(*b*) high sensitivity to a change in dose, measured objectively by the curve relating-dosage and the function of response which over the widest range plots against it as a straight line, (*c*) the definition of potency in terms of a reference standard based upon the reaction to two or more doses of both standard and unknown in each assay, (*d*) the partition of potential variation equally between different dosages and between standard and unknown and (*e*) the determination of its experimental error as an integral part of each assay. Statistical procedures depend in part upon the nature of the response. Those based upon an all-or-none reaction show a sigmoid dosage-effect curve mirroring the individual variation in susceptibility. Since this follows the normal distribution, the curve can be rectified and used effectively for biological assay by transforming dosages to logarithms and percentage effect to probits or their equivalent. Similar procedures are of value when reaction time is used as an assay criterion, providing a biologically stable end-point for each dose. The dosage-response curve for graded reactions is often linear over a manageable dosage range. Especially in these cases the newer statistical designs and methods of analysis extend considerably the precision that can be attained from a given amount of biological material and time.

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Influence of Sex Life upon Resistance to Nostal and Pentobarbital*

By Harald G. O. Holck and Lewis D. Fink†

Agduhr and his collaborators have reported that sex life increases resistance in mice, rats and rabbits to such varied substances as methyl alcohol (1), ethyl alcohol (2), soluble barbital (3) and arsenic trioxide (4). Small, very gradually increasing daily doses were administered until the animals died. Mated female mice showed an increased resistance even though they failed to become pregnant (2). In general, mated female mice gained more in resistance than did the corresponding males (1, 2, 3). However, in the case of arsenic trioxide, mated male rats showed greater resistance. In all of these experiments the animals were divided into three groups: 1, two males together; 2, two females together; 3, male and female together.

EXPERIMENTAL

PART A. OBSERVATIONS WITH A SINGLE DOSE OF ISOPROPYL BROMALLYL BARBITURIC ACID (NOSTAL)

In this phase of the study 81 male and 120 female albino rats, bred from Wistar stock, were divided

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into suitable groups so as to study the effects of living with a member of the same sex (Groups I–IV), sufficient sex life for one male to impregnate one female (V), sex life *ad libitum* for the males (IX), delivery of 1 litter (X), delivery of 2 litters (XI) and partial hysterectomy (VIII, at eight to nine weeks under ether anesthesia), in case of which the finding of semen in the vagina by the method of Frank (5) with at least a two-week interval proved persistence of libido as expected (6) and in which an examination showed presence of normal ovaries at the end of the experiment. In all cases at least twenty days had elapsed between the last delivery and Nostal administration. As far as possible, litter mates were distributed among the various groups. The diet consisted of Purina Dog Chow supplemented by cod liver oil. The rats were kept in air-conditioned quarters at approximately twenty-five degrees Centigrade. The final experiments were conducted on three separate days and each rat was kept in an individual container and well covered; the temperature was twenty-seven degrees C.; a rat was considered recovered when it would promptly retract either hind foot from an extended position. Inasmuch as no consistent definite trend could be found on any of the three days, the results have been combined into one table.

Table I.—Summary of the Results from the Intraperitoneal Administration of 50 mg./Kg. of Nostal (Given as the Sodium Salt) to $4^1/_2$ to $7^1/_2$ Months Old Albino Rats which Had Been Raised under Various Conditions of Sex Life. ϵ = Standard Deviation of the Mean

Group	No. of Rats	Average Hours for Recovery	ϵ of Recovery Time	Percentage of Delayed Death	Combination of Groups	Average Hours for Recovery
		Males				
I—Single males	14	3.4	±0.2	86	I and III	3.1
III—Two males	27	3.0	0.1	81		
IX—Polygamous males	12	3.2	0.2	83	IX and VII	3.3
V—*Male* and F.	15	3.2	0.1	80		
VII—*Males* with F. of Group VIII	13	3.5	0.2	100		
		Females				
II—Single females	15	3.7	±0.2	100	II and IV	3.6
IV—Two females	29	3.6	0.1	100		
VIII—Females with uteri excision	13	4.0	0.2	100		
X—1 Delivery F. with VI *Female* and M.	36	3.4	0.1	86	VI, X and XI	3.3
XI—2 Delivery females	27	3.3	0.1	89		

The differences in recovery time and percentages of delayed death are all within the limits of normal variations for this dose of Nostal (7). Hence there is no indication that any of the experimental conditions of sex life have an effect upon either of these criteria. A closer examination of the complete data shows that even when the time between delivery and injection was only three to six weeks, there still was no certain benefit. Yet, it is possible to influence the resistance to Nostal, as can be seen from the following data. Six doses of an average of 10 mg./Kg. of Nostal were administered at three- to four-day intervals to 18 hybrid rats (10 males, 8 females); four days after the last of these small doses, the regular dose of 50 mg./Kg. of Nostal was given; none died delayed death; of 17 non-treated rats (8 males, 9 females), 83 per cent died within a few days following the 50 mg./Kg.

In view of the fact that Agduhr has reported an increase in the secretory mass of the thyroid gland (8) and Carlson *et al.* (9) have found rearrangement of the adrenal cortex during pregnancy with increased lipoid and diminished vitamin C contents, we decided to fix the thyroid, adrenal and pituitary glands thoroughly in 4 per cent formaldehyde solution, to weigh them and to compare their relation to body-weight, although the body-weights had decreased to a variably marked degree when the rats had died from pulmonary complications following Nostal administration or had been killed on the fifth day (7). Within the groups of either sex, no significant differences could be detected in the average percentages of the weights of any of these glands; however, the proportion of the female thyroid to body-weight was slightly greater, that of the pituitary about twice and that of the adrenal about three times as great as in the corresponding glands of the male. A check upon five male and five female controls (killed acutely with chloroform) gave approximately the same sex-difference, using the weights of either the fresh or the fixed glands. These last two sex-differences in our stock were distinctly greater than have previously been reported by Donaldson (10) and Hatai (11).

PART B. OBSERVATIONS WITH GRADUALLY INCREASING DOSES OF SODIUM PENTOBARBITAL (ETHYL 1-METHYL-BUTYL BARBITURIC ACID; NEMBUTAL) GIVEN EVERY 90 MINUTES

In these experiments we used 74 male and 103 female albino rats. The mated pairs (Group III) and pairs of the same sex (II, VIII) were kept together constantly as in Agduhr's tests. To study the effect of coexistence of the male and female without sexual intercourse, a small wound clip was carefully fastened under ether anesthesia around the vaginal tract just under the surface of the skin

through a lengthwise incision a centimeter and a half to the side of the vaginal opening, with subsequent closing of the incision. Although in 13 of the 18 females no clip was found at the time of barbiturate injection, adhesions had closed the vaginal opening and none of the females in this group (VII) with the vaginal "chastity" clips became pregnant, although caged for about two months with males, each of which had (*a*) impregnated one female (Group V) and (*b*) had had repeated coition with a female with partial uteri excision (VI).

that part of the time they ate and apparently became normal between injections until later on they again became depressed and ultimately died. In a few cases the rats were found to be normal before each subsequent injection for 60 or more periods. Development of tolerance to Pentobarbital in the rat is in agreement with the findings of Moir (12) and Stanton (13), who gave this drug in repeated small doses over a period of several weeks.

The results of these tests have been summarized in Table II. They show that sex life had no in-

Table II.—Summary of Results from Albino Rats, 5 to 6 Months Old, Injected Subcutaneously Every 90 Minutes with Gradually Increasing Doses of Nembutal until Death

Group	No. of Rats	Average Total Dose (mg./Kg.)	Percentage of Rats Developing Tolerance	Chi Square Method Compared with Group	Factor	Remarks
			Males			
I—Males with F. of V, VI and VII	22	1372	77	All 74 M. with the 39 "pregnant" females	20	"Pregnant" = were or had been pregnant
II—Two males	31	1475	77			
III—Male and F.	21	1465	81			
			Females			
IV—Female and M.	21	771	30	All 64 "non-pregnant" F. with the 39 "pregnant" females	17	IV 38% pregnant and 43% lactating at time of injection
V—1 delivery F.	18	716	44			
VI—Females with uteri excision	23	202	4			V 6% pregnant and 94% lactating at time of injection
VII—Clip vagina females	18	235	6			
VIII—Two females	23	198	4			

We administered doses of Sodium Pentobarbital which were very slowly increased logarithmically. The first dose was 12.16 mg./Kg.; this was increased by 5 per cent for two more doses, and from then on by 2.24 per cent. The highest dose given was 94.3 mg./Kg., or 7.7 times the initial dose. Twelve sites of injection under the skin were used in rotation. The 1 per cent solution was freshly prepared 33 times during the experiment which lasted eight days and nights. The sample of Nembutal used was lot 75952, concerning which it was known that delayed death did not occur and to which our male rats showed a distinctly higher resistance than the females. General care of the rats was the same as in Part A, and the final test again was conducted at twenty-seven degrees C. At the end of every 90 minutes the condition of each rat was recorded, except when it had died between injections.

By this technique it was possible: 1, to observe constantly each experimental rat; 2, to record more exactly the time of death; 3, to notice whether tolerance was developed during the experiment; 4, to avoid the possible killing by a cage mate of a rat enfeebled by the drug. Two main types of responses occurred. In one type the rat became deeply depressed after a few doses of Pentobarbital, thence continued to lie on the side and died without having shown any visible signs of improvement. In the second type of response the rats in the beginning became depressed and lay on the side, but after a varying number of injections improved so

fluence upon the male resistance, that living with the male without copulation, or sexual intercourse without pregnancy did not influence the female resistance, but that this was raised distinctly in females that were or recently had been pregnant, although the resistance was not brought up to that of the males.

PART C. FURTHER STUDIES UPON THE EFFECTS OF GRADUALLY INCREASING DOSES OF SODIUM PENTOBARBITAL GIVEN EVERY 90 MINUTES

The main object of this experiment was to establish more firmly that sex life has no influence upon male rat resistance (Groups I, II), but that pregnancy and recent pregnancy (III, IV, V) notably increase the resistance to Pentobarbital. A group of 10 young virgin females (VI) was added to make possible a comparison with the older females; in case of 6 of these young rats which had received more than fifty doses of Pentobarbital and had shown visible tolerance, the administration was discontinued to see if permanent recovery would occur. Another group of 13 rats (VII) was included to observe if tolerance produced by preliminary injections of suitable doses of Sodium Pentobarbital over several weeks (as was previously done with Nostal) would raise the resistance appreciably. Finally, one control rat was given 60 doses of one and another rat the same number of doses of one and one-half times the amount of saline used as a solvent for the barbiturate used in these tests.

The care of the rats and general technique were the same as in the previous experiments, except that we started with a smaller dose, 11.7 mg./Kg., that the increases were uniformly 3.25 per cent, which somewhat shortened the experiment, that only 10 sites of subcutaneous injection were used in rotation, and that we used another sample of Pentobarbital (Lot No. 3032X947503), of which we also knew that sex-difference existed in our stock.

The main results are summarized in Chart 1. Again, there was no significant difference between mated males and males kept in pairs. On the other hand, as before, pregnant or recently pregnant females developed tolerance about ten times more often than did the virgins and the average total fatal dose was five times greater in the former and approached male fatal dosage more closely than in Part B. However, young virgins also developed tolerance about ten times more frequently than did older virgin females; hence, age is an important factor in this type of study. This is of interest in connection with Agduhr's observation (2) that resistance of mice to alcohol seems to be the greatest during the period of most rapid growth; also in connection with our own studies upon Evipal (15), in which the female rat response to single doses gradually increased between the ages of one and four months; however, the opposite occurred in the males. We are unable to state whether the average fatal dose of Sodium Pentobarbital for the young virgins would have exceeded that for the older males, because we ceased injections in the case of 6 of these females which had developed tolerance to see whether they would recover permanently from doses varying from 1572 to 1653 mg./Kg. Uneventful recoveries took place and these rats apparently were in good health and growing two weeks later. Likewise, an older control female recovered readily from 60 doses of the saline solution used as a solvent for the barbiturate, and another one from 60 doses of saline solution one and one-half times that large. The preliminary administration of small doses of Pentobarbital at intervals of several days for several weeks prior to the main experiment, the technique so eminently successful in preventing delayed death when Nostal was used, failed to raise the average resistance of virgin females to a level near that of rats that were or recently had been pregnant, although there was a measurable increase in the average fatal dose over that for virgin rats

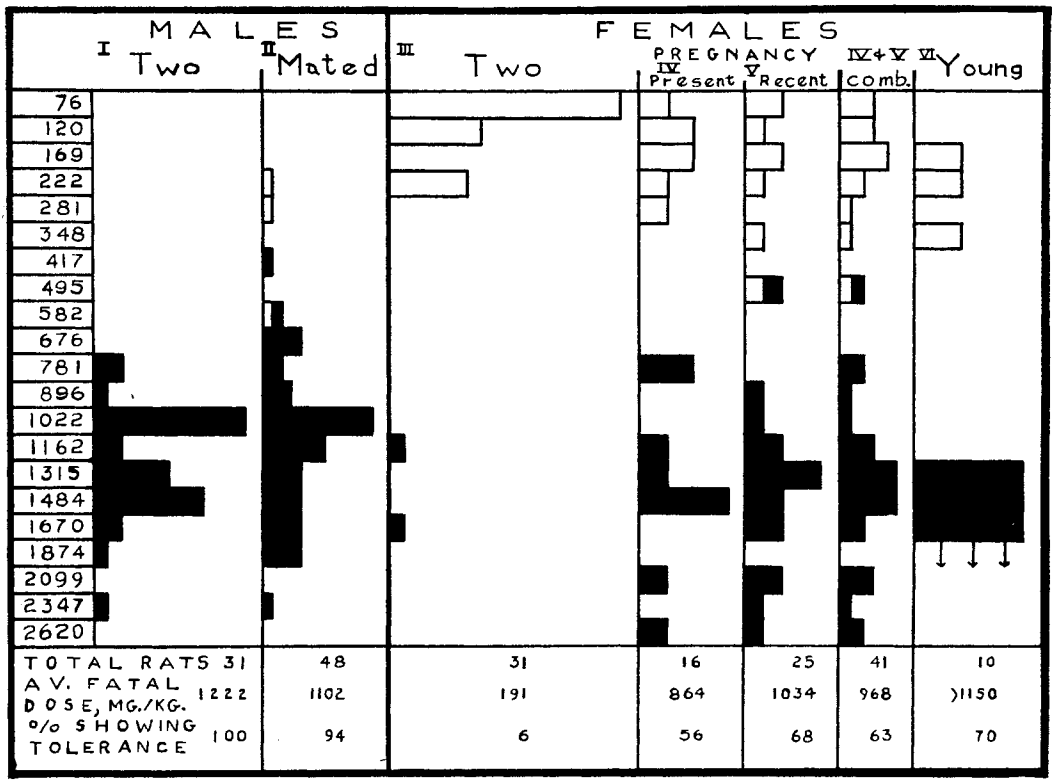

Chart 1.—Percentage Mortality Distribution and Tolerance (in Black) of Various Groups of Albino Rats Injected Subcutaneously with Gradually Increasing Doses of Sodium Pentobarbital. Successive Three Doses Are Combined. Because of Absence of Mortalities on the First Three Doses, They Are Omitted. The Rats Were Five to Seven Months Old Except the Young Which Were Two to Two and One Half Months Old. Recently Pregnant Means up to 39 Days after the Last Delivery. Downward Arrows and > Show That the Total Fatal Dose Is Higher Than Indicated, Due to Discontinuation of Injections to See Whether These Rats Would Permanently Recover.

which had received no treatment. However, it must be borne in mind that the result of a single rat that develops tolerance exerts a much greater influence upon the average when the group is small (only 13 rats constituted this group; 1 developed tolerance and the average total fatal dose was 279 mg./Kg.).

DISCUSSION

The outstanding result of our experiments with gradually increasing doses of Sodium Pentobarbital (Nembutal) is that among the varying conditions of sex life in both sexes of the albino rat, only pregnancy markedly increased the incidence of tolerance and, consequently, the average total fatal dose, and that this effect persists at least during the period of lactation. Just how permanent this effect is will be settled by future tests. It should be noted that our technique of Pentobarbital or of Evipal (methyl cyclo-hexenyl *N*-methyl barbiturate) administration exaggerates any existing sex-difference so that by proper adjustment one may kill nearly all of the females selectively (14, 15). In past experiments with this Pentobarbital sample, the male L.D. 50 was only about sixty per cent higher than that of the female when single doses were administered to groups of our stock, whereas in the present study it was about seven times as great. Hence, to be certain of a significant difference by the technique of daily administrations as carried out by Agduhr or by our 90 minute method, one must secure much greater differences in the average total fatal dose than by determining the average single fatal dose. Therefore, we have stressed the large increase in animals showing visible tolerance, rather than attempting to interpret statistically the combined results of rats showing tolerance and those not showing tolerance in the same group.

Although the literature reports internal changes in the thyroid, adrenal and pituitary glands as a consequence of pregnancy, we were unable to find that the varying conditions of sex life in our study modified the average proportion of the weights of these glands to body-weight; however, there was a greater sex-difference in case of the adrenal and pituitary glands in our stock than has been previously reported by several authorities. A further analysis would seem to necessitate studies upon the various hormones, the amounts of which are changed during pregnancy. It is of interest in this connection that an anti-narcotic action has been reported for both androgens and estrogens (15, 16).

It should also be noted that young virgin female rats showed a resistance at least similar to that of pregnant or recently pregnant older female rats. That such a relation may hold for all drugs seems *a priori* improbable because young rats do not show higher resistance to all drugs, but to some an equal and to others a distinctly lower resistance than the adult; and, as mentioned, even with the same substance there may be a difference in this respect in the two sexes (15). In case of nicotine it has been reported that tolerance to small doses was developed by growing rats only (17). A summary of the effect of age in the rat has recently been prepared (18). The same caution holds in regard to drawing too far-reaching conclusions from the effect of pregnancy in this case. It is true that pregnant rats tolerated doses of thyroid and thyroxin which in non-pregnant rats caused rapid loss of weight and death (19). But, on the other hand, pregnant rats have been reported especially sensitive to combined administration of tyramine and phenylethyl amine (20). It is also an open question what effects pregnancy would have upon female rats in those cases in which the female rat has a higher resistance than the male, as in case of morphine (21), ouabain (22) or even the amino acid, tyrosine (23).

SUMMARY

1. Various conditions of sex life did not significantly influence male or female albino rat recovery time or percentage of delayed deaths from 50 mg./Kg. of Nostal (isopropyl bromallyl barbituric acid), administered intraperitoneally.

2. No difference in resistance to gradually increasing subcutaneous doses of Sodium Pentobarbital (Nembutal; ethyl 1-methylbutyl barbituric acid) could be demonstrated in male rats raised under varying

conditions of opportunity for copulation, or in females caged with males with sexual intercourse established but pregnancy prevented through partial hysterectomy, or in normal females coexisting with males but with coition prevented by a vaginal "chastity" clip.

3. The resistance to such medication was markedly raised in females which were or recently had been pregnant, but was not brought up to that of the males.

4. Young virgin females developed tolerance to gradually increasing doses of Sodium Pentobarbital about ten times more often than did older virgins. Six young rats recovered permanently from medication every 90 minutes for three days and nights.

5. Small doses of Nostal administered at three- to four-day intervals for several weeks completely prevented delayed deaths from 50 mg./Kg. of Nostal, which killed 83 per cent of the controls.

6. Similar preliminary treatment with Sodium Pentobarbital raised the average resistance of virgin female rats only moderately.

7. Varying conditions of sex life did not alter the average percentage weights of the thyroid, adrenal or pituitary glands. The higher proportion of the latter two glands in the females was greater than usually has been reported for the albino rat.

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A Phytochemical and Histological Study of *Purshia tridentata* (Pursh) D.C.*

By Charles V. Netz, Charles H. Rogers and Glenn L. Jenkins

Purshia tridentata (Pursh) D.C., family Rosaceæ, is a diffusely branched shrub grow-

* Presented to the Scientific Section, A. PH. A., Richmond meeting, 1940.

Abstract of thesis presented to the Graduate Faculty of the University of Minnesota by Charles V. Netz in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

From the laboratories of the College of Pharmacy, University of Minnesota.

This is a report in a coöperative research project connected with the Indian Medicinal Plant study of the Bureau of Plant Industry, United States Department of Agriculture.