

SCIENTIFIC SECTION

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STUDIES ON THE BIOASSAY OF DIGITALIS: FROG METHODS.

BY JAMES H. DEFANDORF.

INTRODUCTION.

Although the frog heart-lymph sac method for the bioassay of digitalis has been official in the United States for almost a decade and a half, it has been the object of considerable criticism (3, 6, 9, 22, 23, 24, 27, 29, 30), and as a result numerous other methods (5, 6, 7, 14, 20, 22, 25, 28, 30, 31, 32) have appeared since its adoption by the United States Pharmacopœia (33). It is probable that the chief reasons for its continued recognition in the pharmacopœia are its simplicity, economy, reasonable accuracy and because it is considered to be a relatively good index of therapeutic activity.

The method of the United States Pharmacopœia limits the observation period to one hour, requires an assay temperature of 20° C., and does not refer to the sex of the frogs, whereas the method of the Geneva Conference of the League of Nations (22) designates an observation period of at least four hours, places no limitations on the assay temperature and specifies male frogs.

With these differences in mind, the following studies dealing with the assay of digitalis on frogs were made:

1. Comparison of absorption in the one- and four-hour lymph sac methods.
2. Influence of temperature and of sex on absorption from the lymph sac.
3. Toxicity of digitalis by the one-hour and four-hour lymph sac, the intramuscular and the intravenous methods.

LITERATURE.

Since the conflicting results of various workers may be due largely to lack of uniformity in the experimental conditions and technique, a review of the literature is given below in classified form which indicates some of the probable causes leading to variable results.

1. *Species*.—The U. S. P. X (33) specifies the use of the grass frog, *Rana pipiens*, while the variety known as *Rana temporaria* is generally employed in Europe. Either of these two varieties of grass frogs may be used in the method of assay described by the Geneva Conference of the League of Nations (22).¹

2. *Health*.—No comment is necessary on the importance of this factor. Health can probably best be maintained by keeping the frogs in running water at a temperature not exceeding 15° C., as required in the U. S. P. X (33).

3. *Size*.—Houghton (16) stated that frogs of nearly uniform size should be used and based the dosage upon body weight, and later (17) recommended 15 Gm. as the standard. Famulener and Lyons (10) used 40 Gm. and Focke (11) 25- to 30-Gm. frogs, while Roth (26) and Smith and McClosky (27) worked with 20- to 30-

¹ Hereafter referred to as "Geneva Conference."

Gm. frogs, the limits specified by the U. S. P. X (33). The method of the Geneva Conference allows animals weighing up to 40 Gm. These marked variations are not surprising in view of the difficulty in procuring large numbers of frogs of uniform weight.

4. *Sex*.—Buhner (4) assaying digitalis on *Rana temporaria* by the frog heart-lymph sac method, concluded that females were more uniform in response than males, and finally employed females exclusively. Focke (11) believed that sex was not a factor in July, August and September. Edmunds and Hale (8) found sex a factor only in the spring months, but later Hale (12) stated that even in the mating season the greater weight of females due to enlargement of the egg sac does not render them more susceptible to digitalis when the dose is estimated on body weight. This observation was corroborated by Sollmann, Mendenhall and Stingell (29) working with ouabain, who nevertheless rejected females during the breeding season. Baker (1) found sex relatively unimportant except when the female contained a very large egg mass, when it appeared slightly more resistant. Roth (26) working with digitalin, observed a more variable absorption with females, but found that the occurrence of eggs bore no relation to the degree of absorption. No mention of sex or the occurrence of egg-masses is to be found in the method of the U. S. P. X (33), but the method of the Geneva Conference (22) specifies the use of male frogs.

5. *Seasonal Influence*.—Seasonal variation is rather intimately bound up with sex and susceptibility. Houghton (16) and Edmunds and Hale (8), believed that frogs could be used throughout the year, if the conditions were identical and if comparisons were made with a standard. Edmunds and Hale (8), however, concluded that summer assays were best. Focke (11) found that susceptibility varied only slightly in July, August and September. Hale (12) concluded that tests of digitalis by the frog heart-lymph sac method did not vary 10 per cent from September to March. Baker (2) found poor absorption most likely to occur in the spring and late autumn. Smith and McClosky (27) observed that when frogs are kept at 20° C. there is little variation in seasonal susceptibility.

6. *Influence of Temperature*.—Houghton (16), Famulener and Lyons (10) and other early investigators did not specify definite temperature limits for their assays. Focke (11) and Edmunds and Hale (8) kept the frogs at a temperature of not over 17° C. Hale (12, 13) later designated 22° C. as the standard for his tests. Baker (1, 2) found a more ready absorption at higher temperatures, the minimum lethal dose varying inversely with the temperature. The results of Baker were confirmed by Sollmann, Mendenhall and Stingell (28, 29) while working with ouabain. They found in addition that the increase of toxicity per degree of temperature was much greater at lower than at higher temperatures between the ranges of 10° and 20° C., an observation that is in harmony with the law of the effect of temperature upon biological reactions. Roth (26) found a better absorption of digitalis preparations at 30° and 20° C. than at 10° C. Smith and McClosky (27) found that the sharpness of the end reaction was best at 20° C. The U. S. P. X (33) requires an assay temperature of 20° C. while the standard of the Geneva Conference (22) does not specify a standard temperature.

7. *Amount of Fluid Injected*.—Houghton (16, 17) using 15-Gm. frogs, injected 0.5 cc. of fluid into the ventral lymph sac, whereas Famulener and Lyons (10) used

the same amount for 40-Gm. frogs. Focke (11) injected 0.3 cc. into each of the two leg lymph sacs. Sollmann, Mendenhall and Stingell (29) specified not more than 1 cc. of fluid. Roth (26) found 0.5 cc. of fluid satisfactory for either leg or ventral lymph sacs. Hale (13) specified 0.015 cc. of fluid per Gm. for 20- to 35-Gm. frogs, as did Baker (1, 2) who stated that a majority of healthy frogs will in one hour absorb that quantity of fluid from the ventral lymph sac. The U. S. P. X (33) requires 0.015 cc. per Gm. for 20- to 30-Gm. frogs. The Geneva Conference (22) specified that not more than 0.3 cc., or with weakly active preparations, not more than 0.5 cc., of fluid should be injected into the ventral lymph sac, any excess being injected into one or both thigh lymph sacs.

8. *Character of Fluid Injected.*—The liquid preparations of digitalis are aqueous or hydro-alcoholic in character; hence dilutions of these liquids for injection into the lymph sac will contain varying proportions of alcohol and water or physiological saline. Houghton (16, 17) dissolved strophanthin in physiological saline. Famulener and Lyons (10) made all their dilutions with physiological saline, being careful to have the same alcoholic content in every case. Baker (2) found that physiological saline was more readily absorbed from the lymph sac than distilled water, 25% alcohol more readily absorbed than physiological saline, but that 50, 75 and 90% alcohol were less readily absorbed than 25% alcohol. Roth (26) found that when not more than 0.5 cc. of fluid of the digitalis preparation was injected into the ventral lymph sac, there was no difference in toxicity whether the solvent was alcoholic or aqueous. Edmunds, Lovell and Braden (9) removed all alcohol from preparations. They pointed out the need for standardization along these lines, having observed that some manufacturers allow 25% alcohol in their dilutions for injection. As a result of recent investigations Munch and Quici (20) claim that an alcohol content up to 30% of the fluid to be injected produces no essential or consistent differences in the assay on frogs, so that it is unnecessary to concentrate and redilute tinctures of digitalis in making assays according to the methods of the present Pharmacopœia. In the standard method of the Geneva Conference (22) most of the alcohol of the preparation is removed by evaporation and replaced with distilled water, while the U. S. P. X (33) allows a maximum of 20% alcohol, dilutions being made with distilled water. It will be observed that neither the U. S. P. X nor Geneva Conference methods specify an exact amount of alcohol in the fluid to be injected.

9. *Length of the Observation Period.*—Most of the frog methods for the assay of digitalis employ one of two end-points, either (a) systolic standstill of the ventricle or (b) death. The smallest amount of the drug producing the effect in (a) is known as the minimum systolic dose (M. S. D.) in (b) as the minimum lethal dose (M. L. D.). Various methods of assay by the lymph sac (and other) methods employ different lengths of time in which these end-points may appear. Houghton (16, 17) advocated a minimum lethal dose frog method with an observation period of twelve hours which in modified form appears to be preferred by others, especially European investigators (3, 6, 22, 23, 24, 25, 30, 31, 32). Famulener and Lyons (10) originated the one-hour minimum systolic dose method in the United States, where it has been most popular since its introduction thirty years ago and, in a modified form, has been the standard for a decade and a half. Probably the main objections which are raised against the one-hour lymph sac method are based on

complaints similar to those of Rowe (23, 24, 25), that not enough time is allowed for absorption, and that systolic standstill at any time previous to death is not an accurate end-point. Edmunds, Lovell and Braden (9) maintain that the one-hour method is satisfactory for digitalis, but that a longer observation period is necessary for the strophanthins. While the U. S. P. X one-hour method (33) presents obvious advantages over other frog heart-lymph sac methods in economy of time, the question is still debatable as to whether a longer period of observation such as four hours, as specified in the Geneva Conference Method (22), might not lead to more accurate results. The length of the observation period is closely allied with the factor of absorption, which is discussed below.

10. *Absorption.*—Absorption of digitalis from the lymph sac depends on many factors, such as the species, sex, size and health of the frogs, the character of the solvent, the concentration of the active drug, the age of the preparation, the season of the year, the temperature of the assay, the amount of fluid injected and the length of time allowed for absorption, most of which have been discussed above. Focke (11) using his short time method and injecting into the leg lymph sacs, observed systolic standstill from greatly different doses, which he attributed to variable absorption. Hale (13) asserted that "red leg" and poor health delayed absorption. As a result of comprehensive studies on absorption from the lymph sac, Baker (2) concluded that (a) the majority of healthy frogs will absorb 0.015 cc. of fluid per Gm. of body weight in one hour; (b) poor absorption is most likely to occur in the spring and late autumn; (c) light appears favorably to influence absorption; and (d) while absorption apparently is not affected by "red leg" disease, it is largely dependent on the health of the frogs and the conditions under which they are kept and handled. Roth (26), using 25% alcoholic solutions, found that the absorption and toxicity of digitalis bodies increased with the temperature; that while females showed a more variable absorption than males, the occurrence of eggs bore no relation to the degree of absorption. Paranjpe (21) found a wide variation in absorption with 25% alcoholic solutions of digitalis bodies from the lymph sacs of 40-Gm. frogs. Haskell (15) observed that as tinctures aged, their absorbability decreased. Smith and McClosky (27), while finding no constant relationship between the presence of unabsorbed fluid in the lymph sac, the condition of the heart and the size of the dose, agree that the extreme variations in susceptibility are largely accounted for by lack of absorption. Rowe (24, 25) claimed that the irritation produced by digitalin caused the secretion of a large amount of fluid into the lymph sac, thereby lengthening the time of absorption. Edmunds, Lovell and Braden (9) found that digitalis was more readily absorbed from the lymph sac than was strophanthin.

EXPERIMENTAL.

In the autumn of 1931 while making an assay of a tincture of digitalis by the U. S. P. X method, it was observed that absorption from the lymph sac was complete in only four out of twenty frogs. Further, observation showed that absorption was complete in two of three females, but in only two of seventeen males. These results led to a number of assays by various frog methods, the essentials of which are described on next page.

METHODS.

(1) *The U. S. P. One-Hour Frog Heart-Lymph Sac Method.*—This method (33) requires the use of healthy grass frogs, *Rana pipiens*, twenty to thirty Gm. in weight. They must be stored in a cool place in running water, where the temperature preferably does not rise above 15° C. The day before the frogs are to be used, they are placed in a tank containing water at a temperature of approximately 20° C. One hour before the assay, they are weighed to within 0.5 Gm. and placed in water at a depth of about one cm., which is kept at a constant temperature of 20° C. during the assay. The dose of the tincture is calculated according to the weights of the frogs, and is injected into the ventral lymph sac through the floor of the mouth, the amount of fluid being about 0.015 cc. per Gm. of body weight. Dilutions are made with distilled water, and partial evaporation of the tincture is necessary in those cases where the alcohol content of the dilutions would otherwise exceed 20%. Fifth-eight minutes after the injection each frog is pithed; the heart is later exposed and examined at the end of one hour. The correct end-reaction at this time is systolic standstill of the ventricle, with the auricles widely dilated. The amount producing this effect is known as the minimum systolic dose (M. S. D.). If any of the injected drug is found unabsorbed in the lymph sac, the results with the animal must be rejected in recording the assay.

(2) *The Modified U. S. P. Four-Hour Method.*—This assay differs from the U. S. P. X method only in that four hours instead of one are allowed for the absorption of the drug.

(3) *The Intramuscular Frog Method of Dooley and Higley.*—In this method (7) grass frogs, *Rana pipiens*, are used as in the U. S. P. X method. All the alcohol of the preparation is evaporated at a temperature not over 50° C. and enough alcohol is added to make 15% when the preparation is brought up to the original volume with physiological saline (0.7%). Dilutions of this modified tincture are made so that the amount of fluid injected is never more than 0.01 cc. per Gm. of body weight. One-half of the total dose is injected diagonally into the thickest part of each thigh with a fine needle to avoid hemorrhage. Otherwise, the procedures and end-point are the same as those of the U. S. P. X method.

(4) *The Intravenous Frog Method of Smith and McClosky.*—This method (27) also employs the grass frog, *Rana pipiens*. All the alcohol is removed from the preparation by evaporation on a water-bath at a temperature not over 50° C., the evaporation not proceeding to dryness. The residue is taken up with enough physiological saline to make a concentration of twenty to forty mg. of digitalis per cc. After filtration, it is diluted with physiological saline, to from two and one-half to five times the original volume. The frog's brain is then pithed, the animal is fastened on its back and a median incision is made over the ventral lymph sac. The drug is slowly injected (tuberculin syringe, 26-gauge needle), into the right or left musculo-cutaneous vein, which is clamped after the needle is removed. The animal is then returned to the constant temperature tank, and at the end of one hour its cord is pithed and the examination is made in the usual manner. The end-point is the same as in the U. S. P. X method.

TINCTURES EXAMINED.

Tincture "A." A composite tincture prepared by the class in pharmacology (24 preparations of 100 cc. each) during October 1931, differing from the U. S. P. X requirements only in that it was not defatted.

Tincture "B." A defatted tincture made by the author according to the U. S. P. X during the first week of December 1931.

FROGS USED.

Frogs "A.:" *Rana pipiens*, received September 1931, varying in weight from 36 to 69 Gm., stored in water in the basement at a temperature never over 15° C.

Frogs "B.:" *Rana pipiens*, received during November 1931, varying in weight from 35 to 50 Gm., stored similarly to Frogs "A."

Frogs "C.:" *Rana pipiens*, received April 1932, varying in weight from 24 to 67 Gm., stored in running water in the basement at about 15° C.

Frogs "D:" *Rana pipiens*, received May 1932, varying in weight from 24 to 44 Gm. stored in running water in the basement not over 18° C.

Frogs "F:" *Rana pipiens*, received May 31, 1932, varying in weight from 26 to 37 Gm., stored in running water in the basement not over 20° C.

PROCEDURE.

The assays on frogs "A" and "B" in the autumn were run at temperatures varying from 22.5° to 25.5° C. instead of at the standard of 20° C.

Frogs ranging in weight from 24 to 67 Gm. were used.

Frogs "C," "D" and "F" were placed in the constant temperature tank at 20° C. for one hour previous to and during the assay.

All animals were weighed to within one Gm. and the amount of fluid injected was adjusted so as to average about 0.5 cc., with a maximum of 0.7 cc. and a minimum of 0.3 cc. The amount of alcohol in the preparations injected ranged between zero and 20%.

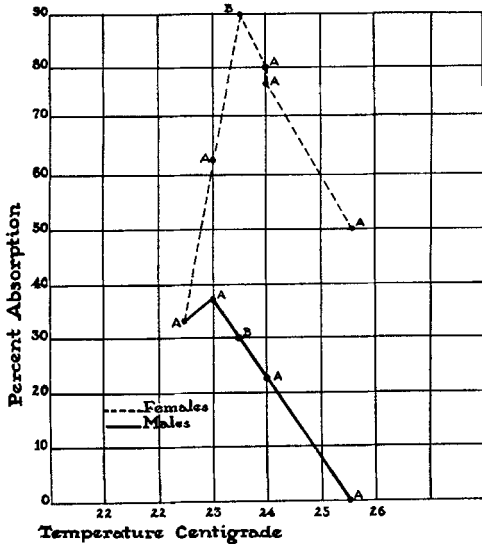


Fig. 1.—Absorption of digitalis tinctures "A" and "B" from the lymph sac of male and female frogs (*Rana pipiens*) at various temperatures by the U. S. P. one-hour method in the autumn of 1931. "-----" = females "———" = males.

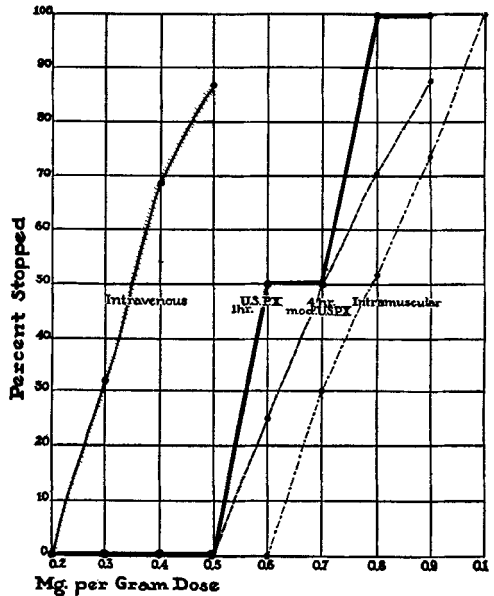


Fig. 2.—Comparison of systolic standstill doses of digitalis tincture "B" on male frogs (*Rana pipiens*) by four assay methods in the spring of 1932, at 20° C.

In the intravenous method of Smith and McClosky the modified diluted preparations were not filtered; in the intramuscular method of Dooley and Higley all of the alcohol was removed.

TABLE I.—THE INFLUENCE OF SEX ON ABSORPTION OF TINCTURE OF DIGITALIS FROM THE LYMPH SAC OF FROGS (*Rana Pipiens*), USING THE ONE-HOUR METHOD.

Tinctures: "A," "B"	Temperature range 22.5° to 25.5° C.		
Frogs: "A," "B"	Date of tests: Autumn, 1931.		
	Number of Frogs Injected.	Number Showing Complete Absorption.	Percentage Absorption.
Males	43	11	25.6
Females	54	36	66.6

Table I shows that absorption of modified tincture of digitalis from the ventral lymph sac in one hour was much better in female than in male grass frogs (*Rana pipiens*), in the autumn at temperatures ranging from 22.5° to 25.5° C., the ratio being over 2.5 to 1. At this season of the year all females contained egg-masses in about the same state of development; determinations made over this period on 33 females showed that the egg-masses averaged 20% of the weight of the frogs.

THE INFLUENCE OF TEMPERATURE ON ABSORPTION OF TINCTURE OF DIGITALIS FROM THE LYMPH SAC OF MALE AND FEMALE FROGS (*Rana Pipiens*), USING THE ONE-HOUR METHOD.

Figure 1 shows the per cent absorption in males and females plotted against the temperature, and indicates that the increased susceptibility at higher temperatures is at least due partly to the increased rate and degree of absorption. The only assay made with Tincture "B" fits perfectly with Tincture "A" in the graph, with both male and female frogs. It should be noted that while the temperatures are non-consecutive environmental variations, the resulting graphs are almost entirely uniform. It is also significant that in both sexes the best absorption appears between the ranges of 22.5° and 23.5° C.

TABLE II.—EFFECT OF ALCOHOL CONTENT OF MODIFIED TINCTURE OF DIGITALIS ON ABSORPTION IN MALE FROGS (*Rana Pipiens*).

Tinctures : "A," "B"		Temperature: 20° C.			
Frogs: "C," "D," "F"		Date of Tests: Spring, 1932.			
Method of Assay.	Alcohol Per Cent.	Diluent.	Number of Frogs Injected.	Number Showing Complete Absorption.	Per Cent Showing Complete Absorption.
One-hour method	Not over 20%	0.7% saline	30	3	10
One-hour method	None	46	10	21.7
Four-hour method	Not over 20%	9	7	77.7
Four-hour method	None	52	50	96.2

Table II shows the relation between the alcohol content and the absorption of the modified tincture, using the one- and four-hour methods. In neither method does an alcohol content ranging from zero to 20% practically affect the degree of absorption.

TABLE III.—COMPARISON OF THE ABSORPTION OF TINCTURE OF DIGITALIS FROM THE LYMPH SAC OF MALE FROGS (*Rana Pipiens*), USING THE ONE-HOUR AND FOUR-HOUR METHODS.

Tincture "B"		Temperature: 20° C.		
Frogs: "C," "D," "F"		Date of Tests: Spring, 1932.		
Dose Used (Mg. per Gm.).	One-Hour Method.		Four-Hour Method.	
	Number of Frogs Injected.	Number Showing Complete Absorption.	Number of Frogs Injected.	Number Showing Complete Absorption.
0.2	2	1
0.3	4	1
0.4	4	1
0.5	4	0	2	2
0.6	13	2	12	12
0.7	14	2	19	18
0.8	17	3	18	17
0.9	18	3	10	8
Totals	76	13	61	57
Percentage		17.1		93.4

Assays begun in the spring of 1932 with male *Rana pipiens* frogs showed such poor absorption by the one-hour method that it was decided to run parallel series of

assays in which the time allowed for absorption was increased from one to four hours. The results of this comparison are given in Table III. Absorption from the ventral lymph sac was complete in only 17.1% of 76 frogs by the one-hour method, while it was complete in 93.4% of 61 frogs by the four-hour method. (The relation of these two methods of assay to the minimum systolic dose will be discussed later.)

TABLE IV.—COMPARISON OF THE ASSAY OF THE TINCTURE OF DIGITALIS BY FOUR FROG METHODS, USING MALE GRASS FROGS (*Rana Pipiens*).

Dose Used (Mg. per Gm.).	Tincture: "B" Frogs: "C," "D," "F"		Temperature: 20° C. Date of Tests: Spring, 1932.		Intramuscular Method (Dooley-Higley).		Intravenous Method (Smith- McCloskey).	
	One-Hour Method. Number of Frogs Showing Complete Absorption.	"+"	Four-Hour Method. Number of Frogs Showing Complete Absorption.	"+"	Number of Frogs Injected.	"+"	Number of Frogs Injected.	"+"
0.2	1	0					2	0
0.3	1	0					6	1
0.4	1	0					16	11
0.5	0	0	2	0	2	0	15	13
0.6	2	1	12	3	12	0		
0.7	2	1	18	9	20	6		
0.8	3	3	17	12	19	10		
0.9	3	3	8	7	15	11		
1.0					2	2		

"+" indicates systolic standstill of ventricle.

Table IV indicates that the minimum systolic dose is between 0.7 and 0.8 mg. per Gm. by either the one-hour or the four-hour method (see also Fig. 2). It would appear from these results that the minimum systolic dose is practically the same by the one-hour and four-hour methods; however, the four-hour method gives more definite results with a smaller number of frogs, and therefore is more economical than the one-hour method. The slight divergence of the one-hour and four-hour curves between 0.7 and 0.8 mg. in Fig. 2 is of little significance since the number of frogs in which complete absorption was obtained was very small in the one-hour method.

Assays made on 70 frogs by the intramuscular method of Dooley and Higley (7) gave rather uniform results as shown in Table IV and Fig. 2, and indicate approximately the same minimum systolic dose as the one- and four-hour lymph sac methods, instead of the 18.6% smaller dose observed by Dooley and Higley (7).

Thirty-nine assays made by the intravenous method of Smith and McClosky (27) show a minimum systolic dose of 0.4 mg. per Gm., or about one-half that obtained by the three other methods, a finding which is in accord with the results of other investigators. The effective dose is sharply defined (Table IV) and the toxicity curve is remarkably uniform (Fig. 2).

DISCUSSION.

The better absorption of modified tincture of digitalis by female frogs as compared with males is not easily accounted for, particularly in the autumn. The presence of the egg-mass may be accompanied by metabolic changes which affect absorption from the lymph sac, and it would appear that such influences exert a relatively marked effect in the spawning season (spring).

The influence of the weight of the egg-mass upon the size of the dose is probably an important factor. Observations made in the autumn showed that all the females contained masses of partially developed eggs, averaging 20% of the body weight. If the distribution of the absorbed digitalis in the egg-mass is proportional to that in the body tissues, no correction in dosage will be necessary. However, if the distribution in the egg-mass is greater or less than in the tissues, the smallest amount producing systolic standstill will not represent the true minimum systolic dose.

CONCLUSIONS.

1. Absorption of tincture of digitalis preparations in the autumn is much better in female than in male frogs (*Rana pipiens*) at temperatures ranging from 22.5° to 25.5° C.
2. Within the temperature ranges of 22.5° to 25.5° C., absorption was best at 23° C.
3. It appears that "increased susceptibility" occurring with a rise of temperature may be due in many instances to the increased absorption.
4. Absorption of tincture of digitalis in the spring in male frogs (*Rana pipiens*) by the U. S. P. X method is much more complete when the period of observation is increased from one to four hours.
5. From a practical standpoint an alcohol content varying from zero to 20% produces no marked change in the rate or amount of absorption and toxicity.
6. During the spring months the minimum systolic dose of tincture of digitalis appears to be approximately the same with the one-hour, the four-hour and the intramuscular methods, while by the intravenous method of Smith and McClosky it is about half that of the three other methods.
7. The value of the U. S. P. X method would be augmented by limiting the assay to male frogs and by increasing the period of observation from one to four hours, both of which measures were recommended by the Geneva Conference (22) of the League of Nations in 1925.

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DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, SCHOOL OF MEDICINE,
 THE GEORGE WASHINGTON UNIVERSITY,
 WASHINGTON, D. C.

A CHEMICAL EXAMINATION OF THE OIL OF ERGOT.*

BY GEORGE W. FIERO.

Oil of ergot is obtained by extracting the drug with petroleum ether prior to the preparation of fluidextract of ergot, U. S. P. X. The fatty oil used in this investigation was contributed by Parke, Davis & Co. It was very dark colored, had a slight green fluorescence and a rancid odor.

Five lots consisting of 10 litres each were saponified by refluxing each lot with 15 litres of a 70 p. c. alcohol solution containing two Kg. of potassium hydroxide (2350 Gm. of KOH, U. S. P. X which contains 15 p. c. of water). This quantity was based upon the saponification value (182) established by saponifying a sample according to the U. S. P. X process. An additional 10 p. c. of potassium hydroxide was allowed to insure complete saponification. After refluxing 8 hours, as much as possible of the alcohol was removed by distillation. Since the soapy mixture had a tendency to froth and foam, it was possible to obtain only approximately one-half of the total volume of alcohol, the balance remaining in the soap solution.

The saponified oil was dissolved in several volumes of water, and the solution shaken repeatedly with ethyl ether to remove the unsaponifiable matter.

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