THE EFFECT OF AGE ON THE ACTIVITY OF TINCTURE OF DIGITALIS.

BY CHAS. C. HASKELL.

Numerous investigations have been carried out in the attempt to determine the effect of age on the potency of digitalis. The results of the earlier studies, in which frogs were used as test animals, were in agreement, indicating a definite loss in the strength of liquid digitalis preparations in even short periods of time. On the other hand, employing the cat method, Hatcher and Eggleston (1) came to the conclusion that deterioration of the ticture of digitalis occurred to only a negligible extent in several years. Experiments carried out on cats in this laboratory led us to the same conclusion; where a sufficient number of animals was used in the initial and later assays, there was no evidence of deterioration to a significant extent in the course of five years (2). To what are these different results attributable? The most plausible explanation, advanced by Hatcher several years ago (1), is that the tincture of digitalis undergoes some change which interferes with its absorbability from the lymph sac of the frog but does not materially alter its potency after entrance into the circulation. Naturally, this raises the question whether a similar decrease in absorbability from the mammalian alimentary tract does not take place with the lapse of time; a question of the greatest importance in the selection of a method for assaying digitalis preparations. If, with age, a tincture of digitalis becomes less absorbable from the human alimentary tract, it is obvious that this preparation would prove less efficacious when administered orally, and the assay by the cat method, where the drug is injected intravenously, would give misleading figures. On the other hand, the employment of the frog method for the assay of an old preparation intended for intravenous injection might lead to disaster, since it would indicate too low potency for the preparation.

In the winter of 1922, there were available three tinctures of digitalis which seemed well suited for the investigation of his question. One of these tinctures, No. 36, had been percolated in the winter of 1916 from leaf grown in the vicinty of Richmond. In the early part of 1917, it was assayed by the one-hour frog method and by the cat method of Hatcher and Bordy. By both methods, the preparation seemed unusually active; unfortunately, the frogs were not standardized with ouabain, but the animals were in good condition and the assay was carried out at temperatures from 18° to 22° C. In the spring of 1922, the assays by both methods were repeated; the result by the frog method indicating a considerable loss in activity; while the slight differences in the figures obtained at the initial and later assays on cats is well within the limit of error. The results of these assays are given in Table I.

TABLE I.—ASSAYS OF TINCTURE OF DIGITALIS 36 AT FIVE-YEAR INTERVALS.

Date of Assay.	Cat Assay mg. × Kg.	Frog Assay cc. × Gm.
1917	42.2	0.0015
1922	41.1	0.0035

The second tincture was percolated in the spring of 1922; Virginia-grown leaf being used for its preparation also; the third tincture was percolated at the same time from leaf procured from a commercial grower. These two tinctures were

numbered 45 and Z-1 respectively. The assays of all three of these tinctures, carried out in the spring of 1922, are given in Table II.

TABLE II.—ASSAYS OF TINCTURES Z-1, 36, AND 45 IN SPRING OF 1922.

No. of Tr.	Cat. assay mg. × Kg.	Cat ratio.	Frog assay cc. × Gm.	Frog ratio.
Z-1	38.44	100.0	0.0025	100.0
36	41.10	93.6	0.0035	71.4
45	74.19	51.8	0.0035	71.4

It is apparent that Z-1 is the strongest preparation by both assay methods; by the cat method, 36 is practically as strong as Z-1; and both of these tinctures are almost double the strength of 45. By the frog method, the old tincture, 36, appears to be decidedly weaker than Z-1, and is just equal in potency to 45.

The first absorption experiments were carried out on cats, utilizing the procedure suggested by Hatcher. The animals were deprived of food for twelve hours and were then given 1 cc. of the selected tincture per kilogram body weight orally, the tincture having been diluted with nine parts of water. In exactly one hour after administration of the tincture, 0.2 Gm. of chloretone per kilogram was injected intraperitoneally, the drug being dissolved in cotton-seed oil. Within thirty to forty minutes, the majority of the animals were sufficiently anesthetized to permit of the operative procedures incident to the insertion of the cannula into the femoral vein; occasionally, it was necessary to supplement the chloretone with a little ether. In exactly two hours after administration of the tincture, the intravenous injection of ouabain, 1 to 100,000 in saline, was commenced, the rate of injection being 1 cc. every two and a half minutes until the death of the animals. Assuming that the average fatal dose of ouabain is 0.1 mg. per kilogram, it is obvious that the difference between this amount and that actually required to cause death may be ascribed to the digitalis which has been absorbed from the alimentary canal. The amount of absorption may be expressed as a fraction of a lethal dose, and, since the same volume of each preparation was administered orally, the proportion of a lethal dose would indicate the potency of the preparation by this method of administration.

There are several possible sources of error in such experiments; individual difference in absorbing power; individual variations in resistance to the digitalis after absorption; and, finally, individual differences in resistance to the injected ouabain. It is to be expected, therefore, that the results in the different cats would show divergent figures for absorption; an expectation which was fulfilled. Thus, of the 23 cats used with 36, the indicated absorption varied from zero to 62% of a lethal dose; of the same number of animals used for 45, from zero to 54% of a lethal dose; of the ten cats used for Z-1, from zero to 51%. Nevertheless, the averages for all the cats seem clearly to show that the fresh tinctures are more readily absorbed from the intestine of the cat than is the case with the old tincture. The averages are given in Table III.

TABLE III.—ABSORPTION OF TINCTURES Z-1, 36, AND 45 FROM THE INTESTINE OF THE CAT.

No. of Tr.	No. of cats used.	Per cent of L. D. absorbed.
Z-1	10	30.0
36	23	20.6
45	23	19.4

It is interesting to compare the relative potency of the three tinctures, as judged by the different methods: frog assay; cat assay; and absorption from the intestine of the cat. This is done in Table IV.

TABLE IV.—RELATIVE POTENCY OF Z-1, 36, AND 45 BY DIFFERENT METHODS.

No. of Tr.	Frog ratio.	Cat ratio.	Absorption ratio.
Z-1	100.0	100.0	100.0
36	71.4	93.6	68.6
45	71.4	51.8	64.6

In spite of the many sources of error, there is a rather remarkable agreement between the results of the frog assay and the absorbability from the intestine of the cat; these being quite at variance with the figures of the cat assay.

Hatcher has stated that absorption from the cat's alimentary tract parallels that from the human intestine. It seemed desirable, however, to secure additional evidence regarding the loss of absorbability undergone by the tincture of digitalis with the passage of time; in order to do this the absorbability of a fresh and of an old tincture from the intestine of dogs was investigated. The same procedure was employed as in the case of the cats; the dogs were starved twelve hours; were then given 1 cc. of the tincture per kilogram body weight orally; in one hour, they received 0.2 Gm. chloretone per kilogram body weight intraperitoneally; and the intravenous injection of ouabain was commenced exactly two hours after the oral administration of the tincture. Only two preparations were used in these experiments; 36 (old) and 45 (fresh). The results of these tests are given in Table V; the amount of absorption being expressed as a fraction of the lethal dose; the lethal dose of ouabain being placed at 0.137 mg. per kilogram.

TABLE V.—ABSORPTION OF TINCTURES 36 AND 45 FROM INTESTINE OF DOG.

No. of Tr.	No. of dogs used.	Per cent of fatal dose absorbed.
36	12	24.0
45	11	27.4

Here, as in the case of the cats, the amount of absorption indicated is about equal for the two tinctures. Indeed, although the cat method leads one to infer that 36 is almost twice as strong as 45, the latter is absorbed in greater amount in the two-hour interval. The individual variations seen in the case of the dogs were much less evident than in the case of the cats; of the twelve dogs used for 36, the indicated absorption varied from 8% to 40% of a lethal dose; of the eleven used for 45, from zero to 55% of a lethal dose; the remaining dogs in the two series ran quite close to the averages given.

It is, of course, most desirable that this question should be studied clinically. However probable it is that absorption of tincture of digitalis from the human alimentary tract parallels that from the intestine of the frog and of the cat, it has not been definitely proved. If clinical observations do show that age decreases the absorbability of tincture of digitalis from the human gastro-intestinal tract to the same extent as it does absorbability from the lymph sac of the frog, a feature of the frog method which has been looked upon only as a disadvantage must be considered as very definitely in its favor.

The absorbability of tincture of digitalis from the alimentary tract may be influenced by more than one factor; and there is no justification for the assumption that absorption of this preparation from the frog's lymph sac always parallels that from the mammalian intestine. Certainly, however, it seems proper to conclude that the change in tincture of digitalis which occurs with age and which lessens the absorbability of this preparation from the lymph sac of the frog affects the absorbability from the intestine of the cat and of the dog to practically the same extent; and, consequently, the frog assay method more truly indicates the potency of such preparations which are intended for oral administration.

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STUDIES IN THE GENUS MENTHA. VI. THE VOLATILE OIL OF A STRAIN OF JAPANESE PEPPERMINT GROWN BY THE WISCONSIN PHARMACEUTICAL EXPERIMENT STATION, AT MADISON.[1]

BY G. C. JENISON[2] AND R. E. KREMERS.[3]

The Japanese oil of peppermint holds a unique place in the world's commerce because of the comparative ease with which crystalline l-menthol is obtained from it. Accordingly, attempts have been made from time to time to introduce strains of Japanese plants into other countries. These attempts have been by no means uniformly successful, and taken in conjunction with what is reported concerning the culture of mints in Japan itself, have only obscured the situation both botanically and economically, instead of clarifying it. The propagation of the strain under present consideration belongs in the category of those failures that have resulted in oils "deficient in menthol." Usually the matter has been dropped at this point. The further investigation of this "deficiency in menthol," especially as to what took the place of menthol, has led in this instance to some very interesting results. The theoretical consideration of these results is left to a later paper. For the moment only the actual examination of the oil will be discussed.

Material.—The strain of Japanese peppermint which yielded the oil for these investigations has been grown for some years by the Wisconsin Pharmaceutical Experiment Station in its gardens in the vicinity of Madison, Wisconsin.[4] The

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[4] The Station gardens are under the immediate supervision of Prof. W. O. Richtmann. The authors gratefully acknowledge their indebtedness to him for the large amount of material made available by his efforts.