SCIENTIFIC SECTION

THE QUESTION OF ASSAYING ERGOTOCIN, THE NEW ERGOT PRINCIPLE.*[1,2]

BY EDWARD E. SWANSON, CHESTER C. HARGREAVES AND K. K. CHEN.

Ergotocin is the new ergot principle isolated by Kharasch and Legault (1) in collaboration with Adair and Davis (2). It melts with decomposition at 155° C., has the probable empirical formula of $C_{21}H_{27}O_3N_3$, and forms easily soluble salts such as the maleate. In a pharmacological paper by Davis, Adair, Chen and Swanson (3), evidence has been presented that the action of ergotocin is different from that of ergotamine or ergotoxine, and it should therefore be standardized by newly developed methods. The substance has a specific and prompt oxytocic effect upon the postpartum human uterus, and offers therapeutic possibilities in obstetrical practice. The present paper is concerned with the comparison of six different methods used in our laboratories for the examination of ergotocin—the polarimetric, the colorimetric, the U. S. P. cock's comb, the isolated rabbit's uterus, the postpartum dog's uterus, and the postpartum human uterus. In all instances, ergotocin maleate was employed. As shown in Table I, a total of 35 lots were assayed. Those numbered 1, 2 and 3 were repeatedly purified and set aside as laboratory standards; those numbered 5, 7, 14, 15, 16, 17, 18, 19, 21, 24, 25, 27, 31, 33, 34 and 35 were factory lots for marketing; and the remaining ones were made by modified processes and subjected to similar tests. The majority of the samples were assayed by three or four methods, only four lots being standardized by the six methods. The authors owe their indebtedness to Drs. Fred. L. Adair and M. Edward Davis, Department of Obstetrics and Gynecology, University of Chicago, for the clinical assay which was carried out by a method developed in their clinic (4), to Dr. E. C. Kleiderer for the polarimetric examination, and to Mr. A. N. Stevens for the colorimetric determination.

RESULTS.

1. Isolated Rabbit's Uterus Method.—Unlike ergotamine and ergotoxine, ergotocin stimulates the isolated rabbit's uterus in dilute concentrations. The response of the mature organ appears to be proportional to the dose, which fact may be utilized for the quantitative evaluation of the product, just as the isolated guinea pig's uterus has been used for the assay of posterior pituitary extracts. Figure 1 shows a typical example of the experiments. It may be interesting to mention here that all the lots showing 100 per cent potency by this method proved to be clinically satisfactory.

In a previous communication (3), it has already been emphasized that ergotocin does not antagonize or inhibit the action of epinephrine, so that the well-known

* Scientific Section, A. PH. A., Portland meeting, 1935.

[1] From the Lilly Research Laboratories, Indianapolis.

[2] Eli Lilly and Company introduced this new ergot principle under the name of *Ergotrate*.

Broom-Clark method (5) is useless in determining its potency. On the contrary, the effect of ergotocin is diminished after the previous application of ergotamine, such as on the isolated rabbit's intestines. The same can be demonstrated on the isolated rabbit's uterus after ergotoxine, as shown in Fig. 2. In fact, ergotocin can almost take the place of epinephrine in the performance of the Broom-Clark test.

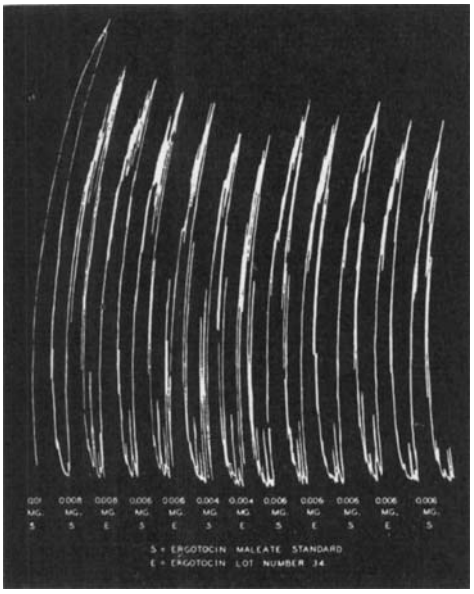

Fig. 1.—An example of ergotocin assay by the isolated rabbit's uterus method.

2. U. S. P. Cock's Comb Method.—Using ergotoxine ethanesulphonate as the standard, the first three lots of ergotocin proved to be 24 to 43 per cent more potent, but subsequent factory lots showed the same activity as the standard, with the exception of one (lot No. 34) which was 12 per cent in excess. In no instance was ergotocin maleate found to be weaker than ergotoxine ethanesulphonate, gram for gram.

3. Polarimetric Method.—In aqueous solution, ergotocin maleate is dextrorotatory and has a specific rotation $[\alpha]_D^{28}$ of about $+52°$. From Table I, it may be noted that lots having a specific rotation $[\alpha]_D^{28}$ less than $+50°$ were correspondingly low in physiological potency. The highest reading ever recorded was $+55.4°$. Only in one case (lot No. 6) did the optical rotation deviate from the biological assay.

4. Colorimetric Method.—The Smith colorimetric method (6) with *p*-dimethylamino-benzaldehyde can be similarly applied to ergotocin. The readings by this method, as shown in Table I, check within 10 per cent with the results obtained by

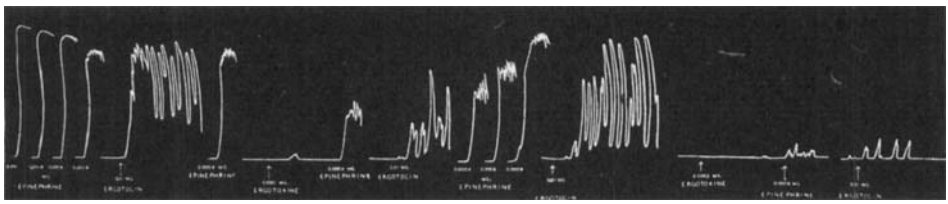

Fig. 2.—Absence of inhibitory action of ergotocin over epinephrine on isolated rabbit's uterus, and diminution of ergotocin action by previous use of ergotoxine.

the isolated rabbit's uterus method; only in one case (lot No. 14) the difference is greater, that is, 17 per cent.

5. Postpartum Dog's Uterus Method.—The technique of this method has been described by one of us (E. E. S.) (7). The results obtained designate the minimal

effective dose given either by vein or by a stomach tube. In the few tests carried out, the data are on the whole confirmatory of those by other methods (Table I).

TABLE I.—ASSAY OF ERGOTOCIN MALEATE BY VARIOUS METHODS.

Lot No.	Isolated Rabbit's Uterus: Lots 1, 2 and 3 = 100. Per Cent.	U. S. P. Cock's Comb: Ergotoxine = 100. Per Cent.	Specific Rotation. $[\alpha]_D^{25}$.	Colorimetric: Lots 1, 2 and 3 = 100. Per Cent.	Postpartum Dog's Uterus. By Vein. 0.01 Mg. per Kg.	Postpartum Dog's Uterus. By Stomach Tube. 0.2 Mg. per Kg.	Postpartum Human Uterus. 0.4 Mg. by Mouth.
1	100	135	+52.0	...	Active	Active	Typical response
2	100	125	+52.0	...	Active	Active	Typical response
3	100	143	+52.0	...	....	....	Typical response
4	0	...	0.0	...	....	....	.....
5	100	...	+51.9	...	....	....	.....
6	80	...	+54.5	89	....	....	.....
7	100	100	+51.4	90	Active	Active	Typical response
8	...	...	0.0	0	....	....	.....
9	5	...	0.0	0	....	....	No response
10	...	...	0.0	0	....	....	.....
11	6	...	0.0	0	....	....	No response
12	...	...	0.0	0	....	....	.....
13	30	...	+19.8	34	....	....	.....
14	100	100	+52.3	83	Active	Active	Typical response
15	100	100	+51.8	...	Active	....	Typical response
16	100	100	+50.9	...	....	....	Typical response
17	100	100	+51.0	...	....	....	Typical response
18	100	100	+52.8	100	....	....	Typical response
19	100	100	+52.8	...	....	....	Typical response
20	...	...	+17.9	...	....	....	.....
21	100	...	+51.5	...	....	....	Typical response
22	6	...	+12.9	...	....	....	.....
23	12	...	+11.5	10	Inactive	....	.....
24	100	...	+50.3	...	....	....	Typical response
25	100	...	+55.4	100	Active	Active	Typical response
26	25	20	+19.1	32	....	....	.....
27	100	100	+51.2	100	Active	....	Typical response
28	40	40	+11.3	...	....	....	.....
29	53	...	+30.5	55	....	....	.....
30	80	...	+43.3	83	....	....	.....
31	100	100	+50.0	...	Active	....	Typical response
32	75	...	+37.1	66	Inactive	....	.....
33	100	100	+52.9	...	....	....	Typical response
34	100	112	+52.0	100	Active	....	Typical response
35	100	..	+50.0	...	....	....	Typical response

6. Postpartum Human Uterus Method.—This constituted the ultimate and specific test for each lot of ergotocin. Although an amount of 0.4 mg. by mouth frequently produced more than the minimal response, this arbitrary dose usually gave rise to definite results in those individuals who were less suitable to the drug. A typical response meant a prompt tetanic contraction in 7 to 8 minutes, followed by rhythmic contractions with an increase in tone, lasting for more than 2 to 4 hours. According to Davis, Adair and their associates (2), about one-half of the multiparous patients can serve as testing subjects.

DISCUSSION.

In all respects, the isolated rabbit's uterus method yields numerical figures which are most helpful to the chemist. The oxytocic action typifies ergotocin and eliminates ergotamine and ergotoxine, and the prolonged rhythmic contractions distinguish it from histamine and tyramine, both of which cause brief responses. One can, therefore, regard the isolated rabbit's uterus method, the simplest laboratory test, with considerable degree of specificity. To insure fully the therapeutic value of the product, a clinical assay should be carried out. The postpartum dog's uterus method appears to be less sensitive than the clinical method. The remaining three tests are essential for confirmatory purposes, although they are of secondary importance on account of their lack of specificity. From the above data, it may be suggested that every lot of ergotocin should be first examined polarimetrically, assayed colorimetrically and physiologically by the isolated rabbit's uterus method, and finally, tested clinically. This will result in a uniform product for medical use.

The authors believe that the same methods, in addition to the analytical and chemical, will settle the question of identity or difference between ergotocin on the one hand and the other newer substances from ergot on the other, such as sensibamine of Chinoin Gyógyszer és Vegyeszeti Termékek Gyára, R. T. (8), ergoclavin of Küssner (9), ergometrine of Dudley and Moir (10), ergostetrine of Thompson (11), and ergobasine of Stoll and Burckhardt (12) and Jacobs and Craig (13). Comparisons should be made, of course, with the same salt and under the same conditions.

SUMMARY.

Thirty-five lots of ergotocin were compared by six methods of testing: the isolated rabbit's uterus, the U. S. P. cock's comb, the polarimetric, the colorimetric, the postpartum dog's uterus and the postpartum human uterus methods. With a few exceptions, the results by the different procedures appear to confirm one another.

Ergotocin may be assayed by the isolated rabbit's uterus method, supplemented by the clinical method. Polarimetric and colorimetric tests may furnish preliminary information concerning the purity of the product, but should always be confirmed by physiological and clinical experiments.

In the light of our present knowledge, the U. S. P. cock's comb method is not specific enough for the assay of ergotocin. The Broom-Clark method is entirely useless for the same purpose.

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DRUG EXTRACTION. V. THE EXTRACTION OF BELLADONNA ROOT WITH GLYCERINIC MENSTRUA.*[1]

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Tests were carried out to determine the efficiency of various glycerin-alcohol-water mixtures in the extraction of belladonna root.

EXPERIMENTAL PART.

100-Gm. portions of belladonna root in No. 40 powder were percolated by Type Process B with four different menstrua, *i. e.*, (A) alcohol 5 vol.—water 1 vol., (B) alcohol 425 vol.—water 100 vol.—glycerin 75 vol., (C) alcohol 325 vol.—water 200 vol.—glycerin 75 vol., and (D) alcohol 200 vol.—water 100 vol.—glycerin 100 vol. The preceding menstrua constituted Menstruum I, 100 cc. being used; a mixture of alcohol 5 vol.—water 1 vol. was used as Menstruum II. In each case the drug was moistened with 60 cc. of Menstruum I, and allowed to macerate for 6 hours before packing. The drug moistened with Menstruum A occupied 250 cc. after packing, while with Menstrua B, C and D the volume after packing was 265 cc. After packing, maceration was allowed to proceed for 24 hours. The percolates were then collected in three fractions, 80 cc., 100 cc. and 300 cc., respectively. The first 80 cc. was collected at the rate of 10 drops per minute, and the remainder of the percolate at 20 drops per minute.

TABLE I.—EFFECT OF VARIOUS GLYCERINIC MENSTRUA ON THE EXTRACTION OF POWDERED BELLADONNA ROOT.

	Grams of Alkaloid in Various Fractions of Percolate. Menstruum.			
Percolate.	A.	B.	C.	D.
80 cc.	0.429	0.420	0.372	0.337
100 cc.	0.052	0.066	0.088	0.092
300 cc.	0.000	0.000	0.018	0.042
Totals	0.481	0.486	0.478	0.471

DISCUSSION OF RESULTS.

The results in Table I show that glycerin retards the extraction of the alkaloids of belladonna root. The retardation increases with increasing concentration of glycerin and with decreasing concentration of alcohol. The results of the tests with glycerinic menstrua uphold the general opinion that glycerin does not aid in the extraction of alkaloidal drugs. Firlas (1) has stated that the presence of glycerin in the menstruum does not affect the amount of alkaloids in fluidextracts of cinchona, hydrastis and ergot. Scoville (2) showed that glycerin in the men-

* Scientific Section, A. PH. A., Portland meeting, 1935.

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

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