SCIENTIFIC SECTION

THE RELATION BETWEEN THE RAT AND THE MOUSE UNITS OF ESTROGENIC ACTIVITY.*[1]

BY L. W. ROWE AND A. E. SIMOND.

The activity of estrogenic preparations must be determined physiologically and by methods which require considerable experience in making the test. The adoption of an international estrogenic reference standard by the London Conference of 1932 and the decision to assign an activity of 10,000 International Units per mg. to this reference standard was undoubtedly a forward step. However, since the comparison between this standard and all such preparations that are manufactured for sale and subsequent therapeutic use must be carried out upon animals, it is not surprising that confusion still exists due to variable experimental ratios reported between rat units and International Units and between mouse units and International Units, whether hypodermic or oral.

Very little can be found in the literature which bears directly upon the relationship between an estrogenic rat unit and a mouse unit; in fact different workers report surprising variations in ratios between the rat unit and the International Unit as noted last year by Rowe and Simond.[2] In an article by Laquer[3] which bears directly upon this subject a mouse unit of the International Standard is found to be practically equal to an International Unit (actually 0.8 I. U.) while a rat unit is about equal to twenty (20) International Units. On this basis the rat unit is 20 times as large as the mouse unit and the ratio is 20 to 1. With the benzoic acid ester of ketohydroxyestrin the ratio was found to be 3.6 to 0.8 or 4.5 to 1 which is a very significant difference.

Since we do not agree with Laquer as to the ratio of the rat unit to the International Unit for theelin—our experimental work having repeatedly shown that 2 to 3 rat units are equivalent to 10 International Units or 1 R. U. is equivalent to 3.3 to 5 I. U., it seemed advisable to compare the mouse unit with the rat unit experimentally. This was done by testing a number of different estrogenic preparations on ovariectomized rats and mice by our regular technique, *i. e.*, 3 subcutaneous aqueous injections within 8 hours and positive estrus in 75 per cent of a group of 20 animals within 56 hours as determined by careful examination of vaginal smears. The following table will show the results as obtained experimentally.

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[1] From the Research Laboratories, Parke, Davis and Company, Detroit, Michigan.

[2] Rowe and Simond, JOUR. A. PH. A., 25, 201 (March 1936).

[3] Laquer, *Klin. Wochschr.*, 14, 339 (March 1935).

TABLE I.

Product.	Label Claim.	Rat Units.	Mouse Units.	Ratio.
Theelin in oil	10,000 I. U. per cc.	3000 R. U. per cc.	15,000 M. U. per cc.	1 R. U. = 5 M. U.
Theelin in oil	10,000 I. U. per cc.	3000 R. U. per cc.	18,000 M. U. per cc.	1 R. U. = 6 M. U.
Ketohydroxyestrin benzoate	250,000 I. U. per cc.	75,000 R. U. per cc.	15,000 M. U. per cc.	5 R. U. = 1 M. U.
Dihydroxyestrin benzoate	50,000 M. U. per cc.	50,000 R. U. per cc.	50,000 M. U. per cc.	1 R. U. = 1 M. U.
Dihydroxyestrin benzoate	50,000 M. U. per cc.	15,000 R. U. per cc.	10,000 to 15,000 M. U. per cc.	1 R. U. = About 1 M. U.

It can be seen from this data that in the case of theelin (ketohydroxyestrin) the rat unit is about 5 times as large as the mouse unit, *i. e.*, 1 R. U. = 5 M. U. This probably accounts for the fact that it became popular in foreign countries to label the activity of such preparations in mouse units, for larger numbers could be assigned than if the activity were stated in rat units.

With the benzoates of both ketohydroxyestrin and dihydroxyestrin the situation is entirely changed so that in the first case 5 R. U. is equal to 1 M. U. and in the second the two units are about equal. Fortunately this situation has been met by the adoption at the 1935 London Conference of an International Benzoate Unit which is again equal to 0.1 gamma (one ten thousandth of a milligram) of a standard benzoate preparation. It is therefore plainly evident that the activity of these two types of estrogenic preparations should be stated in terms of the proper and available international unit.

But this does not take care of the trihydroxyestrin (theelol) type of estrogenic substance which is utilized for oral dosage and for which no international standard or unit has as yet been adopted. It has been shown[2] that for the rat this substance is relatively more effective by *oral* administration than is theelin. In some tests on spayed adult mice 3 hypodermic rat units of theelol were found to be much more than 1.0 oral mouse unit as 95 per cent of the group showed typical estrus. A retest with 1.0 hypodermic rat unit of theelol on the mice orally showed that it was practically equal to 1.0 oral mouse unit as 70 per cent of the group responded positively. Thus mice are about three times as sensitive to theelol orally as are rats.

These results all point to the necessity of stating the potency of estrogenic substances in International Units only, rather than in rat units or mouse units, either hypodermic or oral. They also show by the selectivity of the different types of preparations when tested on both rats and mice, that three international estrogenic standards should be adopted. Two such standards have already been made official but the need for still another of the theelol type is evident.

It is scarcely necessary to state agreement with Laquer[3] that these various relationships as determined upon experimental animals may not be true for the human since it should be definitely understood that bioassay control of any

medicinal is concerned only with the maintenance of uniformly high potency in different lots of the same type of product.

CONCLUSIONS.

1. With the original ovarian follicular hormone ketohydroxyestrin (theelin), one hypodermic rat unit of activity is equivalent to five hypodermic mouse units.

2. With the benzoates of ketohydroxyestrin (theelin) and of dihydroxyestrin, the relationship of the rat and mouse units are found to be 5 to 1 and 1 to 1, respectively, rather than 1 to 5.

3. The desirability of stating the potency of the various estrogenic principles in the proper type of International Unit is pointed out.

EXTRACTION STUDIES ON IPECAC.*[1]

BY SAMUEL W. GOLDSTEIN.

A number of studies on drug extraction have appeared in the literature dealing with the various factors involved in the process of percolation. With regard to the extraction of ipecac, the following menstrua have been used in the studies reported: Alcoholic, hydroalcoholic and acidified hydroalcoholic. Remington (1) pointed out the value of acetic acid as a solvent and as a menstruum for the extraction of drugs, and proposed a new class of galenicals, "acetracts," prepared with acetic acid of various concentrations. In a second paper (2) he reported on the preparation of "acetract of ipecac" with 60% acetic acid as the menstruum, and also that when weaker strengths of acetic acid were used the preparation gelatinized on standing. Roberts (3) recommended that syrup of ipecac be made from a vinegar of ipecac, like syrup of squill.[2] He stated: "acetic acid is a good solvent of the emetic properties of ipecac root." Wayne (4) prepared an acetic syrup of ipecac by macerating the drug in dilute acetic acid for 7 days, expressing and filtering, and then proceeding in the manner prescribed for syrups. Procter (5) prepared a water- and syrup-miscible fluidextract of ipecac by extracting with alcohol, distilling off the alcohol, and pouring the syrupy residue remaining into water. Breddin (6) prepared a fluidextract of ipecac by the process of diacolation (a modified fractional percolation). Other investigators (7, 8, 9, 10) have studied the effects of aqueous, hydroalcoholic and acidified hydroalcoholic menstrua on the extraction of ipecac, using the processes of maceration and infusion. Gstirner (11) studied the extraction of ipecac with water and with acidified menstrua, using hydrochloric and citric acids. He presented the results of other workers in a series of tables. Steiger (12) found, by maceration, that stronger alcoholic menstrua extracted the alkaloids and total solids more readily from finely powdered ipecac than from the coarser powders, but more dilute alcohol gave better results with a coarser powder. Bull (13) found that in the extraction of cinchona and belladonna a moderately fine powder gave the best results. Husa and Huyck (14), working with belladonna, found that within

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[1] From the research laboratories of the School of Pharmacy of the University of Maryland.

[2] The Syrup of Squill official in 1858 was prepared by dissolving the sugar in the Vinegar of Squill, with the aid of gentle heat, and straining the solution while hot.