benefits some types of diabetic patients, it has not been defined what types of diabetes it is best suited for.

The fact that some old patients have felt aphrodisiac stimulation may point out its properties as a general tonic.

It has been believed that *Tecoma mollis* is a diuretic, but facts seem to point to the contrary. Its action is anti-diuretic and we venture to suppose that there may be a possible stimulation to pituitary secretion by the use of the extract.

We have planned experiments to determine the action of the essential oil from the plant as well as to investigate the action of a fraction of proteins or proteoses which can be precipitated from the extract, freed from gums and resins by half saturation with ammonium sulphate.

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G. G. COLIN,
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THE POTENCY OF VARIOUS SAMPLES OF DIGITALIS GROWN IN BRITISH COLUMBIA.

BY M. I. SPARKS.

The wild variety of digitalis has been known for some time to exist in fair abundance throughout the southern parts of the province of British Columbia, Canada. Indeed, the climatic and soil conditions of this district seem very well suited to the flourishing, but uncultivated, growth of this plant. In a report by Dauphinee (3), in 1924, which is referred to more fully below, it was shown that the digitalis content of these wild plants (kept under cultivation for one year) was quite comparable with that of leaves grown in England, Germany and the United States. With these facts in mind an attempt has recently been made to keep these plants under cultivation for commercial purposes; indeed the practicability of growing these plants has been demonstrated.

In the Spring of this year, Prof. V. E. Henderson, while on a tour through the Western Provinces visited and became interested in the work which was being done at this digitalis farm. Since no definite investigation of the potency of these cultivated plants had been made it was deemed advisable that an assay of these plants should be carried out in order to observe how they compared with those hitherto obtained from foreign markets.

On this farm, in addition to the usual *Digitalis purpurea L.* with purple blooms, there were also *Digitalis purpurea* with white blooms, and the botanically distinct species *Digitalis lutea L.* The relative number of plants with white blooms was quite large; if these were of inferior glucosidal content it would materially affect the average quality of the leaf, so samples of leaves from plants having white blooms were also assayed. The potency of *Digitalis lutea* leaves was also determined, as references in the literature to this species are very few. While on

most of the rows of plants no special fertilizer had been employed, a few rows were fertilized with sodium nitrate. Leaves grown from plants in these fertilized plots were also assayed. Since this farm is as yet quite small, all samples of leaves were grown on a relatively small area of land. To further obviate the effect of local conditions on results obtained, the samples were taken from as close parts of the plot as was possible, so that the leaves of different samples might be taken from plants only four or five rows one from the other. Gathering, drying and packing were done under as similar conditions as possible.

The plants used, of all the varieties, were grown from seeds of mixed origin, both Canadian and English, and in every case were of the second year's growth. The leaves were gathered at the midsummer cutting and the average yield per acre was about 1500 pounds of the leaf.

In the case of the plants fertilized with sodium nitrate the fertilizer was added once only, in March of this year (1926). The yield of leaves from these fertilized plants was about 15 per cent larger than the figure quoted above, or about 1725 pounds per acre. The nitrate also resulted in a much better color in the plants.

As comparison for these native-grown products, there were used: digitalis leaf grown in England and standardized for commercial use; a sample of the Standard digitalis leaf as supplied by the International Conference for Standardization of Biological Products, prepared by Prof. Magnus of Utrecht.

All samples of leaves, except the Standard, were obtained through Ayerst, McKenna & Harrison Ltd., of Montreal. The four samples of Canadian leaves were obtained in a dried state and were ground by us to pass a No. 20 mesh. The English leaves, of the 1925 crop, were obtained in the dried and already ground condition, the degree of grinding being much finer than No. 20. The same remarks apply to the Standard powder.

METHODS.

The methods used in assaying these samples were two in number; the one-hour frog method as described in the U. S. P. X; and the Magnus cat method as developed by Wijngaarden (1).

In the experiments with cats, ether anesthesia was used, just sufficient to keep the animal quiet after the preliminary preparation was completed; artificial respiration was used throughout. The leaf to be tested was made up in a one-half per cent infusion, rendered isotonic and filtered. Injections were made at body temperature into the external jugular vein; the first few cc. (3 to 5) at a rapid rate, the remainder at a slow constant rate such that the experiment was completed in not less than thirty minutes and not more than fifty (usually $^1/_2$ to 1 cc. per minute). The end-point was usually quite definite, the heart arrest being determined by palpatation through the thoracic wall and immediately confirmed by thoracotomy. The absence of any beats in the ventricle was considered the test of the end-point though contractions of the auricle, or a few fibrillary movements in the ventricle were allowed.

In the experiments on frogs, 10% tinctures and 5% infusions were used. The frogs were of the species *Rana pipiens* and weighed between 17 and 30 Gm. Prior to use the frogs were kept in cold running water and some time before being used were brought to the laboratory, weighed (to the nearest half Gm.) and

placed in separate glass jars containing a thin layer of water, all set in a water-bath that was kept at a temperature of 20° C. Injections were made by means of a tuberculin syringe through the mouth into the ventral lymph sac. Just before the end of the hour the frogs were pithed, both brain and spinal cord, and opened to expose the heart. The condition at the end of the hour was recorded; frogs showing any trace of the fluid in the lymph sac were discarded; experiments were continued till consistent results were obtained. A positive result was recorded only when the ventricle was so well contracted as to appear a pale pink color, the auricles engorged with blood and no beats occurred; under such conditions, mechanical stimulation still caused one or two contractions.

1. RESULTS WITH ONE-HALF PER CENT INFUSIONS BY CAT METHOD.

In these experiments the infusions were made fresh each morning and used on three (or rarely four) cats consecutively, never later than the afternoon of the same day. The results in most cases lay within the experimental variation allowed by the formula of Wijngaarden, *i. e.*, "the mean of the percentage deviations from the average value of the N estimations carried out is less than 6.67 $\sqrt{n-1}$." Any results exceeding this limit were discarded and an additional animal used. The average minimum lethal dose is calculated in cubic centimeters per kilogram of cat (dead weight); in milligrams of digitalis leaf per Kg.; and in terms of the number of cat M. L. D. in 1 Gm. of digitalis leaves.

	Infusion.	Lethal dose in cc. per Kg. of cat. Cat 1.	Cat 2.	Cat 3.	Average L. D. In cc. per Kg.	In mg. per Kg.	No. of L. D. in 1 Gm. digitalis.	Order of potency.
1.	Dig. purpurea.....	21.83	24.41	23.66	23.30	116.50	8.58	6
2.	Dig. purpurea (Sodium Nitrate).....	14.18	14.00	13.45	13.88	69.40	14.41	1
3.	Dig. purpurea (White Blooms).....	23.38		22.57	22.97	114.85	8.71	5
4.	Dig. lutea.....	16.80	17.52	19.73	18.02	90.10	11.10	2
5.	Dig. purpurea (English)	19.18	18.14	20.61	19.31	96.55	10.36	4
6.	Standard.....	19.09	18.30	18.00	18.74	93.70	10.67	3
		Cat 4.....		19.57				

2. RESULTS WITH 10 PER CENT TINCTURE BY FROG METHOD.

These tinctures were made according to the formula in the British Pharmacopœia, although the description of the method of assay was taken from the U. S. P. X. They were then so treated that the M. L. D. per Gm. of frog would be contained in about 0.015 cc. and that the alcohol content of the injected fluid was less than 20%. Thus 100 cc. of the 10% tincture made with 70% alcohol was evaporated under vacuum to about 70 cc.; made up to 100 cc. with distilled water; then, as required for experiment, diluted 2.5 times and rendered isotonic with sodium chloride.

Typical Final Experiments.

TINCTURE I—*Digitalis purpurea.*

0.015 cc. per Gm. of frog—slow regular beat.

0.016 cc. per Gm. of frog—ventricle contracted, auricles dilated, no beats.

0.017 cc. per Gm. of frog—ventricle contracted, auricles dilated, no beats.

Therefore M. L. D. = 0.016 cc. per Gm. of frog.

TINCTURE II—*Digitalis purpurea*—fertilized with sodium nitrate.

0.009 cc. per Gm. of frog—slow beats.
0.010 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.011 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.012 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
Therefore M. L. D. = 0.010 cc. per Gm. of frog.

TINCTURE III—*Digitalis purpurea*—white blooms.

0.017 cc. per Gm. of frog—slow beats.
0.018 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.019 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.020 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
Therefore M. L. D. = 0.018 cc. per Gm. of frog.

TINCTURE IV—*Digitalis lutea*.

0.014 cc. per Gm. of frog—slow beats.
0.015 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.016 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.017 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
Therefore M. L. D. = 0.015 cc. per Gm. of frog.

TINCTURE V—*Digitalis purpurea*—English.

0.014 cc. per Gm. of frog—slow beats.
0.015 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.016 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
Therefore M. L. D. = 0.015 cc. per Gm. of frog.

TINCTURE VI—Standard.

0.012 cc. per Gm. of frog—slow beats.
0.013 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.014 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.015 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
Therefore M. L. D. = 0.013 cc. per Gm. of frog.

	Tincture.	M. L. D. Cc. per Gm. of frog mg.	M. L. D. Digitalis folia per Gm. frog.
I.	Digitalis purpurea	0.016	0.64
II.	Digitalis purpurea ($NaNO_3$)	0.010	0.40
III.	Digitalis purpurea (white blooms)	0.018	0.72
IV.	Digitalis lutea	0.015	0.60
V.	Digitalis purpurea (English)	0.015	0.60
VI.	Standard	0.013	0.52

As in all standardization by this method, rough approximations were first used and then series at limited intervals such as the above. In some cases individual frogs gave variant and not typical results and in some cases when several series with the same tincture were compared, the exact end-point was hard to determine; but the amount of tincture which arrested the ventricle in over 50 per cent of the cases was taken as the M. L. D.

3. RESULTS WITH 5 PER CENT INFUSIONS BY FROG METHOD.

In order that infusions might be used on frogs, it was necessary that they be more concentrated than $^1/_2\%$ strength. To concentrate a $^1/_2\%$ infusion occupied too great a length of time to avoid deterioration effects, therefore an attempt

was made to make use of a 5% infusion. These were made exactly the same as in the method described by Winjgaarden for $^1/_2$% infusions only using a proportionately larger percentage of leaf.

This method with 5% infusions was tried on all samples of leaf, but in all except the first two samples, the results of which are given below, so great difficulty was experienced in getting complete absorption that it was abandoned.

INFUSION I—*Digitalis purpurea*.

0.015 cc. per Gm. of frog—slow beats.
0.016 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.017 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.018 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
Therefore M. L. D. = 0.016 cc. per Gm. of frog = 0.80 mg. per Gm.

INFUSION II—*Digitalis purpurea* ($NaNO_3$ fertilization).

0.009 cc. per Gm. of frog—slow beats.
0.010 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
0.011 cc. per Gm. of frog—ventricle contracted, auricle dilated, no beats.
Therefore M. L. D. = 0.010 cc. per Gm. of frog = 0.50 mg. per Gm.

4.—TABLE OF RESULTS BY ALL METHODS.

	Sample.	M. L. D. per Kg. of cat in mg.	Order of potency.	M. L. D. in mg. per Gm. frog. 10% Tincture.	Order of potency.	5% Inf.
I.	Digitalis purpurea.....	116.50	6	0.64	5	0.80
II.	Digitalis purpurea ($NaNO_3$).....	69.40	1	0.40	1	0.50
III.	Digitalis purpurea (white bloom)....	114.85	5	0.72	6	
IV.	Digitalis lutea.....	90.10	2	0.60	3	
V.	Digitalis purpurea (English).....	96.55	4	0.60	4	
VI.	Standard.....	93.70	3	0.52	2	

DISCUSSION.

1. The Standard Digitalis powder gave an average M. L. D. for cats of 18.74 cc. per Kg. This result is somewhat higher (*i. e.*, the infusion was more potent) than the average result obtained by a number of workers of 19.63 (± 1.36), as given by Knaffl-Lenz (2), but is close to values obtained by Dale, London, 18.11, and Magnus, Utrecht, 18.11, 18.65.

The average M. L. D. obtained with frogs using alcoholic extracts, of 0.52 mg. per Gm. is again definitely at the more potent end of the range of values that have been obtained by several investigators. Knaffl-Lenz gives the average of these results to be 0.68 mg. (± 0.16).

No explanation can be given of why this Standard should by both these methods assay slightly but definitely higher in digitalis content than the average of results obtained by others. Of interest, however, in this connection is the result of Voegtlin (taken from the article by Knaffl-Lenz), the only American worker quoted, who obtained a M. L. D. of 0.48 mg. per Gm. of frog (*R. pipiens*). This result is the most potent value obtained and is definitely higher than any result obtained in Europe. There is, therfore, the barest suggestion that assay in America tends to show a higher digitalis content than those performed with the same powder in Europe.

2. Table IV gives the results by both the frog and cat methods; examination of these shows that while the results are superficially the same, yet they are not

strictly comparable. Thus the sample which assays the strongest by both methods is II; the weakest is I by the cat, III by the frog method. Further comparison is shown by the figures in Table IV. We thus see that comparing the two methods, while the order of magnitude is roughly proportional, yet the comparison can be pushed no farther than this. This discrepancy between the two methods had been noted by other workers, most recently by Knaffl-Lenz in the case of Standard, A and B, powders (as prepared by Magnus).

3. Comparison of the samples of Canadian-grown digitalis inter se reveals the following facts. *Digitalis purpurea*, purple blooms and white blooms, by the cat method show a negligible difference, if anything slightly greater potency in favor of the white blooms; the frog method, on the other hand, shows the plants with white blooms to be definitely weaker than their coloured relatives. If, as has been suggested, the one-hour frog method indicates more the content of digitalin, rather than that of digitoxin, the above results would indicate that the plants with white blooms have a smaller content of digitoxin than those with purple blooms but that the total content of digitalin plus digitoxin (and other glucosides) is slightly greater in the white flowered than in the purple flowered plants.

Digitalis lutea by both frog and cat methods is stronger than either of the *Digitalis purpurea* samples; the difference, however, is more striking by the cat than by the frog method.

The values obtained for the sample of English Digitalis shows that by the cat method it is definitely more potent than the Canadian Digitalis purpurea, but weaker than the *lutea* sample; by the frog method the *Digitalis purpurea* is again weaker (though not so markedly) while the *lutea* assays at the same strength.

White and Morris (4), could find no difference clinically or by assay between the strength of *Digitalis lutea* and the ordinary variety. In a recent paper, Konnerth and Pickering (5) have duplicated these results. We can confirm these observations when comparing *Digitalis lutea* and the English sample, but when comparison is made with the native-grown *Digitalis purpurea*, the lutea shows a definitely higher assay. Pratt and Morrison (6), also found that *Digitalis lutea* assayed higher than the great majority of their *Digitalis purpurea* samples. No difference such as the first observers remark, between *lutea* and *purpurea* (*i. e.*, absence of vomiting, convulsions, emptying of bowels with lutea but present with purpurea) were observed. We saw no indication of any of these (the bladder was usually emptied) in cats using infusions of either *Digitalis purpurea* or *lutea*.

4. *Effect of fertilizers.*—Dauphinee in his report, claims that cow manure was the most effective fertilizer that was used, increasing the yield of leaves, twice, but from his tables it does not appear to have increased the digitalis content of the leaf. Ammonium sulphate was of slight beneficial effect, while superphosphate was, if anything, detrimental to the culture of these plants.

Our experiments show the effect of only sodium nitrate as a fertilizer, but the results demonstrate that this chemical appears to have a very definite and marked ability to increase the digitalis content of *Digitalis purpurea*. The leaves gave the highest content of any examined and are approximately 23% more potent than the Standard by both the cat and frog methods, and 40% than the unfertilized *Digitalis purpurea* by both methods. Not only is the glucoside content of the leaf increased but also the actual yield of the leaf per acre, so that sodium

nitrate has a dual and very definite power to improve the production of digitalis.

It is extremely doubtful if any clinical difference would be observed between these fertilized and unfertilized plant preparations, in spite of the assayed difference of 40 per cent, because no difference has been found clinically between the preparations Standard and A (Magnus) which show a like discrepancy of 40 per cent assayed strength.

Thus the above results indicate that Canadian-grown digitalis, in the mixture of samples used commercially is quite up to the average strength. A sample consisting of equal parts of the *Digitalis purpurea* varieties of Canadian leaf examined should have a M. L. D. for a cat of 100.25 mg. per Kg. and for a frog 0.59 mg. per Gm. as compared with the values 96.55 mg. per Kg. and 0.60 mg. per Gm. found for the English sample.

Further, the results show that the use of infusions with the frog method is attended by two very considerable difficulties; difficulty in preparing an infusion of sufficient concentration which holds all the active glucosides contained in the leaf; and difficulty in obtaining absorption of such infusion from the lymph sac of the frog.

SUMMARY.

1. Using the Standard Digitalis powder by the cat method the M. L. D. was 18.74 cc. per Kg.; by the one-hour frog method 0.52 mg. per Gm. of frog. These are compared with the values obtained by other workers.

2. The results obtained by the cat method are compared with those obtained by the frog method, and the discrepancies that occur between the two methods, are again noted.

3. Comparison of the potency of *Digitalis purpurea*, purple blooms, with those bearing white blooms, shows very nearly equal values by the cat method, while by the frog method the former are definitely more potent.

4. *Digitalis lutea* assays definitely higher in glucosidal content than *Digitalis purpurea*, by both methods. There was no suggestion in the experimental work that *Digitalis lutea* is less toxic in its action than *Digitalis purpurea*.

5. A mixed sample of Canadian *Digitalis purpurea* shows a potency almost equal to that of the English-grown standardized leaf examined.

6. The effect of sodium nitrate as a fertilizer is to increase the yield of leaf per acre by 15 per cent, but more markedly it increases the glucosidal content of that leaf, 40 per cent more potent than the unfertilized *Digitalis purpurea*.

This work was suggested by Prof. V. E. Henderson, and was carried out under his direction, for which my thanks to him are due.

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