The Elimination of Ergotoxine, Ergotamine and Ergonovine*

By Jerome C. Kopet and James M. Dille†

Goodman and Gilman (1) state that "Little is known of the fate and excretion of the ergot alkaloids." This summarizes the general impression which is found from a survey of the literature on ergot. Ordinarily a clue to the fate of the drug can be had by following the rate of elimination of the drug by chemical methods, but the difficulty in this case lies in the fact that such a small quantity of drug is administered as the original dose, that only extremely small quantities of the substance are present in the blood and tissues. In such cases the pharmacological procedure of essential elimination can often be carried out, but in preliminary experiments on frogs, rats, rabbits and white leghorn cocks this was found to be unreliable because of the wide variations in the reactions of the animals to a given dose and because of the occurrence of delayed gangrene often appearing a week after the administration of a single dose. These obstacles caused us to reëxamine the possibility of using chemical methods and it was found that by the adoption of special procedures and the use of guinea pigs which have a relatively high fatal dose it was possible to secure data on the rate of disappearance of the three active alkaloids of ergot from the blood and muscle after the administration of sublethal doses.

EXPERIMENTAL

THE ANALYSIS OF ERGOTOXINE, ERGOTAMINE AND ERGONOVINE IN BLOOD AND TISSUES

Principle of the Method.—This is a colorimetric method based on the production of a blue color with *p*-dimethylaminobenzaldehyde as described by Van Urk (2). This reaction was used for the assay of ergot by Smith (3) and adopted as an official method for this purpose in the 1934 British Pharmacopœia.

This procedure as modified for the purposes of tissue analysis involves the extraction of the alkaloid from blood or tissues with ether, separation of the alkaloid from the ether by shaking out with tartaric acid solution, treatment of this solution with the *p*-dimethylaminobenzaldehyde reagent, and the estimation of the intensity of the blue color in a photoelectric colorimeter. The concentration of the alkaloid is then determined from a previously prepared standard-concentration curve.

Procedure for the Treatment and Extraction of Tissue.—Blood: The blood, citrated or coagulated, is carefully measured and placed in a large porcelain evaporating dish. Two cubic centimeters of concentrated ammonia solution (28%) is added for each 10 cc. of blood. After the mixture has been allowed to stand for five minutes, anhydrous sodium sulfate is added in small portions with constant stirring in the proportion of 20 Gm. of the salt to each 10 cc. of the blood. Fifteen to twenty minutes are allowed for the mixture to dry completely. The dry cake is then broken up by means of a glass pestle and mixed with about 2 Gm. of shredded asbestos for each 30 Gm. of the dry powder. The material is then carefully removed from the dish and transferred to a Soxhlet thimble of the proper size. Extraction is then carried out with ether for five hours. At the end of this time, the ether is removed and saved. The marc is removed from the thimble, again broken up, realkalinized with the original quantity of ammonia, returned to the Soxhlet apparatus, and extracted for five hours with a new portion of ether. The contents of the flask are removed and saved. The procedure is repeated for the third time. The three ether extracts are combined, concentrated on a water bath to about 35 cc., transferred to a separatory funnel and shaken out with a 1% aqueous tartaric acid solution, 2 cc. being added each time for five washings. The combined tartaric acid portions are heated at 40° C. to remove the dissolved ether and made up to a volume of 10 cc. This solution is then ready to be treated as described for the colorimetric determinations.

Other Tissues: The procedure differs from that for blood in that no sodium sulfate is necessary. The tissue is completely ground in a meat grinder, weighed, alkalinized with ammonia and mixed with asbestos. The same proportions are used as in the case of the dried blood and the material is transferred directly to the Soxhlet thimble. Three five-hour extractions are then made with ether as described above. The combined ether extracts are concentrated as described for blood and shaken with several portions of a very dilute ammonia solution which are withdrawn and discarded. This removes extracted fats. The ether is then shaken out five times with 2-cc. portions of a 1% aqueous solution of tartaric acid. The combined tartaric acid portions are heated at 40° C. to remove the dissolved ether and made up to a volume of 10 cc. This solution is then ready to be tested colorimetrically by the following method.

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Colorimetric Determination of Ergot Alkaloids in Extracts of Blood and Tissues.—The 10-cc. volume of tartaric acid extract obtained above is treated by carefully adding 20 cc. of *p*-dimethylaminobenzaldehyde reagent solution to the tartaric acid extract while the temperature of the mixture is kept at 10° C. The reagent is a 0.125% solution of *p*-dimethylaminobenzaldehyde in a 65% sulfuric acid solution containing, in addition, 0.1 cc. of a 5% solution of ferric chloride (British Pharmacopœia, 1934) for each 100 cc. of the reagent. The tartaric acid extract and reagent are thoroughly mixed and allowed to stand in the light for one hour to insure a maximum color and to permit the solution to come to room temperature. At the end of this time, 25 cc. of the blue-colored solution is transferred to the cell of the colorimeter and a reading of the photoelectric current reduction is made. This reading is then referred to the previous prepared graph to give the amount of alkaloid present. From this value the amount of alkaloid recovered is calculated in terms of the alkaloidal salt and can be most conveniently expressed in terms of milligrams per cent.

Evaluation of the Colorimetric Assay.—Specificity: The blue color is specific for the indol group with the 2-position unsubstituted. In lysergic acid this position is free and this gives the characteristic color reaction of the ergot alkaloids (4). It does not distinguish between the individual alkaloids of ergot but this is unimportant for the purposes of this work since only one known alkaloid is administered, but if this colorimetric method is used for toxicological purposes it would not indicate specific ergot alkaloids.

Sensitivity: The sensitivity of the color reaction was fixed by Van Urk at $^1/_{400}$ mg. of ergotamine or ergotoxine. In the preparation of the colorimeter reading-concentration curve the lowest detectable color was found with a concentration of 0.0005 mg./cc. of standard solution of each of the salts of the three alkaloids. This reading was made on a total volume of 25 cc. of standard solution and reagent. Therefore, since 10 cc. of solution is required, the smallest quantity which can be detected by this test is 0.005 mg.

Accuracy: Small quantities of each of the three ergot alkaloids were added to freshly secured blood, muscle, liver and kidney. The tissue was then analyzed by the above procedure. Results are shown in Table I. It will be seen that there is considerable loss in certain cases. About 50% of the alkaloid was recovered from muscle tissue and about 83% from blood. Liver and kidney tissue gave very poor recoveries and were therefore not used in the experiments. While these recoveries are not perfect they can be regarded as satisfactory in view of the small original doses and in view of the checks between recoveries from each kind of tissue. However, in view of these recoveries in control experiments, care was taken to make any interpretations of results on a relative rather than an absolute basis.

Table I.—The Recovery of Ergotoxine Ethanesulfonate, Ergotamine Tartrate, Ergonovine Tartrate, Added to 10 Cc. of Blood or 50 Gm. of Tissue

Alkaloid	Tissue	Amount Added, Mg.	Amount Recovered, Mg.	Per Cent Recovery
Ergotoxin ethanesulfonate	Muscle	0.300	0.157	53
	Muscle	0.300	0.140	49
	Muscle	0.150	0.078	52
	Muscle	0.100	0.055	55
	Muscle	0.100	0.050	50
	Blood	0.100	0.080	80
	Blood	0.085	0.070	82
	Blood	0.070	0.057	82
	Liver	0.165	0.055	33
	Liver	0.165	0.048	29
	Liver	0.165	0.062	37
	Liver	0.165	0.050	30
	Kidney	0.165	0.040	24
	Kidney	0.165	0.040	24
Ergotamine tartrate	Muscle	0.700	0.330	47
	Muscle	0.700	0.355	51
	Muscle	0.350	0.160	46
	Blood	0.070	0.060	86
Ergonovine tartrate	Muscle	0.700	0.370	53
	Muscle	0.700	0.325	46
	Muscle	0.350	0.175	50
	Blood	0.113	0.097	86
	Blood	0.070	0.057	82
	Blood	0.070	0.060	86
	Blood	0.056	0.450	82

THE RATE OF DISAPPEARANCE OF ERGOTOXINE, ERGOTAMINE AND ERGONOVINE FROM BLOOD AND MUSCLE

Adult male guinea pigs were used in all these experiments. The solutions of the drug were administered intracardially in all cases. No serious difficulty was encountered but if there was any question of faulty technique the animal was not used. The solutions were prepared for injection by patiently triturating the carefully weighed alkaloidal salt in an agate mortar with a small amount of 1% aqueous tartaric acid solution until solution was effected. This was then transferred to a volumetric flask and made up to volume. Solutions of each alkaloid were approximately 0.3% of the alkaloidal salt. The following doses were used: 16 mg. of ergotoxine ethanesulfonate per Kg., 25 mg. of ergotamine tartrate per Kg., and 25 mg. of ergonovine tartrate per Kg.

At the end of the proper time interval after the injection, the animal was rendered unconscious by a blow on the head and killed by exsanguination. Ten to fifteen cubic centimeters of blood was collected in a citrated vessel and used for the anlysis. About 50 Gm. of muscle tissue was obtained from the thigh and saddle. The liver was excised and analyzed for some experiments but the results were not sufficiently reliable to include. The results are summarized in the tables and figures. Each point represents one animal and there is consequent variation due to individual differences, but the curves indicate the general concentration of these three alkaloids in the blood and muscle at various times after administration.

Table II.—Concentration of Ergotoxine, Calculated as Ergotoxine Ethanesulfonate, in Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 16 Mg./Kg. of Ergotoxine Ethanesulfonate

Animal Number	Weight, Kg.	Total Dose, Mg.	Elapsed Time between Administration and Sacrifice of Animal, Min.	Recovery Muscle Mg./100 Gm.	Recovery Blood Mg./100 Cc.
48	0.43	6.88	15	0.14	5.40
53	0.49	7.84	30	0.18	3.80
62	0.64	10.24	60	0.37	0.90
55	0.50	8.0	120	0.24	1.0
65	0.53	8.48	180	0.12	0.23
87	0.46	7.36	240	0.10	0.18
66	0.49	7.84	300	0.09	0.25
70	0.57	9.12	420	0.07	None

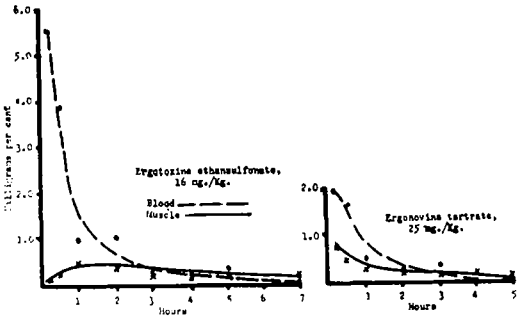

Fig. 1.—The Concentration of Ergotoxine (as Ethanesulfonate) or Ergonovine (as Tartrate) in the Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 16 Mg. of Ergotoxine Ethanesulfonate per Kg. and 25 Mg. of Ergonovine Tartrate per Kg.

Table III.—Concentration of Ergotamine, Calculated as Ergotamine Tartrate, in Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 25 Mg./Kg. of Ergotamine Tartrate

Animal Number	Weight, Kg.	Total Dose, Mg.	Elapsed Time between Administration and Sacrifice of Animal, Min.	Recovery Muscle Mg./100 Gm.	Recovery Blood Mg./100 Cc.
28	0.56	14.00	15	0.16	5.0
22	0.52	13.00	30	0.18	4.0
23	0.62	15.63	60	0.20	3.30
25	0.66	16.50	90	0.21	1.09
44	0.49	12.25	120	0.35	0.63
27	0.59	14.70	180	0.15	0.19
61	0.46	11.50	240	0.285	0.44
56	0.44	11.00	300	0.25	0.38
71	0.46	11.50	420	0.17	None
72	0.53	13.25	720	0.05	None

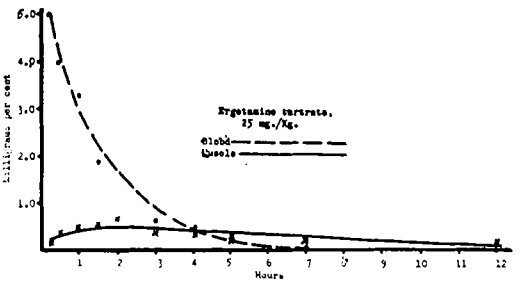

Fig. 2.—The Concentration of Ergotamine (as Tartrate) in the Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 25 Mg. of Ergotamine Tartrate per Kg.

Table IV.—Concentration of Ergonovine, Calculated as Ergonovine Tartrate, in Blood and Muscle of Guinea Pigs after the Intracardiac Administration of 25 Mg./Kg. of Ergonovine Tartrate

Animal Number	Weight, Kg.	Total Dose, Mg.	Elapsed Time between Administration and Sacrifice of Animal, Min.	Recovery Muscle Mg./100 Gm.	Recovery Blood Mg/100 Cc.
59	0.58	14.50	15	0.74	1.91
60	0.53	13.25	30	0.40	1.75
57	0.46	11.50	60	0.12	0.27
36	0.51	13.55	60	....	0.27
38	0.45	11.25	90	0.10	....
88	0.54	13.50	120	0.08	0.17
49	0.48	11.90	180	0.07	0.25
40	0.47	11.75	240	0.06	None
58	0.44	11.00	300	None	None

ROLE OF THE LIVER AND KIDNEYS IN THE ELIMINATION OF ERGOT ALKALOIDS

The animal organism may eliminate a drug by either detoxication or excretion. In the former case the liver is presumed to play a leading role, while the kidneys are the principal organs of excretion. In an attempt to indicate the part these organs play in the elimination of the ergot alkaloids two kinds of experiments were carried out.

Bilateral nephrectomy was performed on three guinea pigs under light ether anesthesia. Twenty-four hours later 16 mg. of ergotoxine ethanesulfonate per Kg. was administered intracardially to one animal and 25 mg. of ergotamine tartrate per Kg. to the other two. After a suitable interval, indicated from the previous experiments, the animals were killed and the blood and muscle analyzed for the alkaloid. Table V shows the results. Comparison of these results to those in Tables II and III shows no significant difference in the blood and muscle concentrations of the alkaloid. Hence it is apparent that the diminution of the alkaloids in the blood and

Table V.—Concentration of Ergot Alkaloids in the Blood and Muscle after Bilateral Nephrectomy or the Production of Liver Damage

Animal Number	Weight, Kg.	Nature of Damage	Drug	Dose, Mg./Kg.	Minutes after Administration	Concentration Muscle Mg./100 Gm.	Concentration Blood Mg./100 Cc.
164	0.57	Bilateral nephrectomy	Ergotoxine	16	180	0.10	0.26
182	0.52	Liver damage with chloroform	Ergotoxine	16	180	0.20	0.14
183	0.38	Liver damage with chloroform	Ergotoxine	16	180	0.22	0.25
184	0.42	Liver damage with phosphorus	Ergotoxine	16	180	0.15	0.39
191	0.47	Liver damage with phosphorus	Ergotoxine	16	180	0.49	0.35
193	0.43	Liver damage with phosphorus	Ergotoxine	16	180	0.25	0.46
167	0.59	Bilateral nephrectomy	Ergonovine	25	120	0.075	0.20
168	0.45	Bilateral nephrectomy	Ergonovine	25	120	0.065	0.23
181	0.72	Liver damage with chloroform	Ergonovine	25	120	0.174	0.15
189	0.37	Liver damage with phosphorus	Ergonovine	25	120	1.23	0.38

muscle is not dependent upon the intactness of the kidneys.

Hepatectomy is difficult to perform and is accompanied by undesirable effects from the surgery but fairly complete damage to the liver may be produced by prolonged administration of chloroform or by the administration of phosphorus. Three animals were treated by two hours of chloroform administration and four were administered two doses of 7 mg. of phosphorus in 0.35% solution in olive oil per Kg. subcutaneously twenty-four hours apart. Twenty-four hours after the administration of the hepatatoxic agent, 16 mg. of ergotoxine ethanesulfonate per Kg. was administered to five animals and 25 mg. of ergonovine tartrate per Kg. was administered to two animals. The animals were killed at the end of a definite time and the blood and muscle analyzed for the alkaloid. Results in Table IV indicate that, while there is considerable variation in these values which can be attributed to the difference in the extent of damage produced, in animals with damaged livers the rate of diminution of the ergot alkaloids is slowed and that, therefore, the liver plays a part in the elimination of these alkaloids.

DISCUSSION

The concentration of each of the three active alkaloids in the blood decreases rapidly after administration. This is probably due to the passage of the alkaloid into the tissues because there is a rise of concentration in the muscle during this period. This decrease in blood concentration becomes much less rapid by the end of two hours, and the last period is characterized by a slower rate of decrease. The concentration of the alkaloid in muscle is also decreasing until after seven hours for ergotoxine, twelve hours for ergotamine and five hours for ergonovine the alkaloid has practically disappeared. These times cannot be accepted as absolute, but it is obvious that the alkaloids must disappear from the body with considerable rapidity. This rate of elimination of these alkaloids is not in agreement with Burn (5) who found the blood pressure response to adrenalin the same twenty-four and forty-two hours after oral administration of powdered ergot. However, it agrees with Ifuku (6) who found by the same method that the action of 1 mg. of ergotoxine intravenously lasted eleven hours.

In view of this relatively rapid elimination of these alkaloids it becomes difficult to accept the belief that they are the direct cause of the gangrene which may occur after a single dose but which does not appear for several days or a week. The means by which the ergot alkaloids produce gangrene is not clear. McGrath (7) in verifying the effect of ergotamine in rats, described by Rothlin (8), states that there are symptoms of acute intoxication for two hours, after which the animals appear normal. At the end of the third to the fifth day the tail becomes pale and by the sixth to ninth day is cyanotic with sloughing of the distal portion and healing of the stump by the sixteenth to the twentieth day. Pathological changes are most marked in the small arteries and arterioles. There is cellular proliferation and swelling of the intima with final occlusion of the lumen. The important point, however, is that these extensive pathological changes do not occur until, as we have shown, long after the ergot alkaloid has been eliminated. This leads us to postulate that these alkaloids initiate the changes which result in gangrene and that these changes once started run their course without the necessity of the presence of the alkaloids.

The ergot alkaloids appear to be detoxicated in the body rather than excreted since bilateral nephrectomy results in no significant difference in the concentration at the end of equivalent periods. The liver seems to play a part in the detoxication

since the production of liver damage results in a higher concentration of ergotoxine and ergonovine at the end of selected intervals.

The method described for the determination of ergotoxine, ergotamine and ergonovine in blood and other tissues should be useful for toxicological purposes. The ordinary toxicological texts such as McNalley (9), Peterson, Haines and Webster (10), and Autenrieth and Warren (11) give no method for this purpose if the amount of alkaloid is small. Rosenbloom and Schildecker (12) report that they were able to isolate a crystalline material which gave color reactions and melting point of ergotinine from stomach, intestine, kidney and liver analyzed together. The method described here is sufficiently sensitive but is not specific for a particular ergot alkaloid. The method of drying the blood proved to be so effective that we suggest it for other alkaloids.

SUMMARY

The color reaction of ergot alkaloids with *p*-dimethylaminobenzaldehyde can be used to make quantitative estimation of ergotoxine, ergotamine and ergonovine in blood and muscle.

The three pharmacologically active ergot alkaloids, ergotoxine, ergotamine and ergonovine, disappear quite rapidly from the blood and muscle of guinea pigs after the administration of these drugs. Thus the late appearance of gangrene cannot be explained by the long-continued presence of these drugs in the body.

The alkaloids appear to be detoxicated rather than excreted and the liver appears to take part in this process.

We wish to thank Sandoz Chemical Works, Inc., for supplying ergotamine tartrate (Gynergen) and ergonovine tartrate (Basergin); and Burroughs Wellcome and Co., U. S. A., for supplying the ergotoxine ethanesulfonate used in these experiments.

REFERENCES

(1) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," The Macmillan Company, New York, N. Y., 1941.

(2) Van Urk, H. W., *Pharm. Weekblad*, 66 (1929), 473.

(3) Smith, M. I., *Pub. Health Rep.* (1930), p. 1466.

(4) Barger, G., *Hand. der Exper. Pharmakol.*, 6 (1938), 90.

(5) Burn, J. H., *Pharm. J.*, 118 (1927), 565.

(6) Ifuku, D., *Japan J. Med. Sci.*, 8 (1935), 148.

(7) McGrath, E. J., *Arch. Intern. Med.*, 55 (1935), 492.

(8) Rothlin, E., *Arch. intern. de pharmacodynamie*, 27 (1923), 459.

(9) McNalley, W. D., "Toxicology," Industrial Medicine, Chicago, Ill., 1937.

(10) Peterson, F., Haines, W. S., and Webster, R. W., "Legal Medicine and Toxicology," W. B. Saunders Company, Philadelphia, Pa., 1923.

(11) Autenrieth, W., and Warren, W. H., "Laboratory Manual for the Detection of Poisons and Powerful Drugs," The Blakiston Company, Philadelphia, Pa., 1928.

(12) Rosenbloom, J., and Schildecker, C. B., *J. Am. Med. Assoc.*, 63 (1914), 1203.

Strychnine X. Comparative Accuracies of Stomach Tube and Intraperitoneal Injection Methods of Bioassay*

By Justus C. Ward and D. Glen Crabtree

While carrying out the experiments with strychnine, upon which the earlier papers in this series[1] were based, we found it would be desirable to conduct adequate studies to compare our system of stomach tube bioassay with the better recognized intraperitoneal injection method. In using strychnine as an economic poison, it must be mixed with baits to be taken by mouth by predatory animals and rodents. Consequently, we have adopted the stomach tube bioassay procedure to approximate most closely the conditions under which the poison must act under field conditions.

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[1] "Strychnine I," JOUR. A. PH. A., 19 (1930), 954–957; "II," *Ibid.*, 19 (1930), 1057–1060; "III," *Ibid.*, 23 (1934), 984–988; "IV," *Ibid.*, 25 (1936), 422–426; "V," Thesis, University of Colorado: "VI," JOUR. A. PH. A., 25 (1936), 590–593; "VII," *Ibid.*, 26 (1937), 29–31; "VIII," *Ibid.*, 26 (1937), 129–134; "IX," Unpublished manuscript.