from water at room temperature contains one and one-half molecules of water of crystallization. No evidence of any other hydrate is indicated by these results.

TABLE IV.

Per Cent Water in Sample.	Moles Water in Sample.	Vapor Pressure in Mm.	Per Cent Water in Sample.	Moles Water in Sample.	Vapor Pressure in Mm.
8.15	1.96	23.0	4.56	1.05	7.0
7.61	1.82	21.0	3.71	0.85	7.0
6.80	1.61	18.0	3.20	0.73	6.6
6.57	1.55	9.0	2.96	0.67	7.0
6.22	1.47	7.5	2.07	0.47	6.5
6.06	1.42	7.1	1.49	0.33	6.0
5.53	1.24	7.0	1.18	0.26	5.0
4.84	1.12	7.0			

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THE BIOASSAY OF THE ANTERIOR PITUITARY-LIKE SEX HORMONE (ANTUITRIN S).*

BY L. W. ROWE, A. SIMOND AND W. O. NELSON.

Although several bioassay methods for evaluating the anterior pituitary-like sex hormone from pregnancy urine have been suggested, none of them has been subjected to adequate critical scrutiny for accuracy nor has there been much basis for a comparison of values obtained by different methods. It will be the purpose of this article to present in detail the method which has been used to control accurately the potency of a commercial product (Antuitrin S) as well as to give data comparing activities obtained in the assay of the same preparation by several other recognized methods.

Zondek and Aschheim (1) suggested the use of baby female white mice weighing 6 to 8 Gm., for assaying anterior pituitary sex hormone preparations.

In 1931, Fevold, Hisaw and Leonard (2) published a method for the assay of the anterior pituitary sex hormone using immature female white rats 20 to 25 days old as the test animals. Later, Fevold, *et al.* (3) used 21- to 23-day old rats and the same technique but they also reported that rabbits 12 weeks old were even more

* Scientific Section, A. PH. A., Washington meeting, 1934.

satisfactory, as they were not so susceptible to small amounts of luteinizing hormone as are rats and, consequently, differentiated better between the follicle-stimulating and the luteinizing action.

Wallen-Lawrence and Van Dyke (4) utilized an assay method in which increase in size of the ovaries or seminal vesicles of immature rats is critically determined.

Katzman and Doisy (5) suggest the use of 19-day old female white mice in which a dose given subcutaneously in six equal portions during the course of three days will cause opening of the vagina and *estrus* on the 22nd to the 24th day of age, as judged by vaginal smears, if the dose used contains one or more mouse units. They also used 21-day old rats in which case one rat unit must produce these reactions by the 27th day of age.

Friedman (6) has studied the effect of various agents on ovulation in the rabbit and suggests an assay method for anterior pituitary sex-regulating activity utilizing this physiological response.

Friedman raised the objection to the rat method that rats reach sexual maturity normally at such a variable age that the ovaries of 3-week-old rats will be in such a widely scattered state of development that the same dose of an effective agent could not bring all of them into maturity at the same time. Some attempt has been made recently by Davy (7) to overcome this objection by roughly standardizing the normal uninjected female rats. She found that those animals which weighed between 35 and 45 Gm. at 24 days of age possessed remarkably uniform ovaries, and drew the conclusion that at least one of three such rats may be expected to give a positive response to one M. E. D. of gonadotropic substance injected in 5 daily doses. This will be commented upon further a little later.

Mention might also be made of the contribution by Swezy (8) in which she voices criticism of practically all the assay methods that have thus far been proposed. Nothing of a constructive nature is offered.

EXPERIMENTAL.

In the early work in this laboratory thirty-day old female white rats, taken from a colony the members of which showed an average sexual maturity age of 50 to 60 days, were used for the potency control of experimental extracts containing the sex-regulating activity of the anterior pituitary as found in pregnancy urine. The unknown solution was tested usually at five levels using only two rats at each level. It was soon realized, however, that a method utilizing a relatively small number of animals is of qualitative value only but does not meet the rigid requirements of a commercial assay method. It was also found that more accurate results could be obtained with the use of rats twenty-six days of age. The details of our present method are as follows:

Immature female white rats *26 days* old at the beginning of the test, from a colony with an average normal sexual maturity age of 55 days, are dosed subcutaneously twice a day for thee days and autopsy done 96 to 100 hours after the first dose. At least *5 rats* and *usually 10* are used at each dosage level and a majority (6 out of 10) must show the definite development of one or more corpora lutea for the test to be positive at this level. The weight of the rats and the condition of the vagina and uterus is also carefully noted at the end of the test but the real criterion is the formation of corpora lutea on the ovaries for we have often

found corpora lutea without the vagina being open and sometimes vice versa. Usually four levels of dosage varying 10% to 20% with each other are selected and the smallest dose determined which meets the rigid requirements of the test. A typical protocol is given herewith in detail.

TABLE I.

Antuitrin S, B# 849774.

Age.	Dose.	Dil.	Total Volume.	Wt. 30 Da.	Corpora Lutea.	Vagina and Uterus.		Rat U.
26 days	0.83 cc. × 6	1–500	0.01 cc.	40 Gm.	2	Open	Congested	100
...	.....	...	....	42 Gm.	2	Closed	Congested	100
...	.....	...	....	48 Gm.	6	Open		100
...	.....	...	....	44 Gm.	3	Open		100
...	.....	...	....	50 Gm.	7	Open		100
...	0.67 cc. × 6	...	0.008 cc.	48 Gm.	4	Open		125
...	.....	...	....	50 Gm.	4	Open		125
...	.....	...	....	50 Gm.	4	Open		125
...	.....	...	....	50 Gm.	5	Open		125
...	.....	...	....	40 Gm.	2	Closed	Congested	125
...	.....	...	....	40 Gm.	3	Closed	Congested	125
...	.....	...	....	40 Gm.	4	Open	Congested	125
...	.....	...	....	50 Gm.	0	Closed	Congested	125
...	.....	...	....	50 Gm.	4	Open		125
...	.....	...	....	44 Gm.	1	Closed	Congested	125
...	0.55 cc. × 6	...	0.006 cc.	50 Gm.	5	Open		150
...	.....	...	....	48 Gm.	2	Closed	Congested	150
...	.....	...	....	50 Gm.	3	Open	Congested	150
...	.....	...	....	44 Gm.	3	Open	Congested	150
...	.....	...	....	40 Gm.	2	Closed	Congested	150
...	.....	...	....	48 Gm.	6	Open		150
...	.....	...	....	48 Gm.	3	Open	Congested	150
...	.....	...	....	38 Gm.	2	Closed	Congested	150
...	.....	...	....	40 Gm.	3	Open	Congested	150
...	.....	...	....	50 Gm.	0	Closed	Congested	150
...	0.47 cc. × 6	...	0.0057 cc.	48 Gm.	0	Closed	Congested	175
...	.....	...	....	46 Gm.	2	Closed	Congested	175
...	.....	...	....	40 Gm.	1	Closed	Congested	175
...	.....	...	....	40 Gm.	2	Closed	Congested	175
...	.....	...	....	50 Gm.	6	Open	Congested	175
...	.....	...	....	50 Gm.	0	Closed	Congested	175
...	.....	...	....	34 Gm.	0	Closed	Congested	175
...	.....	...	....	46 Gm.	2	Open	Congested	175
...	.....	...	....	50 Gm.	3	Open	Congested	175
...	.....	...	....	50 Gm.	0	Closed	Congested	175
...	0.42 cc. × 6	...	0.0050 cc.	50 Gm.	0	Closed	Congested	200
...	.....	...	....	48 Gm.	0	Closed	Congested	200
...	.....	...	....	50 Gm.	0	Closed	Congested	200
...	.....	...	....	50 Gm.	0	Closed	Congested	200
...	.....	...	....	44 Gm.	0	Closed	Congested	200

Activity is 175 rat units per cc. (6 out of 10 positive).

In addition to the successful quantitative use of the method outlined in the assay of almost 1000 samples both experimental and commercial, we have conducted a rather intensive study of the stability of a particular experimental lot,

℞ 095286-B, and also of its activity by several methods. By our regular method using immature rats this lot has been tested as follows:

TABLE II.

1.	May 2, 1933	175 rat units per cc.	
2.	May 9, 1933	175	
3.	June 6, 1933	150	5 weeks old
4.	July 6, 1933	150	9 weeks old
5.	Aug. 8, 1933	125	14 weeks old
6.	Aug. 29, 1933	75	17 weeks old
7.	Sept. 19, 1933	75	20 weeks old
8.	Oct. 17, 1933	75	24 weeks old

Careful tests on this lot in September 1933 upon baby mice for results comparable to the Zondek and Doisy mouse units gave data as follow:

TABLE III.

℞ 095286-B. Female, 15- to 18-day old mice.

Weight. 9/26.	Weight. 9/30.	Dose.	Dil.	Total Volume.	Smear Stage.	Corpora Lutea.	Vagina and Uterus.		Rat Units.
9 Gm.	12 Gm.	0.6 cc. × 6	1–30	0.12 cc.	5	2	Open		8
7 Gm.	11 Gm.	.....	....	....	4	3	Open		
8 Gm.	11 Gm.	.....	....	....	2	2	Open	Congested	
8 Gm.	11 Gm.	.....	....	....	2	1	Open	Congested	
6 Gm.	9 Gm.	.....	....	....	4	1	Open	Congested	
8 Gm.	7 Gm.	.....	....	....	2	0	Open	Congested	
8 Gm.	Died				–				
6 Gm.	Died				–				
10 Gm.	14 Gm.	.....	....	....	5	2	Open		
Male									
6 Gm.	7 Gm.	0.4 cc. × 6	....	0.08 cc.	5	1	Open		12
8 Gm.	10 Gm.	.....	....	....	3	5	Open	Congested	
7 Gm.	11 Gm.	.....	....	....	2	2	Open	Congested	
8 Gm.	12 Gm.	.....	..	....	–	0	Closed		
8 Gm.	10 Gm.	.....	....	....	5	1	Open	Congested	
8 Gm.	7 Gm.	.....	....	....	1–2	4	Open	Congested	
7 Gm.	9 Gm.	.....	....	....	1–2	1	Open	Congested	
6 Gm.	6 Gm.	.....	....	....	1–2	0	Open	Congested	
8 Gm.	10 Gm.	.....	....	....	2	1	Open	Congested	
8 Gm.	Died				...	..			
8 Gm.	10 Gm.	0.33 cc. × 6	....	0.066 cc.	2	1	Open	Congested	15
6 Gm.	Died				...	..			
10 Gm.	11 Gm.	.....	....	....	2	2	Open	Congested	
8 Gm.	10 Gm.	.....	....	....	1–2	2	Open	Congested	
7 Gm.	9 Gm.	.....	....	....	2	2	Open	Congested	
7 Gm.	9 Gm.	.....	....	....	2	0	Open	Congested	
9 Gm.	13 Gm.	.....	....	....	1–2	4	Open	Congested	
6 Gm.	9 Gm.	.....	....	....	4	0	Open		
7 Gm.	10 Gm.	.....	....	....	1–2	1	Open	Congested	
7 Gm.	Died				...	..			
8 Gm.	11 Gm.	0.25 cc. × 6	....	0.05 cc.	1–2	2	Open	Congested	20
8 Gm.	12 Gm.	.....	....	....	...	0	Closed	Congested	
8 Gm.	12 Gm.	.....	....	....	...	0	Closed	Congested	
7 Gm.	8 Gm.	.....	....	....	1–2	2	Open	Congested	
9 Gm.	12 Gm.	.....	....	....	2	2	Open	Congested	
7 Gm.	10 Gm.	.....	....	....	...	0	Closed	Congested	

TABLE III.—*Continued.*

Weight. 9/26.	9/30.	Dose.	Dil.	Total Volume.	Smear Stage.	Corpora Lutea.		Vagina and Uterus.	Rat Units.
8 Gm	12 Gm.	.....	....	....	...	1	Closed	Congested	
8 Gm	10 Gm.	.....	....	....	...	0	Closed	Congested	
7 Gm	Died					..			
8 Gm	12 Gm.	.....	....	....	2	2	Open	Congested	
10 Gm.	12 Gm.	0.16 cc. × 6	....	0.032 cc.	4?	0	Open	Congested	30
6 Gm.	8 Gm.	.....	....	....	...	0	Closed	Congested	
8 Gm.	12 Gm.	.....	....	....	1–2	2	Open	Congested	
8 Gm.	14 Gm.	.....	....	....	...	0	Closed	Congested	
7 Gm.	12 Gm.	.....	....	....	...	0	Closed		
8 Gm.	12 Gm.	.....	....	....	1–2	2	Open	Congested	
6 Gm.	10 Gm.	.....	....	....	...	0	Closed	Congested	
8 Gm.	12 Gm.	.....	....	....	...	0	Closed	Congested	
6 Gm.	9 Gm.	.....	..	....	...	0	Closed	Congested	
8 Gm.	14 Gm.	.. ..	....	....	2	1	Open	Congested	

Five out of 9 were positive at 20 units per cc. and only 3 out of 10 were positive at 30 units, so 20 mouse units per cc. is the activity of ℞ 095286-B. Since this lot assayed 75 and 80 rat units per cc. after August 8, 1933, 1 mouse unit is approximately equal to 4 rat units.

A very recent check test on baby mice at the two critical levels of comparison resulted as follows:

TABLE IV.

℞ 095286-B. Assaying 80 R. U./cc.

Female white mice approx. 16 days old.

Weight. 10/27.	10/21.	Dose.	Dil.	Total Volume.	Corpora Lutea.	Vagina and Uterus.		Mouse Units.	Ratio R. U.:M. U.
8 Gm.	10 Gm.	0.33 cc. × 6	1–30	0.066 cc.	1	Closed	Congested	15	1:5
8 Gm.	11 Gm.	.....	....	...	0	Closed	No effect	15	1:5
7 Gm.	8 Gm.	.....	....	...	2	Closed	Congested	15	1:5
7 Gm.	8 Gm.	.....	....	...	0	Closed	Congested	15	1:5
9 Gm.	10 Gm.	....	....	...	2	Closed	Congested	15	1:5
9 Gm.	11 Gm.	.....	....	...	2	Closed	Congested	15	1:5
7 Gm.	9 Gm.	.....	....	...	2	Closed	Congested	15	1:5
8 Gm.	11 Gm.	.....	....	...	1	Closed	Congested	15	1:5
8 Gm.	8 Gm.	.....	....	...	1	Closed	Congested	15	1:5
7 Gm.	8 Gm.	.....	....	...	0	Closed	No effect	15	1:5
7 Gm.	9 Gm.	0.25 cc. × 6	....	0.050 cc.	0	Closed	No effect	20	1:4
7 Gm.	8 Gm.	.....	....	...	0	Closed	No effect	20	1:4
8 Gm.	10 Gm.	.....	....	...	0	Closed	No effect	20	1:4
8 Gm.	10 Gm.	.....	....	...	2	Open	Congested	20	1:4
9 Gm.	11 Gm.	.....	....	...	1	Closed	Congested	20	1:4
10 Gm.	12 Gm.	.....	....	...	2	Closed	Congested	20	1:4
8 Gm.	10 Gm.	.....	....	...	1	Closed	Congested	20	1:4
8 Gm.	9 Gm.	.....	....	...	0	Closed	No effect	20	1:4

70% were positive at 15 rat units per cc. or 1 mouse unit = 5 rat units.
50% were positive at 20 rat units per cc. or 1 mouse unit = 4 rat units.

Only one mouse was positive out of 18 by the Katzman-Doisy technique and that was with the smaller dose of the two.

Some preliminary work on mice a year previously showed that at 5 and 6 rat units per mouse unit only 50% of the mice were positive but this later relationship is more convincing and probably more nearly correct.

This lot was also tested on 21-day old white rats with observation of vaginal smears at the end of the fourth day after the first injection in order to conform closely to the Katzman-Doisy technique for the purpose of comparing our unit with their rat unit. Results of this test are given in Table V.

TABLE V.

℞ 095286-B. (21-day old rats, Katzman-Doisy technique.)

Weight. Before.	Weight. After.	Dose.	Dil.	Total Volume.	Smear Stage.	Corpora Lutea.	Vagina and Uterus.		Rat Units.
35 Gm.	47 Gm.	0.8 cc. × 6	1–100	0.048 cc.	2	2	Open	Congested	20
32 Gm.	40 Gm.	....	.....	...	3	2	Open	Congested	..
25 Gm.	35 Gm.	....	.....	...	3	3	Open	Congested	..
30 Gm.	38 Gm.	....	.....	...	2	1	Open	Congested	..
20 Gm.	Died				...	...			
35 Gm.	38 Gm.	0.4 cc. × 6	.....	0.024 cc.	2	2	Open		40
36 Gm.	42 Gm.	....	.....	...	2	3	Open		..
28 Gm.	30 Gm.	....	.....	...	...	0	Closed	Congested	..
30 Gm.	35 Gm.	....	.....	...	3	0	Open	Congested	..
30 Gm.	40 Gm.	....	.....	...	...	3	Closed	Congested	..
28 Gm.	30 Gm.	....	.....	...	2	3	Open	Congested	..
30 Gm.	40 Gm.	....	.....	...	2	2	Open	Congested	..
30 Gm.	38 Gm.	....	.....	...	3	2	Open	Congested	..
28 Gm.	38 Gm.	....	.....	...	3	3	Open	Congested	..
25 Gm.	38 Gm.	....	.....	...	2	2	Open	Congested	..
25 Gm.	36 Gm.	0.8 cc. × 6	1–300	0.016 cc.	2	2	Open	Congested	60
36 Gm.	44 Gm.	....	.....	...	1–2		Open	Congested	..
30 Gm.	40 Gm.	....	.....	...	2	1	Open	Congested	..
35 Gm.	43 Gm.	....	.....	...	...	0	Closed	Congested	..
25 Gm.	30 Gm.	....	.....	...	...	0	Closed	Congested	..
30 Gm.	40 Gm.	....	.....	...	1–2	2	Open	Congested	..
36 Gm.	42 Gm.	....	.....	...	...	0	Closed	Congested	..
30 Gm.	38 Gm.	....	.....	...	1–2	2	Open	Congested	..
25 Gm.	33 Gm.	....	.....	...	1–2	1	Open	Congested	..
30 Gm.	35 Gm.	....	.....	...	...	0	Closed	Congested	..
40 Gm.	48 Gm.	0.6 cc. × 6	.....	0.012 cc.	...	0	Closed	Congested	..
45 Gm.	50 Gm.	....	.....	...	...	3	Closed	Congested	..
30 Gm.	36 Gm.	....	.....	...	...	0	Closed	Congested	..
40 Gm.	50 Gm.	....	.....	...	...	0	Closed	Congested	83
34 Gm.	36 Gm.	....	.....	...	...	4	Closed	Congested	..
30 Gm.	38 Gm.	....	.....	...	...	4	Closed	Congested	..
25 Gm.	35 Gm.	....	.....	...	...	3	Closed	Congested	..
25 Gm.	33 Gm.	....	.....	...	...	1	Closed	Congested	..
20 Gm.	30 Gm.	....	.....	...	...	0	Closed	Congested	..
25 Gm.	33 Gm.	....	.....	...	...	0	Closed	Congested	..
47 Gm.	55 Gm.	0.5 cc. × 6	.....	0.010 cc.	...	0	Closed	Congested	100
35 Gm.	43 Gm.	....	.....	...	...	1	Closed	Congested	..
35 Gm.	40 Gm.	....	.....	...	...	0	Closed	Congested	..
25 Gm.	33 Gm.	....	.....	...	...	0	Closed	Congested	..
30 Gm.	40 Gm.	....	.....	...	...	2	Closed	Congested	..

Activity is 60 Doisy rat units per cc.

Activity is 60 to 80 Parke, Davis rat units per cc. on the 21-day old rats or 75 P. D. rat units per cc. on 26- to 28-day old rats.

The Doisy and Parke, Davis rat units are apparently very nearly equal even though the details of the two methods vary materially. To prove further that

these two units are about the same and that assays of the same preparation conducted in different laboratories can agree very closely, the following series of tests made in Dr. Doisy's and in our laboratories is given in Table VI.

TABLE VI.

Antuitrin S.

Sample No.	Our Assay. Date.	Our Assay. Rat Units.	Dr. Doisy's Assay. Date.	Dr. Doisy's Assay. Rat Units.
3008439	5/22/33	175	6/14/33	165
3008440	6/10/33	175	6/26/33	167
3009703	6/10/33	175	7/8/33	166
3009702	6/17/33	175	7/8/33	166
3010507	7/1/33	100	7/29/33	75
3013846	7/22/33	150	8/5/33	166
3013847	7/29/33	150	8/10/33	133
3015303	8/5/33	175	8/18/33	166
3016294	8/12/33	100	9/2/33	133
3014538	8/12/33	150	9/2/33	166
3016295	9/19/33	100	9/12/33	85
3016852	8/26/33	175	9/12/33	166
3017466	9/2/33	125	9/18/33	133
3018030	9/2/33	175	9/18/33	166

The agreement in the above assay values obtained in two different laboratories by methods that are not identical is indeed remarkable. The slight variations are largely due to differences in selection of dosage levels.

THE FRIEDMAN RABBIT OVULATION METHOD.

One of us (W. O. N.) has carefully studied the activity of this particular lot of Antuitrin S, ℞ 095286-B, by the Friedman method with results that may be tabulated as follows:

TABLE VII.

℞ 095286-B. Rabbit Ovulation Tests.
Estrous Animals Including Postpartum Cases.

Weight Kg.	Actual Dose per Kg.	Result.
3.2 pp	0.56 R. U.	−
3.0	0.60 R. U.	−
3.0	0.60 R. U.	−
4.1 pp	0.60 R. U.	−
3.2 pp	0.60 R. U.	−
3.95	0.75 R. U.	−
4.2	0.75 R. U.	−
4.2 pp	0.75 R. U.	−
3.6 pp	0.84 R. U.	−
3.65	0.90 R. U.	+
2.5	0.90 R. U.	−
2.5	0.90 R. U.	−
2.4	0.90 R. U.	−
4.1	0.90 R. U.	−
4.1 pp	0.90 R. U.	+
3.5 pp	0.90 R. U.	+
3.25 pp	0.90 R. U.	−
3.85 pp	0.90 R. U.	+
3.27 pp	0.96 R. U.	+
3.8	0.96 R. U.	−
3.7	0.96 R. U.	+
3.5	0.96 R. U.	+
3.25 pp	0.96 R. U.	+
3.3 pp	0.96 R. U.	+
4.2 pp	0.96 R. U.	+
3.1	1.002 R. U.	+
3.5	1.02 R. U.	+
4.1	1.02 R. U.	+
3.8	1.05 R. U.	+
2.5	1.05 R. U.	+
2.65 pp	1.05 R. U.	+
3.0	1.05 R. U.	+
3.5 pp	1.05 R. U.	+
3.0	1.20 R. U.	+
2.7	1.20 R. U.	+
3.1	1.20 R. U.	+
2.75	1.20 R. U.	+
3.2	1.20 R. U.	+
2.5	1.20 R. U.	+
2.85	1.50 R. U.	+
2.4	1.80 R. U.	+
3.1	2,46 R. U.	+
2.70	3.70 R. U.	+

pp = postpartum animals.
+ = ovulation.
− = no ovulation.

Actual dose means dose figured on the basis of the assay of 75. R. U. per cc. Assay conducted without knowledge of actual potency.

Table VII indicates a slightly greater sensitivity on the part of postpartum animals since at the 0.90 R. U. per Kg. level 3 out of 4 postpartum animals ovulated while only 1 out of 5 of the ordinary isolated females did so, and at 0.96 R. U. per Kg. all 4 of the postpartum animals ovulated while only 2 out of 3 of the others did so. Also a few of the animals were used 2 or more times if they did not ovulate with the first dose, but Friedman has demonstrated that such animals are not rendered more susceptible to subsequent doses.

TABLE VIII.

Non-estrous Rabbits.

Weight Kg.	Actual Dose per Kg.	Result.
3.1	1.2 R. U.	−
2.4	1.2 R. U.	−
2.9	1.2 R. U.	−
2.75	1.2 R. U.	−
4.1	1.2 R. U.	−
2.5	1.8 R. U.	−
3.0	3.0 R. U.	−
2.2	3.0 R. U.	−
2.27	3.0 R. U.	−
2.8	3.0 R. U.	−
2.8	4.5 R. U.	−
2.5	4.5 R. U.	+
2.3	6.0 R. U.	+
3.2	6.0 R. U.	+
2.85	6.0 R. U.	−
2.8	6.0 R. U.	+
2.4	9.0 R. U.	+
2.8	12.0 R. U.	+
2.95	12.0 R. U.	+

These animals were classified as non-estrous because their ovaries contained recent corpora lutea. These corpora lutea had been formed by previous Antuitrin-S injections or the injection of some one of the Antuitrin-L extracts. However, there was every reason to believe that ovulation might be induced, if the dose of Antuitrin-S were large enough, since fairly large follicles were present in the ovaries along with the corpora lutea.

TABLE IX.

Young Animals.

Weight Kg.	Actual Dose per Kg.	Result.
1.85	6.0 R. U.	−
1.80	6.0 R. U.	−
1.9	9.0 R. U.	−
1.85	9.0 R. U.	−
2.05	12.0 R. U.	+
1.90	12.0 R. U.	+
2.1	12.0 R. U.	+

This table gives data on a very limited number of young animals approaching maturity which indicate that about 12 R. U. per Kg. are necessary to induce ovulation.

SUMMARY OF RABBIT OVULATION RESULTS.

TABLE X.

Estrous and Postpartum Animals.

Actual Dose Injected per Kg.	Number of Cases. Positive.	Negative.
0.56 R. U.	0	1
0.60 R. U.	0	4
0.75 R. U.	0	3
0.84 R. U.	0	1
0.90 R. U.	4	5
0.96 R. U.	6	1
1.002 R. U.	1	0
1.02 R. U.	2	0
1.05 R. U.	5	0
1.20 R. U.	6	0
1.50 R. U.	1	0
1.80 R. U.	1	0
2.46 R. U.	1	0
3.70 R. U.	1	0

TABLE XI.

Non-estrous Animals.

Actual Dose Injected per Kg.	Number of Cases. Positive.	Negative.
1.2 R. U.	0	5
1.8 R. U.	0	1
3.0 R. U.	0	4
4.5 R. U.	1	1
6.0 R. U.	3	1
9.0 R. U.	1	0
12.0 R. U.	2	0

TABLE XII.—Young Animals.

Actual Dose Injected per Kg.	Number of Cases. Positive.	Negative.
6.0 R. U.	0	2
9.0 R. U.	0	2
12.0 R. U.	3	0

In each injection for the induction of ovulation the desired quantity of the extract was taken and made up to 1 cc. Injections were given intravenously and the response was determined in 24 to 36 hours by operation or autopsy.

DISCUSSION.

The tabulated experimental data submitted serve to show the practicability of the proposed method using immature white rats (26 to 28 days old for the control of the sex-regulating activity of the anterior pituitary with an accuracy of 10% to 20%. In our opinion the use of rats of this age with autopsy at 96 to 100 hours after the first dose and examination for mature corpora lutea is more definite and practical than the use of younger rats with dependence on the opening of the vagina and upon the estrous stage for a positive reaction, or the use of baby mice where determination of mature corpora lutea is much more difficult.

We do find from assays of the same preparations in different laboratories by our corpora lutea method on 26- to 28-day old rats and by the estrous stage method on 21-day old rats (Doisy) that very good agreement in values can be obtained by the two methods.

As for the methods using baby white mice, we do not find them any more accurate and certainly they are less practical both because of greater difficulty in interpreting results by gross observation, and also because proper test animals cannot be secured very readily.

The rabbit ovulation method appears to be of value and we have provided a basis of comparison between results obtained by it and by the other methods. It is to be noted that our results check closely those reported by Dr. Friedman. However, it does not seem to possess any advantages over the other methods as to accuracy or specificity of the positive reaction and it is more time-consuming and expensive particularly if twenty rabbits are to be used in a test. Friedman's objection to the rat methods that different rats reach sexual maturity at such a variable age is taken care of by the use of a number of rats (5 to 10) at each dosage level and by requiring that only a bare majority be positive at the end of the test, for it is admitted that some individual animals are backward in their ovarian development and could not be brought into sexual maturity by the minimum effective dose for the majority.

SUMMARY.

1. A practical and accurate method is presented for the standardization of the anterior pituitary-like sex hormone from pregnancy urine.

2. The relationship between our rat unit and the mouse unit has been shown experimentally to be about 1 mouse unit equivalent to 4 rat units.

3. The rabbit ovulation unit was found experimentally to be equivalent to 1 rat unit per Kg. body weight of rabbit.

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DRUG EXTRACTION. I. A STUDY OF VARIOUS MENSTRUA FROM THE STANDPOINT OF SWELLING EFFECTS, PENETRATION AND EXTRACTION.[1, 2, 3]

BY WILLIAM J. HUSA[4] AND LOUIS MAGID.

INTRODUCTION.

The extraction of drugs is a time-honored process. However, there has been a feeling in recent years that the fundamental principles involved in this basic pharmaceutical procedure have received insufficient attention. Such factors as the swellings of drugs in liquids and the penetration of menstrua have not been studied quantitatively and many official formulas are based to a considerable extent on empiricism and tradition.

The purpose of the present investigation has been to make a critical study of the fundamental principles of drug extraction with special reference to permeation of cell walls by a selected series of pharmaceutical solvents, to swelling of cellular tissue during maceration with selected menstrua, and to the influence of menstrua upon the structure of vegetable drugs and extraction of the constituents.

SWELLING EFFECT OF SOLVENTS.

Chestnut wood, being a relatively simple vegetable structure, was chosen as the first material to be studied with the idea that the methods evolved and the data collected would be applied in the course of the investigation in the study of various types of drugs. Chestnut wood consists largely of fibrous tissue and one of its constituents is tannin.

Swelling of Strips of Chestnut Wood.—A study of the swelling effect of solvents on thin sections was deemed advisable as the first point of attack. The liquids

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[1] Scientific Section, A. PH. A., Washington meeting, 1934.

[2] This investigation was aided by a grant from the AMERICAN PHARMACEUTICAL ASSOCIATION Research Fund.

[3] This paper is based on a dissertation submitted by Louis Magid to the Graduate Council of the University of Florida in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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