Papers Presented to Local Branches

OBSERVATIONS ON THE KEEPING PROPERTIES OF DIGITALIS AND SOME OF ITS PREPARATIONS.*

ROBERT A. HATCHER, M. D., AND CARY EGGLESTON, M. D.

The opinion is prevalent among both physicians and pharmacists that digitalis and its preparations undergo deterioration with considerable rapidity. Certain manufacturers have made much of this belief in the claims put forth regarding the advantages of their specialties, which, of course, are said not to be subject to such deterioration. In addition, however, to these obviously interested claims we find reports of great loss in activity of the leaf coming from men of such reputation as Focke,[1] who found deterioration amounting to 76 percent of the original value in two and three-fourths months in a leaf containing about 12 percent of moisture. He found a similar loss in one year in a leaf having 6 to 8 percent of moisture; leaves with 6.5 percent of moisture lost from 14 to 53 percent in strength in a year; those having 3 percent of moisture lost 15 percent in activity in the same period; and there was 5 percent loss in a year when the moisture had been reduced to 1.5 percent, the low point recommended by Focke to ensure the keeping properties of the leaf.

Houghton and Hamilton[2] report their results in a series of observations upon the loss of potency of different digitalis preparations. An extract of digitalis made by percolation with fairly strong alcohol showed, on tests of eleven samples, an average loss of activity of about 40 percent in a period of five years—an annual loss of about 8 percent. Eight samples of a fluidextract of digitalis, made according to the U. S. P. VII, with a menstruum of 62.5 percent alcohol, showed an average loss of 25 percent in six years—an annual loss of about 4 percent. Eleven samples of fluidextract of digitalis made according to the U. S. P. VIII, using 48 percent alcohol as the menstruum, showed an average loss of 10 percent per year, or a total loss in activity of 35 percent in three and one-half years. Lastly, six simples of tincture of digitalis made according to the U. S. P. VIII showed a loss in potency of 27 percent in three years—an annual loss of 9 percent. These results would seem to show that the official alcoholic fluid preparations of digitalis undergo deterioration at a rate ranging from 4 to 10 percent per year, varying somewhat in relation to their alcoholic content.

England[3] says of the commercial fluidextract of digitalis, "it is, largely, a concentrated hydro-alcoholic solution of certain proximate principles, or their decomposition products arising from the use of heat." He cites an observation of

*Read before the New York Branch, April 14, 1913.

Roger, giving no reference, however, to the effect that a 5 percent maceration of digitalis, when concentrated by 6.6 percent (sic!) by heat on a water-bath, deteriorated to such an extent that it required sixty times as much after concentration as before to yield its toxic dose.

Hale[4] cites the observations of others on the question of deterioration, and remarks that it would seem to be fairly well established that the leaves should be dried quickly and carefully, and be properly stored so as not to become moist. Hale thus accepts Focke's views, at least to a certain extent. He does not believe, however, that it is necessary to reduce the moisture in the leaves to as low as 1.5 percent, as suggested by Focke, and maintained by certain manufacturers who prepare a specialty along these lines. Hale reports that leaves which had been stored for eight years in a paper bag, and which contained 9.1 percent of moisture, gave a titre of 750 mg. per kilo of frog by the one hour method. Another sample which had been stored in a cloth bag for three years, and which contained 5.8 percent of moisture, required only 500 mg. to kill a kilo frog. A third specimen required 550 mg. per kilo of frog, although it contained 7.8 percent of moisture and had been kept in a paper bag for two years. Leaves kept in a cloth bag for a year, and having a moisture content of 9.4 percent, also gave a frog titre of 500 mg. per kilo. By way of comparison it may be stated that a fresh specimen of select English leaves, having 7.3 percent of moisture, showed 700 mg. per kilo of frog as its titre, thus: three of the old samples showed an activity greater than that of the fresh, high grade sample of English leaves. The fourth showed an activity about equal to that of the fresh English leaf, though it had been kept in a paper bag for eight years, and in spite of the fact that it contained 9.1 percent of moisture.

Hale found that a sample of mouldy leaves showed a deterioration of about 90 percent in one year, and he cites Focke as having found that a specimen which gave a valor of 4.36 showed a valor of only 1.6 a year later, having become mouldy in the interim. It would be a useless waste of time to consider these mouldy specimens further for, of course, they should never be used in any case.

Several observers have contended that heat caused deterioration in digitalis. Some of these are cited by Hale, who then gives some of his own observations which tend to show that temperatures below 120° C. maintained for a moderate length of time do not affect commercial samples of the leaf. This is also borne out by the recommendation of Focke to prepare the leaf for keeping by drying it rapidly with the aid of moderate heat.

Two tinctures of digitalis, made with 70 percent alcohol, in Hale's hands showed a frog titre after eight years which was equal to that of the average fresh tincture prepared from a high grade new specimen of English leaf. On the other hand, assays of a numbr of digitalis preparations obtained in the open market showed a little deterioration in twenty-two months. Three samples of official fluidextract lost 4.3, 6.9, and 8.7 percent, respectively, in this time. Four non-official preparations, obtained at the same time and under like conditions, showed deterioration from 14.3 to 33.3 percent in the same interval of time.

Moran[5] records a number of observations, which include some contradictory results, made upon different samples of tinctures of the same age; thus, one showed no deterioration in four years, while another is stated to have appeared

"to have deteriorated considerably," in the same time. He also tested a tincture which was twenty-four years old and one made from an extract which was nineteen years old. In the case of both of these he says that the activity was probably due to the saponin present, inferring that they retained no digitalis action at all. In the meager details that he gives, however, he states that the perfusion of 20 cc. of the twenty-four year old specimen through the heart of a frog caused "No tonic effect, acceleration of beat; systolic arrest." Of the tincture from the nineteen year old extract only 11 cc. were required to give, "No tonic effect; no slowing; systolic arrest." When the tincture which had not deteriorated was used slowing and tonic effect were observed and systolic arrest was caused by 12 cc. It is true that the typical digitalis action on the frog's heart is early slowing with the so-called 'tonic effect,' and systolic arrest is the typical end reaction. However, it is not infrequent to see a heart poisoned with digitalis react atypically with no slowing, or even with acceleration, and in any case the stage of slowing is usually soon followed by one of acceleration. It is quite possible that Moran's frogs happened to react atpically, or that the stage of slowing was brief and overlooked, the heart passing into that of acceleration. Clark[6] perfused frogs' hearts with digitonin, the saponin body of digitalis, and found that, while it caused "systolic effect," its action was, * * * produced instaneously, but it is not complete, the auricles and part of the ventricles continuing to beat for some hours." Further, he found that in the concentration of 0.01 mg. per cc. of Ringer's solution it has no action, while the action described above is produced when the concentration is raised to 0.1 mg. per cc. of fluid. It is probable that the results reported by Moran were not due to saponin alone, for it is doubtful if this substance is present in the tincture in sufficient concentration to have any effect upon the heart such as that described. This is supported by Kiliani,[7] who states that there are but the merest traces of digitonin in digitalis. Certain it is that the end reaction of systolic arrest is a typical digitalis action, and is not what Moran terms a "saponin effect." If we consider, as we are almost compelled to do, that the systolic arrest seen by Moran was due to digitalis action and not to saponin, then his twenty-four year old tincture still possessed 60 percent of the activity of his undeteriorated tincture, and the nineteen year old extract showed no deterioration.

Moran's own conclusions are to the effect that a tincture should retain its activity for two or three years, but it is difficult to interpret Moran's results.

Goodall,[8] in a note on the keeping properties of the tincture of digitalis concludes that the "tincture of digitalis probably retains its full activity for one year, but that after that period deterioration of its potency to an important extent is likely to take place." His experiments are not given in detail, hence it is impossible to determine the exact value which is to be placed upon his findings, particularly as the information given suggests certain decided defects of technic and control.

Haynes (cited by Goodall without reference) is stated to have found that tincture of digitalis would keep for two years without material change in activity. He kept his specimens in the dark.

We have cited sufficient evidence to show the trend of opinion, and it may be mentioned that the pharmacopoeias of several countries, namely, the French,

Swiss and German, require that the supplies of digitalis leaf be renewed annually. The German pharmacopœia has adopted the recommendations of Focke to the effect that the leaf should be dried over calcined line and kept in small, completely filled glass containers, protected from light and moisture.

In spite of the general consensus of opinion to the effect that age, moisture, light and heat, alone or variously combined, according to the observer, cause marked and rapid deterioration in digitalis leaves and alcoholic preparations, we long since came to a contrary opinion, for we had observed that samples of powdered leaf which had been in the laboratory in cardboard containers for several years, and tinctures prepared from these leaves at different times in the past few years retained their activity almost, if not quite, unimpaired. Stimulated by this apparent anomaly, we undertook an investigation of the question of deterioration of digitalis leaf and some of its preparations.

We began by making new tests of the activity of our own old samples of the leaf and of tinctures made therefrom. Comparing the results of these tests with the records of previous ones, we found that none of the specimens which were four or five years old showed any material deterioration. These samples of leaf and tincture had been kept without any special care, the tinctures being stored in glass-stoppered bottles and exposed to the light and temperature changes of the laboratory. The leaf, as has been mentioned, was kept in the original cardboard containers, and not protected in any way from either heat or moisture changes as these occurred in the atmosphere of the laboratory, but it should be said that the store room is unusually dry for this climate. The cat method was employed for the estimation of the activity of the specimens, and in some few instances we also used the one hour frog method with results quite in accord with those obtained with the cat. We sought to obtain some older specimens than ours, and, through the courtesy of E. R. Squibb & Sons, and Gilpin, Langdon & Company, we were supplied with samples of the leaf, ground and unground, tinctures, extracts, and fluidextracts ranging from less than one to more than thirty years old. With some of these we conducted tests on both cats and frogs.

A sample of German digitalis which had been kept in paper for three years on a jobber's shelf was received in the form of No. 60 powder and was found to contain 7.5 percent of moisture. It gave a cat unit of 110 mg. per kilo of cat weight. A sample of English leaf in fine powder, which had been kept on a shelf in paper for three years gave a cat unit of 128 mg., and it contained 6 percent of moisture. Both of these were considered by the jobbers as being entirely worthless except as specimens. The fallacy of this view is obvious, for each was found to have an activity about equal to that of the average fresh specimen of good quality. By the cat method the average unit for digitalis, in terms of leaf, is 100 mg. per kilo of cat weight; the range of variation in activity of different fresh specimens of good quality runs from 75 mg., for the most active samples, to 120 mg. for the less active. Since these two showed no deterioration we then examined the oldest specimen of leaf which we had obtained.

This was a sample of about 12 gm. of whole dried leaf which had been kept in a glass-stoppered bottle for not less than twenty-five years. The entire specimen was powdered and passed through a No. 60 sieve. After thorough mixing,

10 gm. of this powder were extracted as follows: The powder was moistened with 40 cc. of diluted alcohol (U. S. P.) and allowed to stand for twenty-four hours in a cylindrical percolator; it was then packed tightly and percolation was started; this was allowed to continue until about 30 cc. were obtained; percolation was then interrupted, maceration continuing until the following day, when percolation was again allowed to proceed until 100 cc. had been obtained.

Three tests by the cat method gave the following units: 74 mg., and 95 mg., and 82 mg., an average cat unit of 87 mg. per kilo. Perfectly fresh samples of the most active leaf which we have been able to procure have not shown a lower cat unit than 65 mg. per kilo. This twenty-five year old leaf was, therefore, of very high activity, better even than the average fresh specimen. The leaf was very dry and, although we did not determine its moisture content, we may assume, according to the statements of Focke,[1] that it contained much more than his required minimum of 1.5 percent, especially as the specimen had not been preserved with any particular care. This specimen, therefore, had almost certainly undergone no deterioration during the twenty-five years of standing.

The cat has been said, incorrectly we believe,[9] to be unsuitable for the detection of deterioration owing to the toxic nature of the products of such deterioration, but none of our cats showed atpyical effects.

We also examined this specimen by the one hour frog method, and found the fatal dose to lie between 900 and 1000 milligrams per kilo of frog, which is about 25 percent higher than the average as determined by Hale, and by Famulener and Lyons.[10]

It is probable, however, that the results obtained by the cat method are the more nearly correct in this case, for it is well known that frogs vary considerably in susceptibility to the digitalis bodies, such differences have been discussed fully in the article previously cited,[9] and we would refer the reader to that for confirmation of the statement.

Turning to the fluid preparations, we found that a sample of the fluidextract made over ten years ago gave a cat unit of 110 milligrams of leaf per kilo. This specimen was made with 50 percent alcohol as the menstruum, and probably showed no deterioration.

A sample of fluidextract of digitalis which was said to be "not less than thirty years old" was then tested on the cat, three tests giving units of 130, 162, and 153 milligrams per kilo, respectively, an average cat unit of 148 milligrams, the action being perfectly typical of digitalis. As we have no means of knowing the original activity of the leaf from which this fluidextract was made we might assume that it was of the average strength, that is, that it would originally have shown a unit of about 100 milligrams. On this basis we might suppose that in more than thirty years it had declined only about 40 percent in activity. As a matter of fact, it was more active by 32 percent than the average of thirteen specimens of fluidextract obtained in commerce in the present year, the explanation being that it is especially diffcult to prepare a fluidextract of digitalis which represents the full activity of the leaf.

This thirty-year-old fluidextract, having been made according to the Pharmacopoeia of 1870, had a menstruum composed of about 70 percent alcohol, 20 percent glycerin, and 10 percent water. Tests of this specimen by the one-hour

frog method gave a fatal dose of about 1300 milligrams per kilo of frog. This is almost certainly too high a figure, and may be attributable to the presence of glycerin in the preparation. Glycerin often delays absorption from the lymph-sac of the frog and makes the specimen which contains it seem weaker than it actually is[9], but this is without influence in the case of tests made on the cat by our method.

This specimen of fluidextract of digitalis had, therefore, probably undergone no deterioration in thirty years, since, as stated, it was far more active than the average *fluidextract* of digitalis now in use.

England[3] contends that heat, even when moderate and applied for a comparatively short time, causes enormous loss of activity in the fluid preparations of digitalis. Focke controverts this statement by the results of his experience in the concentration by heat on the water bath of aqueous infusions of digitalis when they are too weak to be tested on the frog. He recommends concentration by 50 percent, and finds that the process causes no reduction in activity. In this country nearly all of those who use the frog method of standardizing digitalis preparations employ heat to reduce the amount of alcohol before testing such preparations as the tincture.

To these statements with regard to the influence of heat we may add that we found a sample of solid extract of digitalis, which was made in 1908, and which was said to represent two and one-half times the weight of leaf, to have a cat unit of 52 mg. per kilo (that is, 128 mg. of the leaf). There was no obvious loss in activity, although the preparation had been reduced to the consistency of a solid extract by means of evaporation in the presence of heat.

At this point we decided to stop further testing of the dried leaf and of those pharmacopoeial preparations of digitalis made with a menstruum containing 50 percent or more of alcohol, for it was evident that deterioration does not occur to any considerable degree in such forms of the drug under ordinary conditions.

It is unnecessary to mention the infusion further than to state that frequent observations confirm the well-known fact that it is prone to undergo rapid deterioration even in the presence of a small amount of alcohol, such as is now used.

Deterioration of digitalis in the presence of water is further well illustrated by the following experience: We diluted a tincture of digitalis of known strength with nine parts of normal saline solution and set it aside, closely stoppered, for seventeen days. It was exposed to the light during this time, and for the most part was in an unheated room, though on some days it was exposed to a temperature of 70° F. for as much as five hours at a time. On the seventeenth day after dilution we tested this solution on cats and found a unit of 81.5 mg. of leaf per kilo. (Three tests, 84.6, 71.0, and 89.0 mg. per kilo, respectively). On the same day we tested the tincture from which the dilution had been made and found it to have a cat unit of 62.2 mg. of leaf per kilo (two tests, 61.8, and 62.7 mg., respectively). In a period of seventeen days, then, this aqueous dilution of a tincture of digitalis had lost 31 percent of its original activity. It is remarkable that it had not lost more than this, and the low temperature of the room may be partly responsible for its comparatively moderate deterioration.

The deterioration of aqueous preparations of digitalis has long been recog-

nized and this fact has recently been recalled by Cushny,[11] who says of strophanthus, squill and digitalis that "Their active principles readily undergo decomposition when the tincture is diluted with water. * * *"

We are disposed to remark that it is irrational to dispense the tincture of digitalis already diluted with water, or with an aqueous vehicle. The physician should order the necessary dilution to be made by the patient each time that he takes the prescribed dose, or should employ a vehicle containing a sufficient amount of alcohol.

There is one other preparation which deserves notice, only to be condemned. This is the acetic fluidextract. A sample of this preparation which was made in 1901 was found to be practically without digitalis action. In order to avoid the disturbing influence of the acetic acid present in the specimen 5 cc. were neutralized with an excess of sodium bicarbonate and evaporated on the water bath to a soft extract. This was treated several times, while still on the water bath, with strong alcohol, the alcoholic extract was decanted and evaporated. It was then taken up with 5 cc. of diluted alcohol, making a clear solution. This was further diluted with normal salt solution to make 50 cc. This solution was then tested on a cat in the usual way. At the end of an hour the animal had received a quantity which represented 1000 mg. of digitalis leaf per kilo. As the animal showed no perceptible effect save slight slowing of the heart (due, in all probability, to the fluid injected), it was released. Five hours later it had still shown no positive digitalis effect.

This same preparation—acetic fluidextract—was injected into the ventral lymp-sac of each of three frogs. The first weighed 14.5 gm. and received 0.25 cc. total, the second 21 gm. and received 0.5 cc. total, and the third weighed 21.5 gm. and was injected with 1 cc., an amount equal to about 5000 mg. per kilo of frog. None of the frogs died.

A second sample of acetic fluidextract of digitalis was tested to see if a fresh preparation was active. This sample was made on January 16, 1913, and was tested on the 29th of the same month, only thirteen days after its preparation. It was found to have a cat unit of 925 mg. per kilo, or, roughly, it had only about 10 percent of its supposed activity.

From the foregoing it is obvious that this preparation is worthless. This is only what is to be expected, for the decomposition of glucosides by dilute acids is universally recognized.

In addition to these tests of the leaf and galenical preparations we have tested some of the proprietaries with reference to their deterioration. One of these, which has been claimed to be permanent, namely, Digalen (liquid), gave the following results:

Two specimens obtained in 1912 were tested at the same time and one gave a cat unit of 1.52 cc. per kilo, while the other gave a unit of 2.45 cc. per kilo. A specimen obtained in 1908, and kept sealed as originally sent out, gave a cat unit of approximately 3 cc. per kilo when tested in November, 1912. In the case of the first two specimens, obtained fresh at the same time, the stronger was almost 100 percent more active than the weaker. The specimen of 1908 was only about half as active as the one of 1912. It is fair to assume that all of the batches of digalen are originally made of the same activity, and if this assumption be correct

this preparation is subject to far more rapid deterioration than either the digitalis leaf or its galenical preparations, which contain 50 percent or over of alcohol. The examples cited are but representative of our results with many different specimens of digalen.

It remains for us to discuss briefly some of the opposed findings here recorded.

All whose observations have been cited used frogs exclusively as the test animals in their determinations. Cloetta has contended that fresh digitalis contains little or no digitoxin, but that this constituent is developed during storage. It is known that digitoxin is irregularly and relatively slowly absorbed from the lymph spaces of the frog. If Cloetta's contention is correct the development of digitoxin during keeping would have a tendency to make the drug appear to have undergone deterioration when tested on the frog. On the other hand, such a change would not materially affect the activity of the drug when tested by the cat method, for in this the factor of absorption is entirely eliminated. The statement of Focke, that it is in the first few weeks after harvest that digitalis deteriorates most rapidly, and to the greatest extent, exactly coincides with the explanation just offered.

CONCLUSIONS.

1. Commercial digitalis leaves of good quality do not undergo any deterioration in many instances as the result of age; in a few cases they do appear to have deteriorated, but only with extreme slowness—at a rate probably not exceeding 1½ to 2 percent a year.

2. The same statement holds for the Pharmacopoeial preparations made with a menstruum containing at least 50 percent of alcohol.

3. Heat below 120° C., applied for a reasonable length of time, does not cause deterioration in digitalis leaves, aqueous infusions, or alcoholic preparations; in the latter case even though the preparation be reduced to a soft solid.

4. The acetic fluidextract is worthless.

5. Liquid Digalen is decidedly inferior to the alcohol-containing galenical preparations of digitalis in so far as permanency is concerned.

[1]Arch. d. Pharm., 1903, cxli, 128.
[2]Am. Jour. Pharm., Oct., 1909.
[3]Phil. Polyclinic, Jan., 1897.
[4]Hygien. Lab. Bull. No. 74, 1911.
[5]Medical Chronicle, No. 55, 1911-1912, p. 1.
[6]Brit. Med. Jour., 1912, II, p. 687.
[7]Arch. der Pharm., ccxliii, p. 7.
[8]Brit. Med. Jour., I, 1912, p. 887.
[9]Amer. Jour. Pharm., lxxxv, 1913, p. 99.
[10]Proc. Am. Pharm. Association, L, 1902, p. 415.
[11]Brit. Med. Jour., 1912, II, p. 685.

LABORATORY OF PHARMACOLOGY, CORNELL UNIVERSITY MEDICAL COLLEGE, New York City.