Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

A. G. DuMez, Editor, Baltimore, Maryland

VOLUME XXIX *SEPTEMBER, 1940* NUMBER 9
CONSECUTIVE No. 17

The Assay of Ergot*

By Ralph G. Smith†

The purpose of this paper is to call attention to recent work, to point out certain general problems and to make certain suggestions in connection with the assay of galenical preparations of ergot.

Since the discovery in 1935 of ergonovine it has been advocated that the official U. S. P. method for ergot assay is inadequate. This method measures the total active alkaloids of ergot by the Cock's Comb method. The uterine-stimulating action of ergonovine is definitely stronger than that of the ergotoxine group, but the available evidence indicates that a difference of comparable degree is not reflected by the Cock's Comb reaction. The same objection holds for other methods such as the paradimethylaminobenzaldehyde colorimetric method and its modifications which depend on the lysergic acid radical common to both groups of alkaloids. Furthermore, the epinephrine-inhibiting action of ergonovine is so weak that the Broom-Clark method fails entirely to measure the content of this substance in ergot. If it could be assumed that the proportion of ergonovine to the other active alkaloids of ergot is constant in various samples, such criticism would not be valid. However, the evidence based on the isolation of pure alkaloids and on separate assays of water-soluble and water-insoluble fractions does not justify such an assumption. There are statements in the literature to the effect that some ergots contain no ergonovine (1, 2), while the results of certain workers (3, 4) indicate that as much as one-third of the total alkaloids of some ergots are water-soluble. Consequently, in order to assay the therapeutic activity of galenical preparations, definite modifications of the standard methods must be made, or new ones devised.

A logical procedure would seem to be the separation of ergonovine from the ergotoxine group prior to assay. Encouraging efforts in this regard have already been made by certain investigators, although further experience in various laboratories is necessary before the reliability of the suggested methods can be evaluated. Hampshire and Page (3), after removal of water-soluble alkaloids from the ether extract of ergot by dilute ammonia water, assayed the remaining insoluble alkaloids and also the original ex-

* Presented at the thirteenth meeting of the United States Pharmacopœial Convention, Washington, D. C., May 13–15, 1940.

† Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor.

tract containing all alkaloids, the ergonovine or soluble fraction being determined by difference. This method has also been used by others (5). Schou and Tønnesen (6) have effected separation of ergotoxine and ergonovine by ether extraction from aqueous or alcoholic aqueous solutions, the former alkaloid being extracted at a p_H of 4 and the latter at definitely alkaline reactions, the two groups of alkaloids then being assayed separately. Allport and Porter (4) also assayed the water-soluble fraction directly after its precipitation under definitely prescribed conditions by antimony trichloride. Trabucci (7) has accomplished separation by precipitation of ergotoxine with picric acid, leaving the ergonovine in solution, a procedure which has also been applied by Christensen and Reese (8). Two of the above groups of workers (3*b*, 4) have reported little success with the picric acid method, apparently due to incomplete precipitation of ergotoxine, although the actual results recorded by Hampshire and Page (3*b*) appear promising. The assay of the fractions has been carried out in all of this work by the *p*-dimethylaminobenzaldehyde colorimetric method. This method, which is official for the British Pharmacopœia, of course measures total rather than only biologically active alkaloids. It would be theoretically valid if inactive isomers were not present at all, or were present in always the same proportion with the active group, a premise for which there is no evidence. However, it was apparently adopted on the assumption that its superiority to biological methods with regard to experimental error and possibly simpler procedure outweighed its theoretical deficiencies.

It is possible that a biological method assaying only active alkaloids might be similarly applied after separation of water-soluble and water-insoluble principles, and in fact Oettel and Bachmann (9) have reported a procedure for the assay of ergonovine in ergot preparations after precipitation of ergotamine. It concerns the uterine response to intramuscular injections of the drug as recorded from a balloon inserted into the puerperal uterus of a cat or rabbit. Such a preparation, in their hands, has proved extremely sensitive to ergonovine and relatively insensitive to ergotamine so that traces of the latter still present have a negligible effect.

Undoubtedly certain difficulties will be encountered in attempting to apply biological methods to fractions of ergot. It is advisable to keep the following points in mind if possibilities of serious error are to be avoided. Attempts to assay the ergonovine content by difference between the results obtained for total activity and activity after its removal offer little hope of success. By way of illustration it is only necessary to recall that the highest accuracy ever claimed for the Cock's Comb or other biological methods for ergot involves an error of ten per cent. If the estimation of ergonovine depends upon two such assays, the *total* content of this substance in a sample of ergot may well fall within the limits of experimental error. Furthermore, the limited accuracy of methods commonly used in the past is suggested by the results of several workers who have obtained comparatively good agreement between the Cock's Comb method, which assays both ergonovine and alkaloids of the ergotoxine group, and the Broom-Clark method which assays only the latter group. In certain series of comparisons the Broom-Clark method, which theoretically should give lower results, showed in the majority of samples a higher value than did the Cock's Comb method. Consequently it would seem advisable to devise procedures which would assay the ergonovine fraction directly rather than by difference.

Another point which may be made concerns assays involving the uterine action of ergonovine. The possibility exists that alkaloids of the ergotoxine group, if present in appreciable amounts, even though having little effect themselves in producing uterine contractions, may inhibit the uterine stimulating action of ergonovine as suggested by the work of Thompson (10) and Swanson, Hargreaves and Chen (11) on the isolated rabbit uterus.

A further type of research which may well prove to be of value is that which played an important part in the isolation of ergonovine. In certain clinics the technique

has been developed for evaluation of oxytoxic drugs by the intrauterine balloon method on puerperal patients. Since the aim of ergot assay is the evaluation of the therapeutic effect of the drug, it should be helpful in establishing the validity of laboratory procedures to collaborate with clinical investigators whenever possible. Parallel assays of ergot or fractions of ergot on the human uterus and by laboratory methods should also offer additional information concerning the possible effects of one alkaloid or group of alkaloids on the action of another.

Finally, it is suggested that a step might be taken which would solve the problem of assay of galenical preparations, at least as far as the Pharmacopœia is concerned. The useful alkaloids are now available in pure form. They satisfy therapeutic requirements and the problem of assay and stability is much simpler for them than for the fluidextract of ergot. Ergonovine has undoubtedly replaced the galenical preparations to a considerable degree. A survey might supply useful information regarding the extent of such replacement. Is there any reason for retaining ergot and the fluidextract of ergot in the Pharmacopœia aside from the fact that a considerable proportion of the medical profession continue to use them because they are accustomed to do so? Probably this is sufficient reason for their retention, but it is believed that such a suggestion should at least be considered by the Revision Committee.

REFERENCES

(1) Stoll, Arthur, *Wien. klin. Wochschr.*, 49 (1936), 1513, 1552.

(2) Kharasch, M. S., and Legault, R. R., *Science*, 81 (1935), 388.

(3) Hampshire, C. H., and Page, G. R., *Quart. J. Pharm. Pharmacol.*, (*a*) 9 (1936), 60; (*b*) 11 (1938), 57.

(4) Allport, Noel L., and Porter, G. V., *Ibid.*, 11 (1938), 96.

(5) Glycart, C. K., *J. Assoc. Official Agr. Chem.*, 20 (1937), 566. *Rept. Am. Pharm. Assoc. Lab. in Bull. Natl. Formulary Comm.*, 8 (1939), 40.

(6) Schou, Aage, and Tønnesen, Mogens, *Dansk Tids. Farm.*, 12 (1938), 272, 279; *Chem. Abstr.*, 33 (1939), 1444.

(7) Trabucci, Emilio, *Boll. soc. ital. biol. sper.*, 12 (1937), 232.

(8) Christensen, B. V., and Reese, J. A., *Jour. A. Ph. A.*, 28 (1939), 343.

(9) Oettel, H., and Bachmann, H., *Arch. exp. Path. Pharmakol.*, 185 (1937), 242.

(10) Thompson, Marvin R., *Jour. A. Ph. A.*, 24 (1935), 748; 26 (1937), 805.

(11) Swanson, Edward E., Hargreaves, Chester C., and Chen, K. K., *Ibid.*, 24 (1935), 835.

Use of Sodium Pentobarbital for Repeated Anesthesia in the White Rat

*By V. Everett Kinsey**

In the present study the effects of giving sodium pentobarbital (Nembutal)[1] repeatedly to white rats will be compared with those observed previously (1, 2) for the rabbit and guinea pig.

The M. L. D. for rats for intraperitoneal injections of Nembutal has been reported as being 75 mg. per kilogram by Fitch and Tatum (3) and 120 mg. per kilogram by Carmichael (4) and Swanson and Shonle (5). Further disagreement is found in the reports concerning M. L. D. for male and female animals. None of these workers mentions a difference in response of the sexes. Barron (6) states that while the female is more susceptible to amytal than the male, there is no difference between the sexes so far as Nembutal is concerned. Holck and Kanan (7), on the other hand, state that the female rat is more sensitive than the male, as judged both by sleeping time and the M. L. D.; their assertion is confirmed by Moir (8) who found that the adult female rat is definitely less resistant than the corresponding male when the sleeping time was used as a criterion of sensitivity.

It would appear from the work of Carmichael (4), who showed that the M. L. D. in rats under nine months old was only 85–95 mg. per kilogram, as compared with 120 mg. per kilogram for rats older than this, that the comparison of M. L. D. sex differences, etc., must be made with animals of approximately the same age.

* Department of Physiological Chemistry, School of Medicine, University of Pittsburgh.

[1] Kindly supplied by the Abbott Laboratories, North Chicago, Ill.