SOME PROPERTIES OF ERGOSTETRINE.*

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Since reporting the isolation of a new crystalline specific alkaloid of ergot (1), it becomes possible to describe some pharmacological and chemical properties of this "X-alkaloid" in pure crystalline form, earlier publications (2) having dealt with the amorphous substance still containing some ergotoxine as impurity. The history of the discovery of this new "X-alkaloid," named "ergostetrine" by the author, with the relationship of ergostetrine to the subsequently reported "ergometrine" of Dudley and Moir (3) and the "ergotocin" of Kharasch and Legault (4), is reviewed elsewhere (5).

ISOLATION AND CHEMICAL PROPERTIES (6, 7).

A 10-Kg. quantity of good quality ergot was exhaustively extracted with alcohol and the alcohol was removed at a temperature not exceeding 40° C. under reduced pressure. The resulting gummy mass was thoroughly washed with many small portions of acidified water to remove the impure ergostetrine. The aqueous washings were concentrated under reduced pressure, alkalinized to litmus with ammonia, and exhaustively shaken out with many small portions of chloroform. Removal of the organic solvent *in vacuo* resulted in crystallization of ergostetrine. The crystals were washed with chloroform and dried *in vacuo* over calcium chloride. The yield was 983 mg. These crystals melted with decomposition at 148° to 150° C., and exhibited a specific rotation[2] (0.1% solution in chloroform containing 5% alcohol to increase solubility) of $[\alpha]_D^{25} = -45°$ to 55°. One recrystallization from hot benzol raised the decomposition point to 152° to 154° C. Repeated recrystallization (5 times) from benzol resulted in white radiating, doubly refractive needles up to $1^1/_4$ inches in length, which, when placed in a bath at 154° C. and slowly heated, melted with decomposition at 160° to 162.5° C., and whose specific rotation (0.2% solution in ethyl alcohol) was $[\alpha]_D^{30} = +50°$ ($\pm 10°$ because of weak solution used). Aqueous solutions were alkaline in reaction and also dextro-rotatory, as were solutions in methyl alcohol. Chloroform and benzol solutions were lævo-rotatory. Solutions of either the base or the salts exhibit a high degree of fluorescence. The base is sparingly soluble in water, chloroform and benzol, and is considerably more soluble in ether, ethyl and methyl alcohols. The salts are uniformly highly soluble in water, but much less so in common organic solvents. More precise determinations must await the availability of larger quantities of ergostetrine. Its presence in ergot to the extent of 0.05 to 0.2 mg. per Gm. introduces great difficulties in securing working quantities from small-scale laboratory production.

As to the molecular composition of ergostetrine, analyses mentioned in an earlier report (1, 2) showed the substance to consist of C, H, O and N, and that the ratio of these elements to one another was roughly similar to that of hitherto identified alkaloids (ergotoxine, ergotamine, sensibamine, etc.) but that the size of the molecule was definitely smaller than that of the known alkaloids. Due caution must be observed in assigning an empirical formula to the new alkaloid because,

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[2] I am indebted to Prof. G. L. Jenkins for coöperation in determinations of optical activity.

though simpler in composition than the other alkaloids of ergot, it is still relatively complex. The crystals form in combination with solvent of crystallization, and the extreme difficulty with which the solvent of crystallization can be safely expelled attaches a definite element of doubt to any analytical results.

Dudley and Moir (3, 8, 9) presented analyses on their ergometrine, Kharasch and Legault (10) on their ergotocin, Stoll and Burckhardt (11) on their ergobasine, and Jacobs and Craig (12) on their alkaloid, all confirming my analyses showing the qualitative composition of the molecule and that the molecular weight is definitely less than that of either ergotoxine or ergotamine. It is the belief of the writer that ergostetrine, ergometrine, ergotocin and ergobasine are one and the same substance. All were independently obtained by chemical methods which, though differing in details, proceed in the same chemical direction, ultimately obtaining the crystals of the free base from chloroform, benzol, the chlor-ethylenes, etc., taking advantage of the lesser solubility of the new alkaloid as compared to the other alkaloids in these solvents. The pharmacologic and clinical activity, optical activity, decomposition point, solubility and color reactions, all tend to establish that the four names have been applied to the same alkaloid, in spite of the variously reported minor differences which are very probably due to differences in the degree of purity of the crystals studied. The empirical formula indicated by more recent analyses of ergostetrine (base) is in excellent agreement with that of Stoll and Burckhardt for ergobasine ($C_{19}H_{23}O_2N_3$), which is the same as that found by Jacobs and Craig for their product. The formula suggested with reservation by Kharasch and Legault for ergotocin ($C_{21}H_{27}O_3N_3$), as pointed out by Jacobs and Craig (12), differs from the above formula by C_2H_4O (or C_2H_5OH, alcohol?), suggesting that the higher molecular weight may be due to analyses of material still containing some solvent of crystallization. Owing to the difficulties of completely freeing from solvent of crystallization, this lies well within the realm of possibility. The somewhat larger molecule suggested by the analyses of ergometrine by Dudley and Moir probably resulted from the same difficulty, or to some remaining impurity.

COLOR REACTIONS OF ERGOSTETRINE.

As established in a previous report by the writer (1), ergostetrine gives the usual color reactions of ergot alkaloids. The well-known Van Urk color reaction as modified by the quantitative method of Smith appears to be the more sensitive in comparing the various alkaloids of ergot. Consequently the quantitative data presented were obtained by using this reaction. The reagent consisted of 0.125% *p*-dimethyl-amino-benzaldehyde and 0.1% ferric chloride in 50% (by volume) sulphuric acid. Two cc. of a 1–10,000 solution of ergostetrine (base), mixed with 4.0 cc. of the reagent, produced a blue color approximately $2^1/_2$ times as intense as U. S. P. Standard Ergotoxine ethanesulphonate under identical treatment. When 2.0 cc. of ergostetrine (base) 1–20,000 was compared with 2.0 cc. of the standard ergotoxine ethanesulphonate 1–10,000, the color intensity as determined in the colorimeter was essentially identical. Thus, ergostetrine base produced a color reaction twice as intense as U. S. P. standard ergotoxine ethanesulphonate. Calculating both in terms of free base, the color produced by ergostetrine is approximately 1.68 times as intense as that produced by the ergotoxine represented in the U. S. P. standard salt of ergotoxine. The difference in solvent of crystallization would, of course, account

for some deviation from the above figures, the ergostetrine being crystallized from benzol as above described.

These color reactions, therefore, offer no possibility for assaying ergostetrine in ergot preparations without resorting to prior chemical separation of the alkaloids, except as explained in the earlier report (2), in which it was pointed out that simple extracts exhibiting satisfactory color values would necessarily contain all of the ergostetrine present in the parent drug plus variable amounts of the other alkaloids, because of the greater stability and solubility of ergostetrine in the usual menstrua (water or water with alcohol) in the presence of the other alkaloids.

ACTION OF ERGOSTETRINE ON THE COCKSCOMB.

As pointed out in an earlier report (1, 2), ergostetrine is intensely active in producing cyanosis of the combs of cockerels. Adhering to the U. S. P. procedure, perceptible cyanosis was produced in the combs of some cocks in doses as low as 0.05 mg. per Kg. Others were more resistant, but in approximately 50 trials, a dose of 0.1 mg. per Kg. always produced a definite cyanosis. Doses of 0.1 mg. per Kg. and upward also produced the characteristic general depression or narcotic symptoms of ergot in the cockerels. Using birds standardized with U. S. P. standard ergotoxine ethanesulphonate, the pure crystalline ergostetrine base was judged to be approximately 160% (±20%) as potent as the standard ergotoxine salt. In general, it was also noted that the cyanosis developed somewhat more quickly in the case of ergostetrine, and that the cyanosis was characterized by a deeper blue-black color in the combs than that produced by ergotoxine or ergotamine, in which blanching is often but not always observed. It is believed that the more prompt action of ergostetrine may result in the actual trapping of more blood in the combs, thus accounting for the more intense darkening, whereas the action of the other alkaloids being slower, forces much more of the blood out into the general circulation before finally trapping the remainder for subsequent exhausting of oxygen to produce cyanosis. In any case, the difference between ergostetrine and the other alkaloids is by no means well-defined, the observation being purely a general impression formed by experience with many tests.

Thus, the official Cockscomb method is not specific in measuring the more important ergostetrine activity in ergot preparations also containing the other alkaloids. The limitations of the method are the same as those mentioned for the colorimetric method.

ACTION ON THE ISOLATED RABBIT UTERUS.

The action of pure crystalline ergostetrine on the isolated rabbit uterus shows that the amorphous substance previously reported upon (2) still contained some ergotoxine. Studies with pure ergostetrine reveal that, in marked contrast to ergotoxine, ergotamine, sensibamine and ergoclavine, the rabbit uterus is stimulated into strong contractions by ergostetrine. A study of the illustrations in Figs. 1, 2 and 3 shows the stimulative response of the uterine strips to epinephrine and ergostetrine, that ergostetrine inhibits the epinephrine response to only a relatively slight degree, as compared with ergotoxine or ergotamine, and that ergotoxine inhibits or abolishes the stimulative activity of both epinephrine and ergostetrine. This indicates that ergostetrine mainly stimulates sympathetic endings in the rabbit

uterus, gradually giving way to only a slight depression, whereas ergotoxine, ergotamine, sensibamine and ergoclavine are indistinguishable in being mainly depressant to the same sympathetic endings.

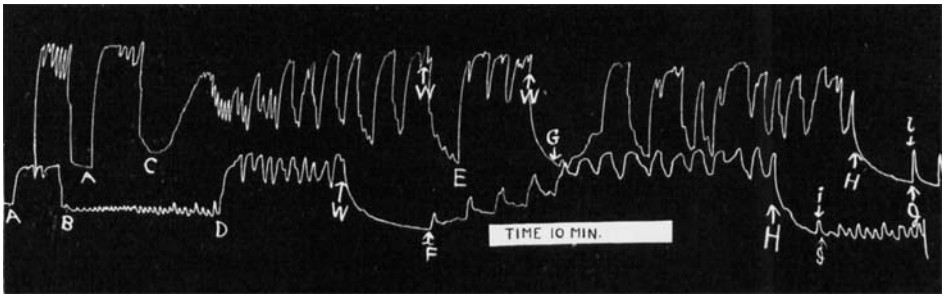

Fig. 1.—Isolated strips of rabbit uterus in 50-cc. tissue chambers. (Used third day after removal from rabbit.)

A—contraction from 0.05 mg. epinephrine.
B—slight stimulation from 0 2 mg. ergostetrine.
C—strong contractions from 0.4 mg. ergostetrine.
D—contraction from 0.05 mg. epinephrine showing no inhibition by the 0.2 mg. dose of ergostetrine.
E—contraction from 0.05 mg. epinephrine showing no inhibition by the 0.4 mg. dose of ergostetrine.
F & G—strong contraction from 0.4 mg. ergostetrine.
H & H—abolition of ergostetrine contractions by 0.05 mg. of ergotoxine.
I—failure of 0.4 mg. ergostetrine to cause typical contractions after the ergotoxine.
J—failure of 0.05 mg. epinephrine to cause typical contractions after the ergotoxine.
W—replacement of drugged Lock-Ringer solution with new solution.

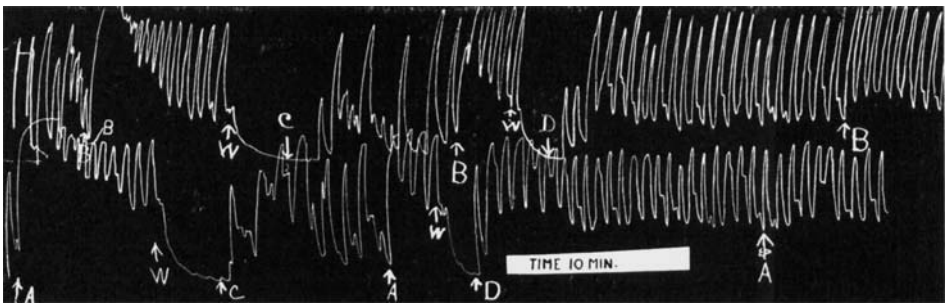

Fig. 2.—Isolated strips of rabbit uterus as in Fig. 1. (Used third day after removal from rabbit.

A—contraction from 0.05 mg. epinephrine (lower tracing).
B—contraction from 0.05 mg. epinephrine (upper tracing).
C—contractions from 0.4 mg. ergostetrine.
D—contractions from 1.0 mg. ergostetrine. Note the definite inhibition of the final epinephrine response produced by these large doses of ergostetrine. Ergotoxine in less than one-tenth this dose of ergostetrine causes as great or greater inhibition of the epinephrine response.
W—wash.

Thus the well-known Broom-Clark method can measure the ergotoxine type of activity in ergot preparations, but the presence of even small amounts of ergotoxine in such preparations eliminates the possibility of the use of the isolated rabbit uterus to estimate ergostetrine unless complete chemical separation is first resorted to.

Considerable variation in sensitivity to ergostetrine was observed in different uteri, as was to be expected. A concentration of 1:3,000,000 caused contractions with an increase in tone with the most sensitive uterine strips, whereas 1:500,000 was necessary to produce definite stimulation in others. Uteri used immediately after removal from the rabbit were the most sensitive. The longer the uterus is kept in the refrigerator before use, the less sensitive become strips from that uterus. After the fourth day in the refrigerator, it has become customary in this laboratory to discard any remainder of the uterus, because ergostetrine in a concentration of 1:50,000 or higher is then often found to be necessary to evoke satisfactory response.

The isolated rabbit uterus provides an excellent method of assaying different

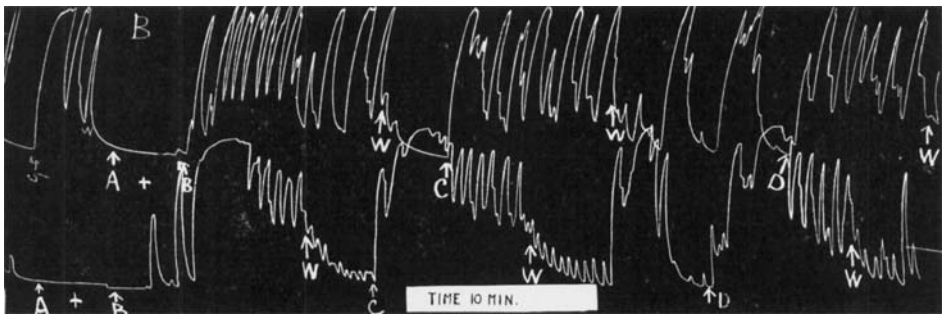

Fig. 3.—Similar strips of rabbit uterus as in Fig. 1 and 2. (Used third day after removal from rabbit.)

A—absence of response to 0.1 mg. ergostetrine.

B—strong contractions when additional 0.4 mg. ergostetrine added.

C—strong contractions from 0.5 mg. ergostetrine.

D—weaker stimulation from 0.4 mg. ergostetrine.

W—wash.

lots of ergostetrine in terms of a lot preserved *in vacuo* as the standard, since repeated doses produce satisfactorily constant responses in proportion to size of dosage.

ACTION ON ISOLATED GUINEA PIG UTERI.

Immature virgin guinea pig uteri (as used in Pituitary assays) are much more sensitive to ergostetrine than rabbit uteri (as used in the Broom-Clark procedure). Strong contractions are produced in all types of guinea pig uteri, and this tissue is, therefore, serviceable in assaying different lots of ergostetrine in terms of a lot preserved as a standard. The presence of histamine, tyramine, etc., and the other alkaloids in ergot preparations makes the method worthless for assay purposes, unless such crude preparations are subjected to chemical procedures for separating the various constituents.

ACTION ON CAROTID BLOOD PRESSURE OF ANESTHETIZED CATS AND DOGS.

Given orally, the blood pressure is not raised by ergostetrine. Intravenously, the substance usually produces a pressor effect, as described earlier (2), but relatively

enormous doses of crystalline ergostetrine failed to produce the well-known "vasomotor reversal" or reversal of the effect of epinephrine. The production of the "reversal" by the amorphous alkaloid reported earlier (2) shows that the amorphous product still contained appreciable amounts of ergotoxine. Perhaps acutely toxic doses of pure ergostetrine might inhibit the pressor effect of epinephrine to some extent (as observed with ergostetrine and epinephrine on the rabbit uterus) but doses of 2.0 mg. per Kg. to dogs or cats failed to demonstrate the "epinephrine-reversal" or indeed a definite inhibition of epinephrine response. The pressor effects were feeble when compared with ergotoxine or ergotamine, and a depressor effect was occasionally observed. Chloretone was used as the anesthetic.

ACTION ON PREGNANT CATS.

This was described in the earlier report (2), the chief characteristic being that ergostetrine acts much more promptly and powerfully than ergotoxine, ergotamine, etc. The crystalline product was effective in smaller doses than the amorphous product. This method has little quantitative value.

ACTION ON PUERPERAL HUMANS.

This was described elsewhere by Koff (13) for the amorphous product. Studies on the clinical effect of crystalline ergostetrine are described elsewhere by Tuck (14) and others (3, 15). The clinical results now available have caused the obstetricians involved to refer to the new alkaloid as "the true active principle" of ergot. It appears to account for the greater part of the traditional oxytocic activity of whole ergot, being far more effective than any constituent of ergot hitherto investigated. The ergotocin of Kharasch and Legault and the ergometrine of Dudley and Moir are apparently indistinguishable from ergostetrine in clinical effectiveness (3, 13, 14, 15, etc.), supporting the view that all three are identical.

The sensibamine used in these studies was obtained in the form of "Ergone," distributed by Parke, Davis & Co. The ergoclavine was kindly supplied by Dr. Joseph Rosin, Merck & Co.

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