hour and fifteen minutes after the last dose and was growing well one week later.

In these earlier tests we started with 11.7 mg./Kg. and each dose increase was 3.22%. To avoid an excessive number of doses, we started with 10.9 mg./Kg. in the experiments in Parts III and IV, and employed 4.5% dosage increases; now only a very few of the young rats survived 60 doses, the maximum being 66 doses.

The results of our 14 experiments pertaining to Part III are presented in Table VI. The data of Group 3, Part *A*, Table VIII verify our previous finding that castration of adult male rats diminishes the percentage of rats which develop tolerance, but once tolerance has been established, the castrates detoxify pentobarbital as well as the normals. Similarly, we verified that spaying of adult female rats somewhat increased the percentage which develop tolerance (Group 10, Part *B*, Table VIII). However, spayed female rats were far below the normal males in their ability to develop tolerance.

Heavy dosage of testosterone propionate for three days did not influence the normal male rat response, but with the 10-day administration all male rats developed tolerance. Doubling the hormone dose was no more effective than the single dose of 1 mg./Kg. of body weight daily for ten days. In castrate males the 10-day medication again was somewhat more effective than the 3-day medication in distinctly increasing the percentage of rats that developed tolerance. The hormone did not increase the ability of tolerant castrated rats to de-

Table VI.—Summary of Experiments upon the Effects of Castration and of Varying Doses and Durations of Time of Administration of Testosterone Propionate (Perandren) upon the Ability of the White Rat to Build Up Tolerance to and to Detoxify Sodium Pentobarbital

The hormone was administered as a 1.19% or 2.39% solution, in sesame oil; 1 or 2 mg./Kg. of body weight, calculated as testosterone, was injected subcutaneously daily for the specified number of days prior to the pentobarbital administration, at least 90 min. before the pentobarbital medication, and thence daily until each rat had died. The barbiturate was given subcutaneously in 1% solution. Starting with 10.9 mg./Kg. of body weight, the dose was increased by 4.5% every 90 min., and thus continued until each rat had died. The animals averaged 7 months of age (range, 6 to 9 months), except the females which received 2 mg./Kg. of the hormone, which were 2 months older (8 to 10 months).

Groups	No. of Rats	Per Cent Developing Tolerance	Average Total Fatal Dose, Mg./Kg.	Tolerant Rats Only: No. of Rats	Tolerant Rats Only: Average Total Fatal Dose, Mg./Kg.	Tolerant Rats Only: Average Maximum Dose Detoxified in 90 Min., Mg./Kg.	Tolerant Rats Only: Standard Error	Critical Ratio
Males								
1. Normal (*N*)	19	84	1081	16	1255	52	3.2	1.4 (1 & 2)
2. *N* + 3 day testosterone (T), 1 mg./Kg.	21	81	1173	17	1429	60	4.9	....
3. N + 10 day T, 1 mg./Kg.	22	100	1406	22	1406	..	...	....
4. N + 10 day T, 2 mg./Kg.	15	87	1228	13	1398	..	...	....
5. Castrates (C)	23	52	731	12	1284	..	...	....
6. C + 3 day T, 1 mg./Kg.	23	83	1063	19	1254	..	...	....
7. C + 10 day T, 1 mg./Kg.	22	95	1273	21	1329	..	...	....
Females								
8. Normal	23	9	206	2	895	..	...	....
9. N + 3 day T, 1 mg./Kg.	20	15	292	3	1192	..	...	....
10. N + 10 day T, 1 mg./Kg.	23	65	955	15	1386	..	...	....
11. N + 10 day T, 2 mg./Kg.	20	55	856	11	1436			
12. Spayed (S)	23	35	490	8	1202	52.2	2.1	3.0 (12 & 14)
13. S + 3 day T, 1 mg./Kg.	23	74	898	17	1162	48	2.1	4.1 (13 & 14)
14. S + 10 day T, 1 mg./Kg.	24	83	1353	20	1602	65	3.5	....

Table VII.—Summary of the Results from the Subcutaneous Administration 100 Mg./Kg. of Sodium Evipal (Sodium Methyl-Cyclohexenyl-*N*-Methyl Barbiturate, 2% Solution) to Two Groups Consisting of 11 Albino Male Rats Each, and to Two Groups Consisting of 14 Albino Female Rats Each, 3 to 4 Months Old

The hormone was administered as a 0.67% solution of estradiol dipropionate in a sesame oil; 1 mg./Kg. of body weight, calculated as estradiol, was injected subcutaneously daily for 10 days. The final dose was given 3 hrs. before the evipal.

Group	Average Weight, in Grams	Sodium Evipal, Average Hours of Recovery	Treatment (10 Days)	Weight	Hours of Recovery	Weight	Hours of Recovery	Weight	Hours of Recovery
Males		10[a]			24[a]		14[a]		
1	222	1.5	Sesame oil, 2 cc./Kg. daily	227	1.3	258	1.5	256	1.9
2	204	1.5	Estradiol, 1 mg./Kg. daily, 11 doses	172	2.2	192	2.7	201	3.0
Females									
3	159	2.5	Sesame oil, 2 cc./Kg. daily	159	2.6	179	2.8	...	...
4	159	2.5	Estradiol, 1 mg./Kg. daily, 11 doses	150	2.6	173[b]	2.6	...	...

[a] Time interval in days.
[b] One rat died two days previously; the average gain of the 13 rats was 24 Gm.

toxify pentobarbital. Concerning normal female rats, again the 10-day hormone treatment was much more effective than the one of only three days, and again 2 mg./Kg. were no better than 1. As to spayed female rats, the 10-day was somewhat more effective than the 3-day administration in markedly increasing the percentage of rats that developed tolerance. Whereas the average maximum dose detoxified in 90 min. was not influenced by the 3-day medication, it was significantly higher in the spayed females when the hormone was given for ten days.

PART IV. EFFECTS OF AGE, OF CASTRATION AND OF VARYING DOSES AND DURATIONS OF TIME OF ADMINISTRATION OF ESTRADIOL DIPROPIONATE (DI-OVOCYLIN) AND TESTOSTERONE PROPIONATE (PERANDREN) UPON THE ABILITY OF THE ALBINO RAT TO DETOXIFY EVIPAL AND TO BUILD UP TOLERANCE TO AND TO DETOXIFY PENTOBARBITAL

In the experiments upon the effect of 10-day administration of a large, daily dose of estradiol dipropionate (di-ovocylin) upon the response to sodium evipal, summarized in Table VII, there was a weight loss in both sexes, but the reduction was much greater in the males. Concerning the effect of this hormone upon the recovery time from sodium evipal, this was not altered in the females; but with the males it was markedly lengthened, even though the evipal dose was based upon the reduced body weight. Twenty-four days after discontinuing the hormone medication, all rats showed good gains in weight, but the males were not yet back to their original weights. Whereas now the recovery times from evipal were practically identical in the two female groups, with the treated males it was even longer than at the end of the hormone administration. Two weeks later the hormone-treated males had gained still further in weight, but their recovery time still was much longer than that of the controls. At the end of this last test with evipal all of the males were killed with chloroform.

Smear tests from epididimus material showed numerous sperms in all of the controls; this was true in only four of the hormone-treated rats; in four of the latter no sperms could be found and in three only very few. Inasmuch as a marked reduction in the size of testes was observed in six of the eleven hormone-treated males, we weighed all the testes. In the 11 controls, the average weight of testes was 0.81% of body weight; one of these rats had only one testis, and in two others the glands were of moderate size. In the hormone-treated rats the average was only 0.49% of body weight, and only four of the eleven rats had nearly normal percentages. However, there was no correlation between the size of the testes and the recovery time in these rats.

Again administering gradually increasing doses of sodium pentobarbital (see Part III), the effects of varying doses of estradiol dipropionate were compared in the two sexes. The first hormone dose was given ten days prior to the first sodium pentobarbital administration; the effect of this hormone dose was then followed up by taking vaginal smears; the second dose was given five days before beginning with pentobarbital, and smear tests again carried out twice; the third hormone dose was injected on the day prior to the pentobarbital medication and was followed by a vaginal smear test before the fourth hormone administration given three hours before the pentobarbital tests were commenced. Finally, the hormone was continued once daily in rats which developed tolerance to the barbiturate. All results are summarized in Table VIII, Parts *A* and *B*. All female rats gave positive vaginal smear tests (only one was doubtful) on the day the pentobarbital medication was started. The spayed females gave positive tests sooner with the 30 γ/Kg. of body

Table VIII.—Summary of Experiments upon the Effects of Age, of Castrating and of Varying Doses and Durations of Time of Administration of Estradiol Dipropionate (Di-ovocylin) and Testosterone Propionate (Perandren) upon the Ability of the Albino Rat to Build Up Tolerance to and to Detoxify Pentobarbital

The estrogenic hormone was administered as a 1.33% solution in sesame oil; 1 mg./Kg. of body weight, calculated as estradiol, was injected subcutaneously 10 days, 5 days, 1 day, and 3 hrs. prior to the pentobarbital administration, and thence daily until each rat had died. The testosterone propionate was administered in corresponding concentration in sesame oil and the dosage calculated as testosterone. It was administered daily beginning 10 days prior to, 3 hrs. before the pentobarbital administration, and thence daily until each rat had died. The barbiturate was given subcutaneously (1% solution). Starting with 10.9 mg./Kg. of body weight, the dose was increased by 4.5% every 90 min., and thus continued until each rat had died. The adult rats were from $3^1/_2$ to 8 months old, and the young rats were 7 to 10 weeks old. Comparing only the rats which developed tolerance the average maximum dose of sodium pentobarbital detoxified in 90 min. was 98 mg./Kg. for the 15 young normal female rats and only 74 mg./Kg. for the 11 young female rats treated with estradiol dipropionate.

PART A.—MALES

Groups	No. of Rats	Average Weight in Grams 10 Days Prior to Pentobarbital Administration	Day of Pentobarbital Administration	Per Cent Developing Tolerance	Tolerant Rats Only: Average Total Fatal Dose, Mg./Kg.	Tolerant Rats Only: No. of Rats	Tolerant Rats Only: Average Maximum Dose Detoxified in 90 Min., Mg./Kg.	Seminal Vesicles, All Rats Average, Mg./100 Gm.
1. Normal (*N*) adults	21	300[a]	308[a]	85	1087	18	51	306
2. *N* + estradiol (E), 30 γ/Kg., 4 doses	25	281	270	36	547	9	51	125
3. Castrate (C) adults	24	270	276	29	475	7	46	50
4. C + E, 30 γ/Kg., 4 doses	23	276	273	25	444	6	42	59
5. C + E, 30 γ/Kg., 4 doses + testosterone, 1 mg./Kg., 11 doses	24	278	284	83	1233	20	57	390
6. Normal young (Y)	12	154	176	100	2517	12	95	152
7. Y + E, 300 γ/Kg., 4 doses	14	155	155	85	1522	12	66	84

[a] Due to a misunderstanding only ten of the rats were weighed ten days before the pentobarbital experiment. The weights here given represent these ten rats. The average weights of all the 21 rats on the day of the pentobarbital administration was 294 Gm.

PART B.—FEMALES

Groups	No. of Rats	Average Weight in Grams 10 Days Prior to Pentobarbital Administration	Day of Pentobarbital Administration	Per Cent Developing Tolerance	Average Total Fatal Dose, Mg./Kg.	Uterus and Tubes, Average Mg./100 Gm. of Rat	Remarks on Vaginal Smear Tests before and on Pentobarbital Test Day
8. Normal (*N*) adults	26	197	192	0	113	330	Usual varied picture on and before test day
9. N + 30 γ/Kg. estradiol (*E*), 4 doses	26	188	188	4	149	283	Nine—four days after first dose of E, 15—day before, all + on test day
10. Spayed (S) adults	27	209	217	15	312	61	All negative
11. S + 5 γ/Kg. E, 4 doses	27	213	211	4	191	105	One—four days after first dose of E, one on day before and doubtful on test day
12. S + 30 γ/Kg. E., 4 doses	25	203	201	0	143	182	All + third day after first dose of E, all + on test day
13. Normal young (Y)	19	119	132	79	2020	164	Usual varied picture on and before test day
14. Y + 300 γ/Kg. E., 4 doses	19	122	130	58	1169	249	One—third day after first E dose, one—day before, all + on test day

weight than the normals. That our dosage was effective is also seen by the increased average weights of the uterus and uterine tubes in the spayed rats, varying with the dose, and that the large dose of 300 γ/Kg. increased these weights in the young normal females. The two junior pharmacy students who did our organ weighing had previously practiced the correct procedure upon four normal rats. In spite of this limited experience, we believe that the large average trends certainly can be considered significant. In the male rats the estradiol ester diminished the average size of the seminal vesicles markedly in both adult and young normal males, but had no influence upon these in castrated adult males.

As to body weight, estradiol caused a loss in the normal adult male rats, inhibited the growth of the young males, but had no certain effect upon any of

the female groups, or upon the male castrates. Concerning the effect upon resistance to pentobarbital, the estrogenic hormone markedly lowered the percentage of normal adult males which developed tolerance, clearly diminished the capacity of the young males to detoxify pentobarbital, decreased the percentage of spayed or of normal young female rats which developed tolerance, and, consequently, the average total fatal dose; it had no certain effect upon castrated males or upon normal adult females. Comparing only the young female rats which developed tolerance, the hormone decreased the average maximum dose of pentobarbital detoxified within 90 min. (see end of legend to Table VIII). When a dose of testosterone propionate (Group 5, Part *A*), previously found to be effective, was given together with a smaller dose of estradiol dipropionate to castrated adult male rats, the testosterone effect dominated in that the percentage which developed tolerance was markedly raised, the maximum dose detoxified within 90 min. by the tolerant rats was somewhat greater, and the seminal vesicles were increased markedly in size.

DISCUSSION

We have here established that testosterone acetate or propionate, when given daily in suitable doses for one or two weeks, causes significant shortening in the depression time in normal female rats and in castrated rats of either sex to the two barbiturates, pernoston and evipal; only with the propionate did we succeed in shortening the hypnosis by evipal in the normal male. Equally important is the observation that such prolonged hormone administration markedly increases the percentage of normal female and of castrated male or female rats which develop tolerance when pentobarbital is administered in very gradually increasing doses every 90 min. until each rat dies, and that in spayed females the hormone medication also increases the capacity to detoxify pentobarbital once tolerance is established; this latter needs confirmation.

The exact mechanism by which these changes in the rats are produced by the male sex hormone, and that by which effects of opposite nature are produced by castration in the two sexes must await future investigation. These involve the question of possible changed resistance of the nervous system to the barbiturates evipal, pernoston and pentobarbital. They necessitate further studies upon changes in the composition of the blood, especially in regard to calcium (6), directly or indirectly produced by the testosterone, because reports in the literature are conflicting concerning such changes. One may need to examine in more detail the ability of the liver to detoxify the mentioned barbiturates under the various conditions given. This plan is suggested by the reports of Deuel, *et al.* (9, 10, 11, 12), that the glycogen content of the liver of the normal males and spayed females was higher than in the normal adult females, that immature rats showed no sex difference in the glycogen content of the liver, and that testis hormone administration caused an increased glycogen content in this gland, whereas estrone had the opposite effect. In this connection we may note that estradiol, wherever any action could be detected by us, lowered the capacity to develop tolerance and to detoxify pentobarbital. Also that Holck, *et al.* (1), failed to accentuate the sex difference of the rat to evipal by a preliminary inanition period of 36 hrs., which causes faster depletion of liver glycogen in the female rat. A possible protection of the liver cells by testosterone is also in point (13).

In any case we have the additional problems of explaining: (*a*) why the male sex hormone failed to change the resistance of the rat to the related barbital (1) and nostal; (*b*) why castrated males, once they have developed tolerance, detoxify pentobarbital as well as normal male rats do; (*c*) why the synthetic male sex hormone failed to influence the resistance of female mice to evipal, toward which the resistance of female rats is easily increased. One should try different doses in mice before drawing a final conclusion, especially so because it has been reported recently that testosterone medication greatly increased their resistance to moderate doses of mercuric bichloride (13).

SUMMARY

1. Testosterone acetate in a dosage of 125 γ per rat daily for two weeks or 500 γ for one week significantly shortened the response of the rat to the barbiturate, pernoston. Eleven doses of 62.5 γ, given twice daily, shortened the response of normal female rats to evipal, but 10-day hormone treatment with twice this dose given daily, or 0.5 mg. daily for ten days, failed to influence the male rat response to this barbiturate. Likewise, 125 γ of the hormone daily per adult female mouse for a week failed to alter the response to evipal.

2. Treatment of about one week's duration with daily doses of 125 γ of testosterone acetate per rat significantly and to about the same extent shortened the response to evipal in both normal and spayed female rats.

3. Treatment for two weeks with 0.25 mg. per rat daily of the more powerful testosterone propionate shortened the response to evipal in adult and young female rats to that of the male level, and even reduced the depression time in the male; treatment of the male rats with double this daily hormone dose for two additional weeks was about as effective as the dose of 0.25 mg. per rat.

4. Ten-day treatment of male and female rats with 1 mg./Kg. of testosterone (given as the propionate) failed to influence the acute depression time and the incidence of delayed deaths following administration of the barbiturate, nostal, to which the untreated rats showed a measurable sex difference for the first time in our experience.

5. Three- or ten-day administration of 1 mg./Kg. of testosterone (given as the propionate) daily or double this dose for ten days did not influence the percentage of male rats which developed tolerance to gradually increasing doses of sodium pentobarbital. However, the lowered percentage of castrated male rats which developed tolerance was brought up to the normal level by either 3- or 10-day hormone administration in the dosage just given. The hormone administration did not increase the average maximum dose of pentobarbital that tolerant normal or castrated males detoxified within 90 min.

6. Whereas 3-day administration of 1 mg./Kg. of testosterone (given as the propionate) failed to increase definitely the percentage of normal female rats which developed tolerance to pentobarbital, 10-day treatment was signally effective in this respect; doubling the dose of the hormone did not cause further improvement. The somewhat increased percentage of tolerance consequent upon spaying of female rats was further increased by 3-day administration of the hormone and brought up to the male level by 10-day treatment. This latter also increased the average maximum dose of pentobarbital detoxified within 90 min. by the tolerant spayed females.

7. When estradiol (given as the dipropionate) was administered in a dosage of 1 mg./Kg. daily for ten days to normal male and female rats, it caused severe loss of body weight in the males and, later on, marked reduction in the size of the testes in most cases. Whereas this hormone did not influence the female reaction time to evipal, it markedly lengthened this in case of the males; this effect persisted at least six weeks after cessation of hormone medication when these male rats were again gaining in weight.

8. Five, thirty, and three hundred gammas, respectively, of estradiol per Kg. (given as the dipropionate) were administered ten, five and one day before, and on the day of beginning medication with gradually increasing doses of pentobarbital, and once daily thereafter to rats which developed tolerance. Vaginal smear tests were positive when the barbiturate was first given; also, definite effects were noted upon the average weights of the uterus and uterine tubes, and of the seminal vesicles. The only change in effects that could be detected was always a lessened ability to develop tolerance to pentobarbital, and, with the highest dose of hormone, a lowered average maximum dose of pentobarbital detoxified in 90 min. by tolerant young rats of either sex. When a heavy dosage of testosterone propionate was given simultaneously with a moderate dosage (30 γ/Kg.) of estradiol, which latter failed to influence the castrated male rat response, the beneficial action of the male hormone upon tolerance was observed in its usual degree.

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The Preparation and Vitamin A and Vitamin D Standardization of the Second U. S. P. Reference Cod Liver Oil by the U. S. P. Vitamin Advisory Board

The U. S. P. Reference Cod Liver Oil No. 2, a standard of reference for vitamin A and vitamin D, is now ready for distribution. This oil contains 1700 U. S. P. units of vitamin A and 115 U. S. P. units of vitamin D per gram. It has been prepared under the direction of the Vitamin Advisory Board and may be obtained from Dr. E. Fullerton Cook, Chairman of the U. S. P. Revision Committee, 43rd and Woodland Ave., Philadelphia, Pa.

Plans for providing this new reference cod liver oil were begun early in 1938 when it became apparent that the supply of U. S. P. reference cod liver oil was being depleted. At that time recognition was given to the principle that a standard for the assay of vitamins should preferably be the pure vitamin or a preparation made therefrom which could be readily reproduced and which might be suitable from the standpoint of stability and convenience. The International Standard for vitamin D now being distributed by the Health Organization of the League of Nations is of such a degree of purity. Some recent studies of vitamin A esters indicate that certain compounds of vitamin A may be suitable from the standpoint of stability and reproducibility as a standard for vitamin A. However, such pure preparations of vitamins A or D have not been available in sufficient quantities to supply the need for reference standards in this country and it was therefore decided to arrange for the preparation and standardization of another lot of reference cod liver oil. Owing to steadily increasing demands for samples of the reference cod liver oil for assay purposes, the stocks of the first lot of oil were depleted more rapidly than had been anticipated and it was necessary to issue samples of the new lot of oil just as soon as the standardization was completed.

In the early plans it was estimated that 200 gallons of destearinated oil would be a sufficient quantity of the new standard. With the equipment that was available for preparation of the oil it was found preferable to use a batch of 400 or 500 gallons for destearination and further processing. The fish liver oil secured was obtained from authoritative officials and is known to be exclusively the oil from the livers of *Gadus Morrhua*. This oil was obtained in lots of 120 gallons from the Norwegian Fisheries Research Station through the courtesy of Dr. Olav Notevarp, from the Fisheries Research Institute of Newfoundland through the courtesy of Dr. W. F. Hampton, and from Messrs. Crooks of London through the coöperation of Dr. J. C. Drummond. A fourth lot of 90 gallons was obtained from The Atlantic Coast Fisheries Company through the kindness of Dr. Harden F. Taylor. This specially selected oil was mixed, destearinated and packaged under carbon dioxide with relatively high pressure under the most ideal conditions obtainable and the oil has been stored under refrigeration continually from the day it was received in this country. The processing and packaging of the oil was done through the coöperation of E. R. Squibb and Sons.

In response to invitation to collaborate in the standardization of this oil more than 18 laboratories agreed to take part in the study. To these laboratories were sent samples of the oil to be standardized as well as International Standards for vitamins A and D to be used for reference in the biological assays. These assays were carried out according to the methods prescribed in the Second Supplement of the U. S. Pharmacopœia XI.

Complete reports from 18 laboratories have been received, 18 submitting results of vitamin D assays and 14 results of vitamin A assays, and interpretations of these records were used to set a value for the potency of the reference oil. A condensed summary of the reports received are presented in Tables I and II. In Table I are the vitamin A potencies assigned by each collaborator to the assay oil as well as the number of animals reported used by each of the laboratories responding. It will be noted that more than 1600 animals were used in the vitamin A assays alone. The arithmetical average of the values reported is 1787. The extreme values are 1000 and 2500. It is evident that the smaller numbers of animals were used by the laboratories reporting the highest potencies for the oil. The value 1700 was given the oil in consideration of the interpretation of the full reports submitted by the collaborators as well as in recognition of the preliminary reports of spectrophotometric collaborative studies which were yet to be completed.