Scientific Section

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A NEW UTERUS-CONTRACTING METHOD OF TESTING ERGOT,—WITH COMPARISON WITH THE BLOOD-PRESSURE METHOD.

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It is well known that non-striated muscular tissue exhibits automatic (spontaneous) rhythmic contractions. It is also well known that certain drugs have the power of stimulating and thereby increasing these normal muscular movements. Various methods of standardizing ergot have been devised and employed by competent pharmacologists utilizing these facts. In most cases, however, the muscle was suspended in oxygenated Ringer's solution, contained in a Harvard muscle warmer with a capacity of about 40 cc. and in practically all cases the Harvard light muscle lever was employed for recording the contractions.

The non-concordant results obtained by other workers with isolated uteri have been ascribed to the interference of spontaneous contractions and the increasing irritability of the muscle tissue under the continued influence of the drug. These two factors form the principal objections to uterine methods. Pharmacologists have therefore endeavored to overcome these objections by selecting the uteri of animals manifesting the *least degree of normal movements*, —preferably those of the cat. To illustrate, Edmunds and Hale, in reporting their observations upon the non-pregnant uteri of cats, state in Bulletin 76, Hygienic Lab. U. S. Pub. Health and Marine Hospital Service: "It is true that the uteri of young cats which may be perfectly quiet in the earlier stages of an experiment after some time may begin to contract spontaneously and increase the difficulty of making comparisons of the effects from successive injections of the drug." In such cases, the authors state, "it may be necessary to employ as a standard the smallest amount which will clearly influence these movements, as, for example, by delay in the relaxation."

The uteri best adapted to our method, on the contrary, are those which manifest a *high* degree of normal spontaneous movements, preferably those of a non-pregnant guinea-pig, weighing between 275 and 325 gms. Instead of employing a Harvard light muscle lever we attach the free end of the uterus by means of a silk thread to one side of an escapement wheel, to the other side of which is suspended a counterpoise bucket for holding shot. By adding the proper amount of shot to this bucket the operator is enabled to weight the uterus down and thus reduce the amplitude of these movements so they can be controlled. Thus the marked spontaneous contractions can be reduced until the uterus is just able to contract under the increased load, or in other words, shot is added until the maximum amount of work that the uterus is normally capable of performing

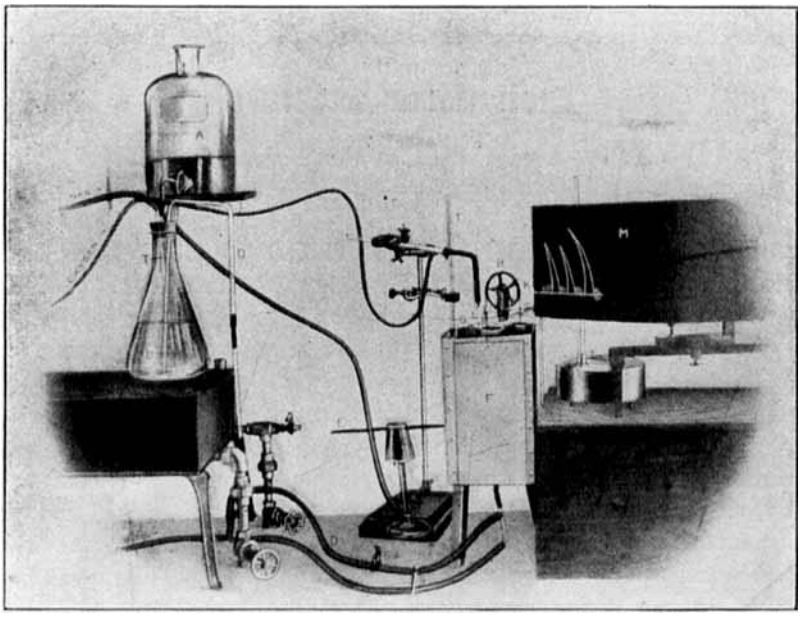

FIGURE 1.—Arrangement of the apparatus employed.

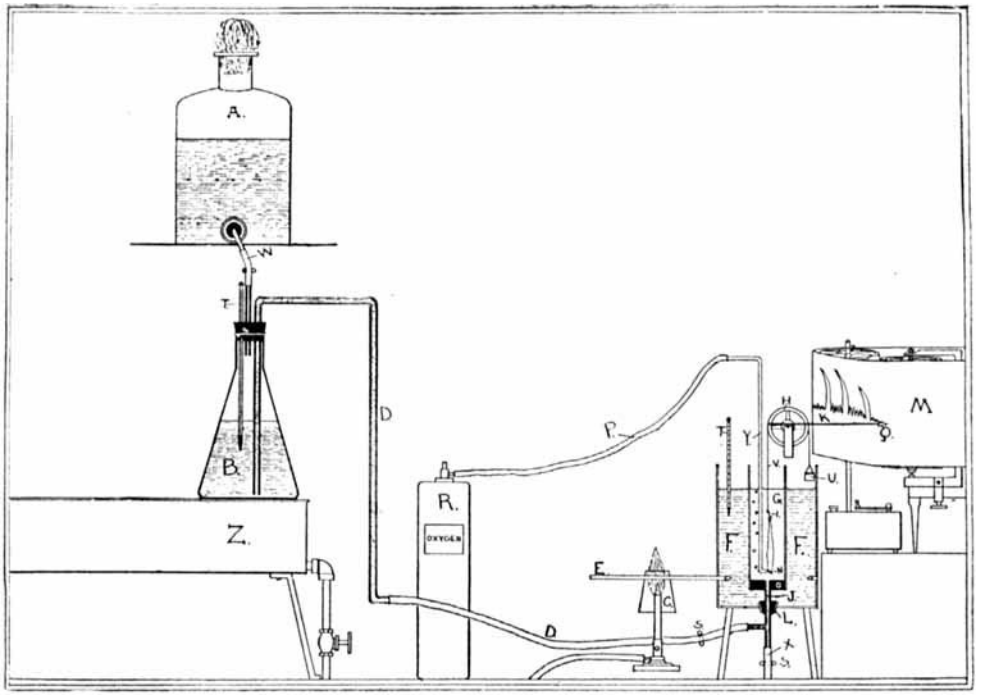

FIGURE 2 is a graphic drawing of the apparatus and will serve to illustrate the following detailed outline of the method which we employ.

is counterbalanced. Any increase in the amplitude of the contraction after the addition of a given drug can now be produced only by that drug.

As is to be expected, the addition of weights necessitates the employment of larger doses of the drug in order to produce marked contractions but, on the other hand, the resultant contractions are more nearly parallel to the doses given. (See Figure 4).

The uterus is suspended in about 250 cc. of Ringer's solution contained in a cylindrical glass vessel (G), the lower end of which is plugged with a rubber stopper (O) having a central bore. Through the latter passes one arm of a wide glass "T" tube (J) which ends flush with the upper surface of the stopper, so that the cylindrical vessel may be completely emptied. This tube passes through a second rubber stopper (L) which fills an opening in the bottom of an outer metallic vessel (F) which forms a constant temperature water jacket.

The temperature of the water in the jacket is kept constant by means of a metallic rod (E) which penetrates the wall of the jacket, passes through the water, and is soldered to the opposite side of the jacket. The portion of the rod external to the jacket is heated by a protected Bunsen burner (C) which slides on the rod. The temperature is regulated by sliding this burner backward and forward until that point is reached where the amount of heat transmitted by the rod to the water inside is sufficient to keep the thermometer (T) suspended in the water at the proper degree (38° to 39° C.)

One of the other arms of the "T" tube is connected by a rubber junction (X) armed with spring clamps (S) to a waste pipe by which the cylindrical glass vessel may be emptied.

The remaining arm is connected by a syphon tube (D) to a flask (B) which holds a small amount of Ringer's solution for refilling the cylindrical vessel. This flask is kept at a temperature between 40° and 45° C. by means of a steam bath (Z). The main supply of Ringer's solution is contained in a large aspirator bottle (A) connected with the small flask by a rubber tube (W), the object being to avoid exposing the reserved solution to prolonged heat. Heat causes Ringer's solution to gradually decompose and lose CO_2.

The Ringer's solution in the small flask should be reduced to 39° C. immediately before admitting it to the cylindrical vessel by allowing sufficient cold solution to run into it from the aspirator bottle.

Into the cylindrical vessel containing the Ringer's solution dips a narrow glass tube (Y). This tube is turned at right angles about half an inch from its lower end. Into this is sealed a platinum pin (N) for attaching the *lower end of the isolated uterus.* The upper end of this tube is connected by means of rubber tubing (P) to an oxygen reservoir (R). A constant stream of oxygen is allowed to bubble through a small vent at the lower end of the tube, thus preserving the muscular irritability of the uterus and at the same time stirring the Ringer's solution.

The other end of the uterus is fastened to a small platinum hook (I) connected to a silk thread (V) which passes over an escapement wheel (H) and is attached to a pin on the opposite side of the wheel. A counterpoise bucket for holding shot (U) is attached to the opposite side of the wheel. To this wheel is soldered an stylet of aluminum (K); the axle of the wheel serving as

a fulcrum. To the end of this stylet a pen point is fixed (Q) for recording the contractions of the uterus on the revolving drum of the kymograph.

Method of Procedure.—The animal is bled by quickly severing the carotid artery with a sharp-pointed scissors. The spinal column is then severed with a strong scissors. One horn of the uterus is then quickly excised together with the ovary which is left attached by means of the fold of broad ligament in which the Fallopian tube runs. This horn is then quickly transferred to the oxygenated Ringer's solution in the cylindrical vessel and attached to the two platinum pins above referred to (the ovary is fastened to the hook suspended from the escapement wheel and the lower end of the horn is fastened to the pin at the lower end of the oxygen tube). The manipulation and exposure followed by the immersion in the warm solution will almost invariably produce a high degree of tonus, which, however, gradually diminishes until the uterus returns to its normal condition. If at this point the uterus does not exhibit *strong* rhythmic contractions it should be discarded and replaced by a new one. The weights are now added by dropping shot into the bucket *until the uterus can make only small rhythmic contractions.*

Conditions are now suitable for determining the activity of the drug to be tested or standardized. The samples to be tested are first freed from alcohol by evaporation on a water bath and then made up to their original volume with water.

A small dose (0.3-0.5 cc.) of the standard preparation is now pipetted into the Ringer's solution in which the uterus is suspended. If all conditions are ideal the uterus which was recording small rhythmic contractions will now forcibly contract and record its contractions by a long sweeping curve. After the curve reaches its maximum and commences to decline (which may require from 5 to 15 minutes) the medicated Ringer's solution is quickly run off and replaced by fresh solution, previously adjusted to the proper temperature. The momentary exposure to the air while changing the solution generally causes the uterus to contract rather forcibly, thus markedly increasing the amplitude of the curve produced by the action of the drug. It is necessary, therefore, in changing solutions to hold the escapement wheel for a few seconds or until the uterus is again covered with the saline solution. This will prevent the record from being interrupted by contractions not produced by the drug. The curve now quickly returns to normal and the uterus containues to record its small rythmic contractions.

Should the uterus chance to be a *very sensitive* one, a dose of 0.5 cc. of the standard preparation may produce a contraction so strong that it will carry the writing pen off the smoked chart. In such cases it is necessary to reduce the dose. If, however, the uterus still continues to give such marked contractions, shot should be added until the contraction can be controlled. On the other hand, should a dose of 0.5 cc. not produce contraction, the dose should be increased to 1 or 1½ cc. If, however, doses of 1.5 cc. do not call out contractions, shot should be removed until a marked contraction is produced by these doses.

After thus adjusting the apparatus two successive doses of equal amounts of the standard solution should be given. If the resultant contractions are equal the uterus is giving concordant results and is ready for assay purposes. In

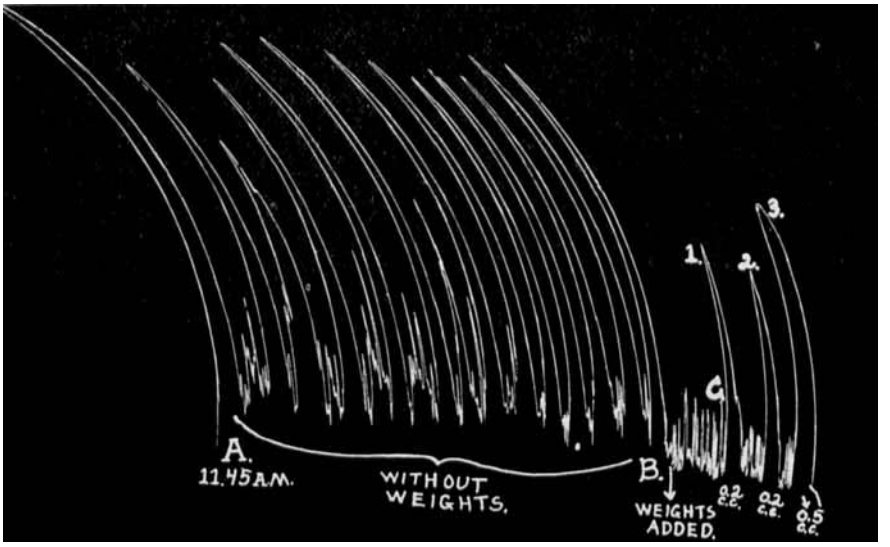

FIGURE 3.—Demonstrates first, the normally acting uterus (A to B); second, the action when weighted down by shot, (B to C); and third, the action of ergot on the uterus when loaded and working against resistance (1, 2 and 3).

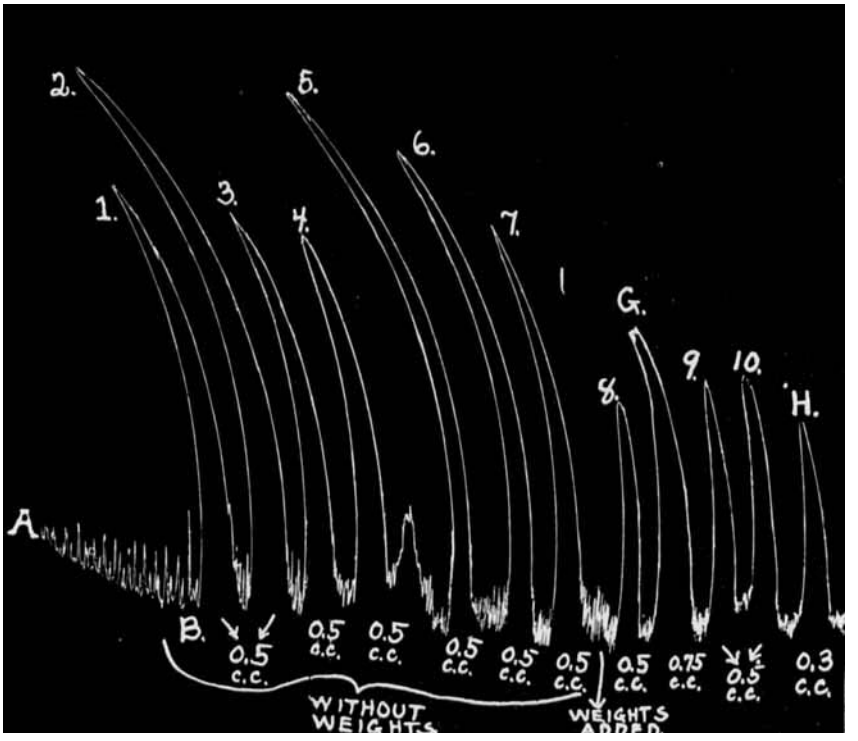

FIGURE 4.—Demonstrates first, the normally acting uterus (A to B); second the *non-concordant* results produced by repeated doses of the same amount of fluid extract ergot (1, 2, 3, 4, 5, 6 and 7); third, the *concordant results obtained after the uterine contractions are controlled by weights* (8, 9 and 10). The curves G and H indicate the quantitative results obtained by a larger and a smaller dose.

order to determine the relative activity of an unknown preparation it is now merely necessary to give progressively increasing or decreasing doses of the unknown preparation until that amount is found which will produce contractions of an equal amplitude as those produced by the standard preparation.

Description of Charts.—The terms "no weights" and "weights added" used in this paper refer to the shot used in inhibiting the normal contraction of the uterus, not to the counterpoise employed to keep the uterus suspended in the Ringer's solution.

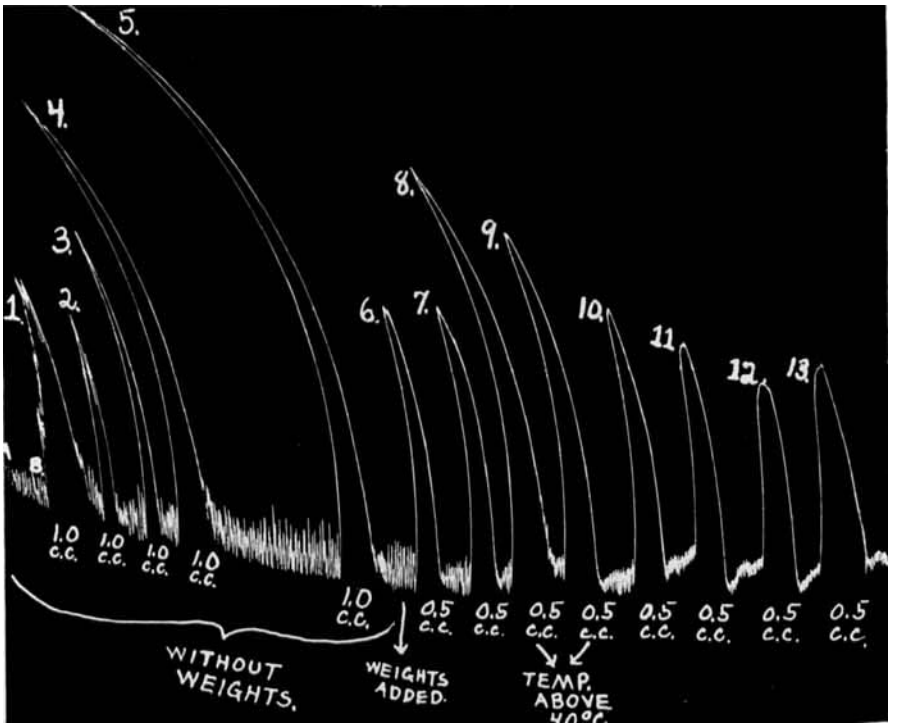

FIGURE 5.—This illustration is similar to Figure No. 2. A to B show normal contractions; 1, 2, 3, 4, and 5, show non-concordant results obtained without weights; 6, 7, 10, 11, 12, and 13, show concordant results obtained after weights were added. The curves 8 and 9 indicate contractions produced under increased stimulation due to a rise in the temperature of the Ringer's solution, and show the necessity for maintaining an even temperature during the experiment.

During our experiment we were greatly impressed by the differences in the uteri as to power and muscular structure, and their mutual relation. Some specimens were greatly deficient in muscular substance and acted feebly. Other specimens showed greater muscular development and contracted strongly. Some of the specimens proved absolutely inert and would not respond at all. After more extended experience, however, we found that the normal activity practically runs parallel to the amount of muscular tissue present; the "stringy" uteri are all deficient in normal activity and in response to stimuli, while the thick, more muscular uteri are practically all active and sensitive. This knowledge enables us to save considerable amounts of time as it renders it pos-

sible for us to distinguish between active and inactive uteri before connecting them with the apparatus.

Having at one time a series of unsatisfactory results which extended over a period of one week, we were at a loss as to the cause of this sudden change in the efficiency of the test. After careful investigation, however, we found that our stock of Ringer's solution was unsterile and showed bacterial growths. New solution was made and placed in sterile containers, after which our results were again concordant. This emphasizes the necessity for keeping both the solution and syphon tubes in a sterile condition.

Due to the marked difference in the sensitiveness of the various uteri, it is

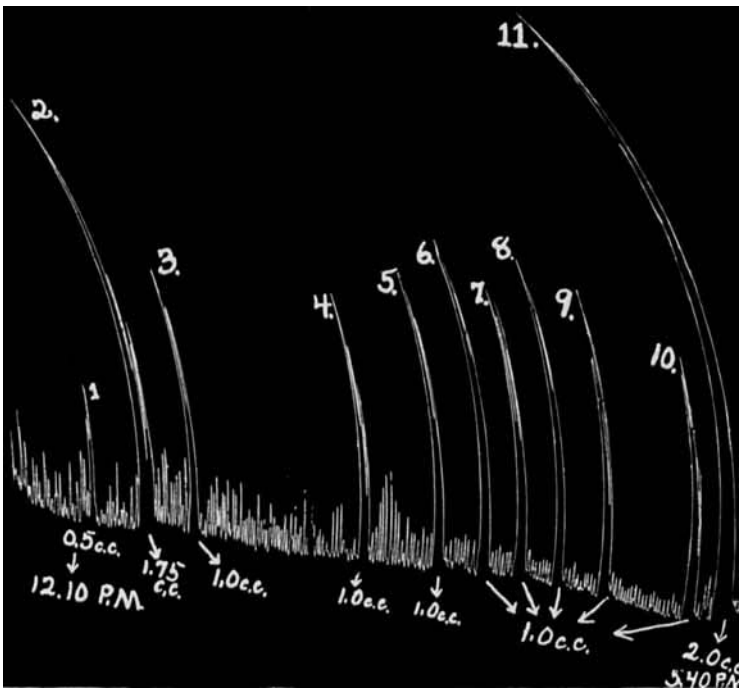

FIGURE 6.—This chart clearly demonstrates the concordant results from repeated doses of an equal amount of fluidextract ergot, 3, 4, 5, 6, 7, 8, 9, and 10; 1, 2, and 11, indicate the quantitative results obtained by varying the doses and demonstrate the accuracy of the test.

necessary to employ a standard preparation with which to compare the unknown preparations. These comparisons must, of course, always be made on the same uterus.

As a standard we employ a fluidextract or ergot of such strength that when injected intravenously into a series of three or more dogs, it produces an average rise in blood pressure of 30 mm. of mercury. After preparing a standard preparation of the above strength it is placed into 4 cc. vacuum ampoules to prevent deterioration. These can then be opened and used as required.

The marked sensitiveness of this method, together with the concordant results obtained by the same, led us to believe that by its use we could perhaps ascertain whether or not the uterine and circulatory actions of ergot run

parallel to one another. This is an important factor, since the blood pressure method is rather extensively used for standardization purposes, its employment being supported by statements that the characteristic effect of ergot is a stimulation of all unstriped muscle tissue of the body, and that the changes in the circulation, in the intestines and in the uterus are but a part of one general action. The employment of this method has been further supported by the fact that all of the substances which have been suggested by various writers as the active principles of ergot have produced stimulation of the blood-vessels as well as of the uterus. So far as we are able to learn, however, no experimental results have ever been published to show whether or not these two actions run parallel.

We are, therefore, now testing every sample of ergot submitted for assay by both the uterine and blood pressure method. By this means we will be able after a sufficient number of samples have been tested to compare the results obtained and thus determine whether or not a parallelism does exist between these two actions. If on completion of our experiments the results show that all the samples which assayed high by the blood pressure method also assayed high by the uterine method we will know that these actions do run parallel. If, on the other hand, some samples run high by one method and low by the other, we will know that such a parallelism does not exist.

Up to the present time we have made comparisons with seven samples with the following results:

Preparation.	Comparative strength by B. P. method.	Comparative strength by uterine method.
No. 1 F. E.	166%	170%
No. 2 Cornutol	100%	90%
No. 3 F. E.	153%	148%
No. 4 F. E. 5 mo. old	51%	100%
No. 5 F. E.	224%	230%
No. 6 F. E.	210%	198%
No. 7 F. E.	118%	105%

The above table would indicate that a parallelism does exist between the action of ergot upon the circulatory system and the action upon the uterus. We will, however, be unable to arrive at a definite conclusion until more data has been compiled.

We will, therefore, continue our investigations along these lines for another year.

RESEARCH LABORATORY OF H. K. MULFORD COMPANY, July 28, 1913.

THE PROPOSED LIST OF USEFUL REMEDIES.*

M. I. WILBERT, WASHINGTON, D. C.

For more than a decade men who are genuinely interested in the development of pharmacy as a recognized and necessary branch of medicine, have viewed with alarm the ever-growing accumulation of disparaging evidence, on the part of national and state food, dairy and drug officials, and have urged upon the fellow-

*Read before the Scientific Section at the Nashville Meeting.