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## THE EFFECTIVENESS OF THEELOL BY ORAL ADMINISTRATION.\*

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Since the isolation of two crystalline estrogenic hormones, Theelin or keto-hydroxyestrin,  $C_{18}H_{22}O_2$ , and Theelol or trihydroxyestrin,  $C_{18}H_{24}O_3$  (Doisy and collaborators, 1, 2, 3, 4, 5 and Marrian, 6, 7) and particularly since the establishment of an International Standard for the control of potency of such substances, a certain amount of disagreement exists as to their relative potencies.

Prior to the adoption of the International Estrogenic Standard at the London Conference of 1932 with the arbitrary agreement that 1 mg. of this standard should be equivalent in estrogenic activity to 10,000 International Units, the literature showed many conflicting statements by different workers as to the relation between the rat unit and the mouse unit and similarly between the potency of Theelin and Theelol. Thus Curtis and Doisy (5) and also Marrian (7) found that in aqueous solution and by hypodermic administration Theelin was just about twice as active as Theelol. Butenandt (8) found that in oily solution Theelin was 5.3 times as potent as Theelol. Burn and Elphick (9) on the other hand found Theelin to be only 85% as active as Theelol when compared in aqueous solutions in four injections but in oil solution with a single injection Theelin was found to be 4.5 times as potent as Theelol. These workers do not agree at all with Curtis and Doisy or with Marrian but they do agree very well with Butenandt.

Even greater discrepancies exist in the literature as to the relation between the estrogenic rat unit and mouse unit. Although we are not particularly concerned with this phase of the subject in this short paper, for convenience we will summarize the various findings as follows: Laquer and de Jongh (10) using aqueous solutions and dividing the dose into six injections found the rat unit to be four times as great as the mouse unit and the ratio therefore to be 4 to 1; Kochmann (11) for aqueous solutions found the ratio to be 4 to 1 and for oily solutions to be 2 to 1; Marrian working in conjunction with Dickens and Dodds (12), using an aqueous solution and both four and six injections found the ratio to be 10 to 1; Coward and Burn (13), Becker, Mellish, D'Amour and Gustavson (14) and Burn and Elphick (9) all find the ratio to be 1 to 1 if oily solutions are used in a single injection method.

### EXPERIMENTAL WORK.

In the series of tests about to be described, the general method of Allen and Doisy (15) and the suggestions of Kahnt and Doisy (16) were followed except that doses were given orally instead of subcutaneously. That is, the oral dose was divided into three parts given at intervals of about four hours to sensitized rats and

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of 20 rats used at a particular dosage level, about 75 per cent must react positively in order to consider that a positive test.

Many comparisons of Theelin and Theelol by subcutaneous injection in our laboratory have rather definitely established the fact that Theelin is three times as potent as Theelol when each is administered hypodermically to spayed rats, since pure Theelin is found to test from 2500 to 3000 Doisy rat units per mg. while pure Theelol tests 750 to 1000 rat units per mg.

A number of hypodermic tests of the International Standard after it became available, have shown it to contain from 2500 to 3000 Doisy rat units per mg. Other laboratories have assigned different experimental values to this standard of reference so that there is absolutely no uniformity between the International Unit and the Rat Unit as stated by various authorities. For instance Hinglais and Hinglais (17) using an oil solution of the International Standard, divided into three hypodermic doses find that one Doisy rat unit is equivalent to 25 or 30 International Units rather than the usual 3 or 4 which we have found experimentally.

#### ORAL ESTROGENIC TESTS (ADULT RATS).

The following series of oral tests were made in the early months of 1935 with Theelin of high potency (2500 and 3000 R. U. per mg.) derived solely from mare's urine of pregnancy which is known to contain no Theelol, and with Theelol of high purity:

TABLE I.

Dose.	No. of Rats.	Positive.	Typical.
6 R. U. (subcu.) in 3 oral doses	10	0	0%
8 " " " 3 " "	10	0	0%
10 " " " 3 " "	10	4	40%
15 " " " 3 " "	18	13	72%
15 " " " 3 " "	20	14	70%
15 " " " 3 " "	20	13	65%
20 " " " 3 " "	20	16	80%

At 15 R. U. (subcu.) 40 out of 58 were positive = 70%.

With Theelol,  $\text{R} \ 869366$ , which assayed 1000 R. U. per mg. hypodermically the following oral tests were made:

TABLE II.

Dose.	No. of Rats.	Positive.	Typical.
2.0 R. U. (subcu.) in 3 oral doses	10	5	50%
2.0 " " " 3 " "	20	12	60%
2.5 " " " 3 " "	17	9	53%
2.5 " " " 3 " "	20	10	50%
2.5 " " " 3 " "	20	11	55%
3.0 " " " 3 " "	10	9	90%
3.0 " " " 3 " "	20	16	80%

At 2.5 R. U. (subcu.) 30 out of 57 were positive = 52%.

At 3.0 R. U. (subcu.) 25 out of 30 were positive = 83%.

From these recent tests on highly purified Theelin and Theelol where the relation by hypodermic test is about 3 to 1 (3000 R. U. per mg. for Theelin and 1000 R. U. per mg. for Theelol) the nearest basis of comparison by oral dosage is 15 R. U. (subcutaneous) of Theelin is equivalent to 3 R. U. (subcutaneous) of Theelol, or that five times as much Theelin is necessary when given orally as would be necessary of Theelol to produce the same effect physiologically. Thus a prod-

uct containing only Theelol and standardized hypodermically to 200 International Units per capsule would be just as effective *orally* as one containing only Theelin which was standardized hypodermically to 1000 International Units per tablet.

#### ORAL ESTROGENIC TESTS (IMMATURE RATS).

The effect of the various estrogenic principles when administered orally to *immature* female rats (30 days old) has been studied experimentally in order to determine which is most rapid and effective in bringing about sexual maturity as evidenced by opening of the vagina (18).

TABLE III.

Product.	Dose.	Results.
	Hypodermic Units.	
	Given orally in 3 doses at 4-hour intervals.	
International Std. (Oil Solution)	30 R. U. (100 Int. U.)	2 out of 10 rats, vagina open on the 4th day. All open on the 5th day
Theelin B 878216 (Oil Solution)	15 R. U.	2 out of 10 rats, vagina open on the 5th day. All open on the 6th day
Benzoic acid ester of Dihydro Theelin (Oil Solution)	30 R. U.	5 out of 10 rats, vagina open on the 3rd day. All open on the 4th day
	15 R. U.	5 out of 10 rats, vagina open on the 3rd day. All open on the 6th day
Dihydro Theelin (Oil Solution)	.15 R. U.	3 out of 15 rats, vagina open on the 3rd day. All open on the 7th day
	15 R. U.	2 out of 10 rats, vagina open on the 4th day. All open on the 6th day
Theelol (Aqueous Solution)	5 R. U.	2 out of 10 rats, vagina open on the 5th day. All open on the 12th day
	15 R. U.	8 out of 15 rats, vagina open on the 4th day. All open on the 6th day

Since control rats in our colony reach sexual maturity normally at about 58 days of age on the average, *i. e.*, 28 days after the above tests were begun, it is seen that large doses of all these estrogenic principles (15 hypodermic rat units given orally in 3 doses) complete this process in 4 to 7 days or less than one-fourth of the normal time.

#### OIL SOLUTIONS VERSUS AQUEOUS SOLUTIONS.

The increasing popularity of oil solutions of Theelin for clinical use intramuscularly, due to the large single doses that can be given with gradual absorption and utilization, has influenced us to report experimental data, both oral and hypodermic, concerning the action of oil preparations upon test animals.

In testing Theelin in Oil by hypodermic administration we find that a single injection is just as effective as when the dose is divided into three parts and injected at four-hour intervals. However, it is impossible to demonstrate experimentally all the activity which is known to be present in an oil solution. That is, if a previously tested lot of crystalline Theelin is accurately dissolved in oil so as to contain 100 rat units per cc. only 75 per cent or 80 per cent of this amount can be found by a careful test of the oil solution due to the reduced rate of absorption of the Theelin from the oil. Thus when the International Standard is dissolved

in oil to contain 1000 international units per cc. and tested at that level, only 30 per cent of the rats showed typical estrus instead of the necessary 75 per cent to make a positive test. When tested at the 800-rat unit level, 70 per cent showed typical estrus which practically met the 75 per cent requirement.

When administered *orally* to spayed rats the several estrogenic principles were relatively less effective *in oil solution* than in aqueous solution. The following table shows this conclusively.

TABLE IV.—OIL SOLUTIONS. HYPODERMIC VERSUS ORAL ADMINISTRATION.

Product. (In Oil. Orally.)	Dose. Orally in Hypodermic Units.	Result.
International Std.	15 R. U.	Much less than 1 oral unit
"	30 R. U.	Less than 1 oral unit. 30% typical
"	60 R. U.	More than 1 oral unit. 90% "
"	50 R. U.	More than 1 oral unit. 90% "
"	40 R. U.	Equal to 1 oral unit. 80% "
Theelin	15 R. U.	Less than 1 oral unit. About 30% typical
No. 877432	50 R. U.	More than 1 oral unit. 90% typical
No. 878216	40 R. U.	Equal to 1 oral unit. 80% "
Benzoic acid ester of Dihydro	15 R. U.	Less than 1 oral unit. No effect.
Theelin	30 R. U.	Less than 1 oral unit. 10% typical
	33 R. U.	Less than 1 oral unit. 20% "
	60 R. U.	Less than 1 oral unit. 30% "
	50 R. U.	Less than 1 oral unit. 40% "
	75 R. U.	More than 1 oral unit. 90% "
Dihydro	25 R. U.	Less than 1 oral unit. About 20% typical
Theelin	50 R. U.	Less than 1 oral unit. About 20% "
	75 R. U.	More than 1 oral unit. About 90% "
Theelol	5 R. U.	Less than 1 oral unit. 40% typical
	10 R. U.	Equal to 1 oral unit. 80% "

From this data it is seen that 40 hypodermic rat units of Theelin given orally in oil solution are necessary to equal 1 oral unit as contrasted with 15 to 1 for aqueous solutions. With Theelol about 10 hypodermic rat units are necessary while with Dihydro Theelin and its benzoic acid ester about 60 to 75 rat units are necessary to equal 1 oral unit.

#### SUMMARY.

(1) The adoption of the International Standard and Unit as a standard of reference has not resulted in uniformity or agreement among various authorities as to the relation between the International Unit and the experimental rat unit.

(2) Using Theelin derived from mare's urine of pregnancy and using a pure Theelol from human urine, the relationship between the two by oral dosage was experimentally established at a ratio of 5 to 1; in other words, five hypodermic units of Theelin are required to produce an effect equivalent to that of one hypodermic unit of Theelol.

(3) All the estrogenic principles when given orally to immature rats in doses comparable to those necessary to produce estrus in adult rats by oral administration, will bring about sexual maturity in less than one-fourth the time required normally.

(4) On the basis of animal experiments Theelol appears to be the ideal estrogenic principle for oral use.

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## THE BACTERICIDAL AND BACTERIOSTATIC VALUE OF COLLOIDAL CADMIUM PROTEINATE.\*

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It has been known for many years that various colloidal silver compounds exert considerable bacteriostatic action and some bacteriocidal action. In more recent years a large number of other heavy metal compounds have been prepared in the colloidal state and found to be somewhat active in these respects. Notable among these were compounds of mercury and copper. Cooper and Nicholas, *J. Soc. Chem. Ind.*, 49, 386T (1930), called attention to the fact that cadmium acetate was germicidal in a dilution of 1:70,000. Since in the cases of silver and mercury, at least, the germicidal action exerted by soluble ionizable salts of the metals is partially retained by colloidal dispersions of otherwise insoluble salts of these metals, it was considered reasonable to expect high bacteriostatic activity and some bacteriocidal activity on the part of colloidal cadmium proteinate. In order to evaluate it for such activity we prepared a reversible colloidal cadmium proteinate, which contained 5.32% cadmium. This preparation was a very satisfactory stable colloidal solution, which after vacuum desiccation, was slowly but completely reversible. It had no bacteriocidal action and only very feeble bacteriostatic action.

## EXPERIMENTAL.

Eighty-five grams of gelatin were hydrolyzed by refluxing for one hour in 300 cc. of water containing 6.8 Gm. of 48% potassium hydroxide. Upon cooling, this

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