

rabbits, but no practical method has been found to insure the selection of anything but a fair proportion of satisfactory uteri. Pregnancy, age of rabbit, and stage of oestrous are the interfering factors.

Thirdly, and perhaps the greatest objection of all, the time required to run an assay compared to that required in the official Cockscomb test, has been far too great. It appears to be a quite general view that an operator must be exceedingly fortunate to obtain a satisfactory assay on more than one sample during a whole day.

The writer has devoted a great deal of time during the past two years in a continuation of previously reported work on Ergot. In the course of this work, the Rabbit Uterus Method has been depended upon almost exclusively for estimations of alkaloidal activity, and a technique has been developed which, at least partially, eliminates hitherto existing objections to this method.

First of all, this technique provides for accuracy and dependability in estimating the alkaloidal activity that cannot be duplicated by any other known biological method in our hands.

Secondly, this technique provides for an assay upon a sample of Fluidextract of Ergot, that is sufficiently accurate for all practical purposes, in a period of time not exceeding two hours.

Thirdly, the necessity for blaming poor results upon unsatisfactory uteri has been practically eliminated.

Lastly, the technique does not require expensive equipment or an unreasonable amount of experience, as has been proven to the writer's satisfaction by the fact that students have demonstrated their ability to secure dependable results after using the technique only a relatively short period of time.

In support of these claims, it is necessary to describe this technique in detail.

(To be continued)

DIGITALIS FAT—THE PETROLEUM-ETHER EXTRACTIVE OF DIGITALIS PURPUREA LINNÉ.*

BY A. JOHN SCHWARZ.

INTRODUCTION.

Reports on the pharmacological action of Digitalis and various preparations of the leaf make mention of the presence of a "fixed oil" or "fat" in the leaf which may or may not render the preparation (particularly the tincture)

- a. Less slightly
- b. Less stable
- c. Less palatable
- d. Less tolerable.

The last three conditions apply also to the leaf.

It is strange that no detailed chemical analysis of the digitalis fat has been recorded since the above-mentioned characteristics have been ascribed to it. Such

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a study is deemed necessary in order to determine whether the fat does or does not possess the properties attributed to it by the various investigators. It is the purpose of this investigation to determine as many as possible of the individual constituents of digitalis fat.

HISTORICAL.

The fat-free tincture of Digitalis was introduced in 1894 by J. W. England¹ who believed the nausea in digitalis administration to be caused by the presence of the fixed oil and its free acids. W. C. Alpers (1915)² made the statement that the fatty oil of digitalis leaves causes nausea. This reported property of digitalis fat has been disproven by the work of R. Hatcher and C. Eggleston (1913).³ They isolated the fat from the leaf by means of petroleum-ether extraction. Large doses of it were fed to cats—the amount contained in 70,000 therapeutic doses—with no emetic or nauseous indications. The digitalis bodies produce the nausea by acting directly upon the vomiting center in the medulla. H. A. Christian (1919)⁴ belittled the fat-free preparations of digitalis, describing the procedure as a fad. In his opinion, results would be more satisfactory if the effort were spent in obtaining good leaves. M. Dooley (1919)⁵ found that the fat-free tinctures produce nausea as well as those which are not defatted.

The instability of digitalis preparations has also been charged against the fat of digitalis leaves. M. Mansier (1901)⁶ believed that the changes in the tincture of digitalis were due to oxidase action at the expense of changes in tannin and in small quantities of the fatty and volatile bodies in the tincture. By the removal of the fat it was thought that the enzyme activity on the active constituents would be checked, thereby preserving the strength of the tincture and preventing a precipitation. G. Roth (1916)⁷ found that the fat-free tinctures of digitalis deteriorated as rapidly, and in some cases more rapidly, than those which had not been defatted. P. Pittenger and H. K. Mulford, Jr. (1918)⁸ also reported deterioration of the fat-free tinctures but the deterioration did not seem to be so rapid as in those which had not been defatted.

The matter of "greater degree of pharmaceutical elegance as a result of the removal of the fat"⁹ has been another one of the reasons advanced for the removal of the fat, since this checks, if not entirely prevents, the formation of a precipitate after a short period of standing.¹⁰

With so much concern about the evil effects of the fat of digitalis leaves, there are only four references to the oil or fat of digitalis leaves, and in none of these reported instances has there been an attempt at an analysis of the fat. Three references have been found to the fat of the seed, in two of which there has been no attempt at analysis, and in the third, only a meagre analysis.

The first oil of digitalis was reported by J. D. Morris (1834).¹¹ This was an empyreumatic oil obtained by the dry distillation of the leaf. The physical properties were described.

¹ *Amer. Jour. Pharm.*, 71 (1899), 333. ² *JOUR. A. PH. A.*, 4 (1915), 715.

³ *Jour. Amer. Med. Assoc.*, 60 (1913), 499. ⁴ *Amer. Jour. Med. Sci.*, 157 (1919), 593.

⁵ *Midland Drug.*, 53 (1919), 5. ⁶ *Brit. & Col. Drug.*, 40 (1901), 175.

⁷ *Bull. Hyg. Lab.*, No. 102 (1916), 30. ⁸ *JOUR. A. PH. A.*, 7 (1918), 236, 435, 1031.

⁹ *Drug. Circ.*, 61 (1917), 25. ¹⁰ *Pharm. Centralh.*, 43 (1902), 425, 660.

¹¹ *Amer. Jour. Pharm.*, 5 (1834), 262.

The second report on oil of digitalis was made by J. England in 1887.¹ The oil was obtained by petroleum-ether extraction of the leaves. He expressed surprise at finding 5 per cent of fixed oil which he described as a dark red-brown transparent liquid of heavy narcotic odor consisting of 5.4 per cent of volatile substance which is lost upon heating the oil at 100° C. for eight hours. Here again there is no analysis of the oil or fat.

In 1917, G. Éwe² reported the physical constants of an oil obtained from digitalis leaves by petroleum-ether extraction, but no mention is made of the chemical composition of the oil. He described the extractive as an orange-yellow liquid. The percentage of oil in the leaves was reported as a "maximum of 12 per cent, although the average amount is much less."

B. K. Whyte (1919)³ found 4.43 and 4.014 per cent of oil in digitalis leaves which she described as a thick green fluid still having the odor of petroleum ether.

Concerning the oil of digitalis seed, A. Buchner, Sr. (1851)⁴ obtained an oil by ether extraction which has merely been referred to as a siccative oil, constituting 40 per cent of the weight of the seed.

I. S. Melanoff (1927)⁵ reported an amber-colored, oily liquid with a fatty odor and bland taste which he obtained from digitalis seeds. He determined a number of constants for it and classified it as a semi-drying oil.

The only attempt at a chemical analysis of the fat of either the leaf or seed of digitalis was made by S. Culter (1930)⁶ when he reported the seed to contain 31.4 per cent of oil from which he isolated glycerol, linolenic and linolic acids.

EXPERIMENTAL.

The Petroleum-Ether Extract.—Digitalis, grown and harvested at the Wisconsin Pharmaceutical Garden, was reduced to a coarse powder in a Hance disintegrating mill and sifted. The ground drug was moistened with petroleum-ether (b. p. 30° to 80° C.) and then packed in a Lloyd's extractor for continuous percolation. Each charge of 9 Kg. in the extractor was subjected to the action of the petroleum-ether for six days, after which time the percolate no longer contained fatty extractive as shown by the evaporation of 10 cc. from a watch glass.

The greater amount of the petroleum ether was recovered from the percolate by distillation on a steam-bath under reduced pressure. The extract was then placed in evaporating dishes and exposed to the air in a warm room, stirred mechanically, and placed in an electric fan air current so as to remove the final traces of petroleum ether. This was carried out in two separate fractions with the following results:

	Fraction I.	Fraction II.
Weight of leaves.....	13.07 Kg.	30.11 Kg.
Weight of petroleum-ether extract.....	0.167 Kg.	0.308 Kg.
Per cent of extractive.....	1.277	1.023

It was a surprise to find that the petroleum-ether extractive was so low, especially after Éwe had reported a maximum of 12%, England 5% and Whyte 4 plus %. In order to verify the above results three 10-Gm. samples were ex-

¹ *Amer. Jour. Pharm.*, 64 (1892), 364. ² "Proc. Penna. Pharm. Assoc.," 41 (1918), 162.

³ *Unpublished data*, School of Pharmacy, U. of Wis. ⁴ "Buchner's Report," 9 (1851), 38.

⁵ *Amer. Jour. Pharm.*, 99 (1927), 549. ⁶ *Ibid.*, 102 (1930), 545.

tracted in Soxhlet extractors, the extraction continuing for fourteen days. The results of these extractions were:

	Sample I.	Sample II.	Sample III.
Weight of leaves.....	10.000 Gm.	10.000 Gm.	10.000 Gm.
Weight of extractive...	0.1124 Gm.	0.1189 Gm.	0.1236 Gm.
Per cent of extractive..	1.124 per cent	1.189 per cent	1.236 per cent

This gives an average of 1.22 per cent of petroleum-ether extractive which compares favorably with the percentages of extractive obtained for the larger fractions in the Lloyd extractor.

On account of the low per cent of extractive in the digitalis leaves, and on account of the difficulties involved in carrying on research with small quantities of material, the coöperation of Eli Lilly & Co. (Indianapolis) was obtained who consented to recover the fat from their gasoline extractions of digitalis leaves used in the preparation of the fat-free tincture. The leaves were of German importation; they were reduced to a coarse powder in a Meade mill, and extracted with a special high grade gasoline (b. p. 60° to 80° C.), a product of the standard Oil Co. of Indiana.

The fat furnished by Eli Lilly & Co. was obtained from the processing of three lots of leaves. The data is as follows:

	Lot No. I.	Lot No. II.	Lot No. III.
Weight of leaves.....	107.000 Kg.	107.000 Kg.	107.000 Kg.
Weight of extractive...	1.123 Kg.	1.875 Kg.	1.419 Kg.
Per cent of extractive..	1.05 per cent	1.75 per cent	1.32 per cent

These three fractions were combined with an average of 1.373 per cent of petroleum-ether extractive. These results compare favorably with those obtained on the Wisconsin grown digitalis but very unfavorably with those previously reported by England, Éwe and Whyte.

The combined extracts had a consistency of cold molasses; not completely solid, yet liquid enough so that it could be poured, the mobility being very sluggish. The color, in mass, is black but upon dispersions shows a decided deep green, chlorophyllaceous color. The odor is pronounced, heavy and faintly characteristic. The extractive or fat is greasy to the feel.

Saponification of the Fat.—1000 Gm. of the petroleum-ether extractive or digitalis fat was saponified with alcoholic potassium hydroxide in 100-Gm. lots according to A. Boemer's method.¹ After saponification and the addition of water, the liquid is allowed to cool to 20° C. and then extracted repeatedly with ether until the ethereal extractions are no longer colored. The ethereal extracts are thoroughly washed with distilled water several times, dried over anhydrous sodium sulphate after which the ether is recovered.

Combining all the fractions, a total of 344 Gm. of non-saponifiable material was obtained or approximately 35%. This has a reddish, chocolate color and possesses a faint odor. The consistency is a little harder than that of cold butter; it is smooth and waxy to the feel, and gives a bright orange-yellow stain to any object with which it comes in contact.

As to the saponifiable material, the combined material of the 10 fractions yielded 731 Gm., part of which weight is due to water held by emulsification.

¹ *Ztschr. f. Untersuch. d. Nahres. u. Genussm.*, 1 (1898), 21.

THE SAPONIFIED FRACTION.—The potassium soap solution which had been extracted with the ether to remove the non-saponifiable matter, was warmed and a stream of carbon dioxide gas bubbled through it until the last traces of alcohol were removed. To each 400 cc. of the resulting solution, 1600 cc. of hot water were added and the solution treated with a slight excess of 1:3 sulphuric acid solution. The mixture was kept hot and under an atmosphere of carbon dioxide until the free fatty acids separated as a distinct green layer. This was then transferred to a warm separatory funnel and washed with several portions of boiling water until the washings were free of acid. The fatty acids showed a strong tendency to emulsify. This was partly overcome by keeping the separatory funnel hot. The various portions, amounting to 731 Gm. were combined and kept in a tightly closed container under carbon dioxide until they were used.

Glycerol.—The aqueous portion obtained in the separation of the free fatty acids was neutralized with hydrochloric acid, and then evaporated at room temperature. After considerable concentration, the potassium sulphate crystallized and was removed from time to time. Finally there remained a syrupy, sticky mass which was extracted with a mixture of alcohol and ether (3:1). This was dried over calcium chloride, filtered and then evaporated at room temperature. When all of the alcohol and ether had been evaporated, there remained a reddish colored syrupy liquid that had a sweet taste. When some of it was heated with potassium bisulphate, it gave a positive acrolein test, indicating glycerol.

The Fatty Acids.—For the purpose of separating the insoluble fatty acids, the 731 Gm. of the green fatty acid material were treated in two portions by a lead-alcohol method, based on those of E. Twitchell,¹ A. Gruen² and R. J. Pauly.³

The procedure consisted in adding a hot alcoholic solution of lead acetate slowly and with continuous, rapid stirring to the nearly boiling alcoholic solution of the green fatty acid material. This was allowed to cool and then placed in an ice box and kept at a temperature of 15° C. for three days. It was then filtered, the filtrate tested for excess lead with alcoholic sulphuric acid and the residue then washed with cool alcohol.

The filtrate and washings were combined, amounting to 10 liters which were of a dark green color by transmitted light and black by reflected light. The residue (300 Gm.) was a powder of medium light green color, with fragments of a very dark greenish color.

This was purified by boiling with 8 L. of alcohol to which 50 cc. of glacial acetic acid had been added to facilitate solution of the lead salts of the fatty acids. The lead salts that dissolved were filtered from those that remained insoluble, and the filtrate was cooled, placed in the ice box at 15° C. for three days and then filtered and repeatedly washed with cold alcohol. As a result of this treatment of the lead salts of the fatty acids, the following four fractions were obtained:

- I. Lead salts insoluble in boiling alcohol (148 Gm.).
- II. Lead salts soluble in hot alcohol, but insoluble in cold alcohol (97 Gm.).
- III. Solution of lead salts soluble in cold alcohol, obtained by repeated washing and purification of Fraction II (8 liters).
- IV. Solution of lead salts soluble in cold alcohol (10 liters).

¹ *Ind. & Eng. Chem.*, 13 (1921), 806.

² A. Gruen, *Analyse der Fette u. Wachse*, 1 (1925), 221. ³ Ph.D. Thesis, U. of Wis. (1931).

(To be continued)