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### DETERIORATION OF THE TINCTURE OF DIGITALIS.\*

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The keeping qualities of the tincture of digitalis has been the subject of many investigations. In one of the earliest papers reporting the results of such a study, Houghton and Hamilton<sup>9</sup> concluded that there was a loss in the strength of the tincture with age. Two years later, Sharp and Lancaster and Sharp and Branson<sup>16,17</sup> presented evidence indicating that there was a definite loss in the activity of certain tinctures, becoming manifest at the expiration of 15 months. Goodall,<sup>3</sup> from the examination of 23 samples, arrived at about the same conclusions as those reached by the authors just mentioned. According to his assays, there was no loss in strength up to 14 months; but, after this, some of the samples showed a decrease in potency; and, at the end of 3 years, one tincture possessed less than one-third of its original toxicity. He cites Hanes as saying that the tincture keeps for two years without material change in activity, while Moran makes the claim that there is no important amount of deterioration up to 3 years. From assays on a number of liquid preparations of digitalis, Hale<sup>4</sup> concluded that the rate of deterioration differed in different cases, but that some occurred with age in all of his samples. O'Brien and Snyder<sup>11</sup> observed a rapid loss in the activity of a tincture, originally of a very high degree of potency, this loss amounting to 55% in the course of 2½ years. Even more remarkable rate of deterioration was reported by Schmidt and Heyl<sup>15</sup>—a tincture tested by them being found to have retained only 40% of its original strength at the end of 15 months. Indeed, all of the preparations examined by Schmidt and Heyl lost strength so rapidly that one is led to suspect that the conditions under which their experiments were carried out differed from the ordinary.

All of the investigators who have been cited employed one of the various frog methods in testing the specimens examined. While their conclusions as to the rate of deterioration are far from being in complete harmony, their results do agree in indicating that the tincture of digitalis loses strength in relatively short periods of time, when judged by the criterion of the frog test. From the meager reports of similar experiments carried out on guinea pigs, the same inference may be drawn. Employing the guinea-pig method, Pittenger and Mulford<sup>14</sup> reported a rate of deterioration surpassing even the unique observation of Schmidt and Heyl; for, after 7 months, a tincture tested by them retained little more than a quarter of its original toxicity. Only one of the specimens which Pittenger and Mulford examined failed to show deterioration; and they state “. . . that, in most cases, tincture of digitalis not only deteriorates, but deteriorates very rapidly.” As Hamilton remarks,<sup>5</sup> the acceptance of such “revolutionary” conclusions as these is tantamount to holding that the tincture of digitalis is a useless preparation;

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its strength, "in most cases," having been so reduced before the patient receives it that therapeutic results can scarcely be hoped for. It is well recognized that the guinea-pig method is accompanied by important sources of error, and it is at least possible that some of these were overlooked by the Philadelphia investigators. Indeed, in a later paper, Pittenger<sup>13</sup> seems to take a more optimistic view as to the keeping qualities of the tincture of digitalis, for he reports a maximum loss of activity amounting to 46% in 12 months, as compared with the former report of almost 75% in 7 months. Even though more experimentation and further reflection may cause a still greater modification of the views held as to the stability of the tincture of digitalis, as judged by the guinea-pig method of assay, it seems justifiable to assume that this method, as well as the frog methods, indicates a definite loss in activity of the tincture in time.

Opposed to the results obtained on frogs and guinea pigs are the findings of Hatcher and Eggleston,<sup>7</sup> when Hatcher's cat method of assay was employed. From numerous experiments on cats, these authors were convinced that deterioration of liquid preparations of digitalis containing 50% or more of alcohol did not occur to any extent worthy of consideration in reasonable lengths of time. The criticism has been offered<sup>14</sup> that these conclusions were based on a single assay of old preparations, the initial strength of which was unknown. The following quotation shows that this criticism is unfounded: "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 the previous ones, we found that none of the specimens which were four or five years old showed any material deterioration.*" (Italics ours.)

In the investigation by O'Brien and Snyder<sup>11</sup> previously referred to, a tincture and fluidextract of digitalis were assayed by the cat method and the assay repeated at the end of 2½ years. So far as can be gathered from their protocols, these authors used a single cat for each of the initial assays and one or two for each of the subsequent ones. From the results of these tests it was concluded that, judged by its toxicity for cats, the tincture had lost about 30% of its strength in the lapse of time mentioned. While their figures check remarkably well, it would seem unsafe to base conclusions as to the strength of a digitalis preparation on a determination of its toxicity for one or even two cats; especially, when these conclusions are so opposed to those drawn by the two men most experienced in the use of the cat method. It was felt that further study would be of interest, not only as regards the rate of deterioration as determined by the cat method, but, possibly, also by shedding some light on the relative value of the cat and frog methods as means of determining the therapeutic worth of digitalis preparations.

In the fall of 1916, the Department of Pharmacy of the Medical College of Virginia was furnished with twelve samples of digitalis leaf, grown in the vicinity of Richmond by Mr. J. W. Wilber. The plants were all of the first year's growth and all of the species *purpurea*; from the appearance of the leaf, however, they seemed to represent twelve distinct varieties. Tinctures were made from these samples, according to U. S. P. IX; but, unfortunately, we were not informed as to the manner of drying the leaf nor the exact date of percolation. (The digitalis was percolated some time during the months of November or December 1916.) Dur-

ing January, February, March, and April 1917 all of the samples were tested by the one-hour-frog method; nine by the cat method, and four by the guinea-pig method. By all three methods, an unusual degree of potency was shown; at present, however, we shall consider only the results obtained by the cat method.

In testing the samples on cats, we followed, in essentials, the directions of Hatcher and Brody.<sup>6</sup> Only adult animals were used; in a few instances, a lactating or pregnant female was included in a series, but only when the reaction of such an animal showed no marked deviation from the others used in the series. It has been our experience, in accord with that of Hatcher<sup>6</sup> and Morris,<sup>10</sup> that lactation or pregnancy may markedly influence the resistance of a cat to poisoning by digitalis, but this is by no means invariably the case. As a rule, ether was used for anesthesia; occasionally, chloretone, 0.2 gram per kilogram body weight, was administered orally or intraperitoneally. The tincture was diluted with either nine or nineteen parts of physiological saline, and the injections were made into the femoral vein from a burette. The extremes for the time consumed in the injections were 28 minutes and a little less than two hours; in most cases it averaged about 45 minutes. In the later tests, a rate of injection of 1 cc of the diluted tincture every 2½ minutes was arbitrarily adopted, regardless of the strength of the sample or weight of the cat. This seemed justifiable, in view of the fact that the samples showed no extreme variations in strength and the weight of the cats employed in most of the tests was fairly uniform. In the original communication of Hatcher and Brody<sup>6</sup> it was stated that a sublethal dose of the tincture should first be injected intravenously and, after an interval of about fifteen minutes, a solution of ouabain should be slowly injected until the death of the animal occurred. In no instance did we use this "combined method;" the diluted tincture alone was slowly injected until death was produced.

After completion of the initial assays, the tinctures were kept in flint, cork-stoppered bottles, in a laboratory frequently unheated for considerable periods of time in the winter and unusually hot in summer, being directly under the roof. From time to time most, if not all, of the bottles were opened, and small amounts of the contents taken out, the frequency with which this was done varying in the different cases. Several of the samples were retested in 1921, but no systematic examination was undertaken until April, May, and June of the present year, when all nine of the samples were again assayed on cats. The results of the initial assays (1917) and the later assays (1922) when considered separately seemed satisfactory, but, when the figures of the different assays were taken together and subjected to critical analysis, it was evident that only those obtained for four of the samples could be relied upon. The unsatisfactory outcome of the assays of the other samples was due to imperfections of the method in our hands; in order to make these clear, a lengthy discussion of the cat method would be necessary. This we do not wish to enter into now.

TABLE I.—SUMMARY OF RESULTS OF CAT ASSAYS IN 1917 AND IN 1922.

| No. of sample. | First assay in<br>mg. per Kg. | Time lapse<br>in months. | Second assay in<br>mg. per Kg. | Percentage<br>change. |
|----------------|-------------------------------|--------------------------|--------------------------------|-----------------------|
| 9              | 55.7                          | 64                       | 62.5                           | -12.2                 |
| 31             | 68.2                          | 61                       | 70.0                           | - 2.6                 |
| 36             | 42.2                          | 63                       | 40.8                           | + 3.3                 |
| 39             | 71.2                          | 64                       | 64.0                           | +11.2                 |

The figures for the assays of these four samples, both in 1917 and in 1922, are given in Table I.

It is evident from these figures that the four samples have undergone no appreciable change in the period of a little over five years. It has been claimed that the method permits the estimation of digitalis preparations with an error not greater than 10%; the changes indicated by the assays practically are within this limit. It would seem unlikely that of nine samples prepared from leaf of the same source and in exactly the same manner, four would prove stable and the remainder show deterioration when all were kept under the same conditions. Unfortunately, as has been mentioned, our results do not justify any conclusions in regard to the remaining five samples; they do show, however, that two closely checking cats do not prove the strength of a tincture of digitalis. This, of course, disproves the contention of O'Brien and Snyder<sup>11</sup> in regard to deterioration of their specimens, because their claim is based upon an entirely inadequate number of animals. On the whole, our results on all of the samples are in harmony with those of Hatcher and Eggleston,<sup>7</sup> and they have convinced us, at least, that tincture of digitalis shows no appreciable loss in activity in reasonable lengths of time, when judged by the criterion of the cat method of assay.

From the review of the literature bearing on the uses of a frog method in aging experiments, it seems fairly conclusively shown that tincture of digitalis loses strength in comparatively short periods of time as judged by this test. Accepting the view that the cat method fails to show deterioration of the tincture while the frog method does indicate such a change, what explanation is to be offered for the different results of the two methods? As is well known, absorption plays an important part in the frog and guinea-pig methods of assay, while in the cat method, since intravenous injection is employed, the question of absorption is eliminated. So far as we can ascertain, Hatcher and Eggleston<sup>7</sup> are the only ones who have remarked on this very obvious explanation for the difference in the results obtained by the different methods. According to these authors, Cloetta has stated that digitoxin is developed in the tincture of digitalis only on standing and, since digitoxin is irregularly and poorly absorbed from the lymph spaces of the frog, a conversion of some of the absorbable active constituents of the tincture into the poorly absorbable digitoxin would make it appear that the preparation had deteriorated when assayed by the frog method. A question of practical importance is whether a similar decrease in absorbability from the human gastro-intestinal tract also takes place in time. Digitalis is generally administered orally, and, if it is poorly absorbed, this is equivalent to a loss in strength, suggesting that the question of absorbability from the lymph sac of the frog, long considered a serious drawback to the method may, in fact, furnish us with important information which is not obtained when the cat method is employed. Attention has recently been called to the occurrence of tinctures apparently active when tested on the cat but unsatisfactory when employed clinically. The suggestion has been made that the failure to obtain therapeutic results with these preparations was due to a delay in or absence of absorption from the human alimentary tract. Though it has been assumed<sup>12</sup> that absorption from the lymph sac of the frog parallels that from the human intestine, this has not been proved; indeed, the investigations of Hatcher and Eggleston<sup>2</sup> incline to the opposite conclusion. Experiments which are in prog-

ress in this laboratory indicate that the change which occurs in the tincture of digitalis as the result of age does not interfere seriously with absorption from the alimentary tract of the cat.

#### CONCLUSIONS.

1. From the published results it seems clearly shown that the tincture of digitalis loses strength rather rapidly, as judged by the frog or guinea-pig method of assay.
2. With Hatcher's cat method, when sufficient numbers of cats are used to justify inferences, no apparent loss of strength in the tincture of digitalis can be detected in a period of as long as five years.
3. The difference in the results obtained by the two methods is probably explainable by the reference to a decrease in the absorbability of the tincture from the lymph sac of the frog.
4. Aging does not cause any marked decrease of absorbability of the tincture from the alimentary tract of the cat.

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