

- (11) Weiss, *Muench. Med. Wochenschr.*, 82 (1935), 748.
 (12) Emmert and Schmidt, *Anesth. & Analag.*, 18 (1939), 274.
 (13) Schmidt, *Journal-Lancet*, 59 (1939), 26.

Further Observations on the Influence of the Anesthetic on the Results of Digitalis Assay by the Cat Method of Hatcher and Brody

By Chas. C. Haskell

In 1936, data were presented, which established that the cat unit for certain preparations of digitalis was materially higher when a non-volatile anesthetic, dial-urethane solution, was substituted for ether in the Hatcher-Brody method (9). The only earlier published reports regarding the possible influence of a non-volatile anesthetic on the size of the cat unit encountered were in the papers of Epstein (4), David and Rajaminickam (2), and Bauer and Fromherz (1), but, with the possible exception of the last-mentioned, the number of experiments performed by these authors seems scarcely to justify the conclusions drawn. This criticism is not applicable to the later publication by Edmunds, Moyer and Shaw (3), in which it was pointed out that, with animals under urethane anesthesia, the cat unit for the International Powder was considerably higher than was the case when etherized animals were used. The practical importance of departing from the original technique of the Hatcher-Brody method by substitution of a non-volatile anesthetic is immediately obvious; in addition, the interesting question arises why etherized cats succumb to smaller doses of digitalis than do those under the influence of dial-urethane or urethane. Does ether in some way directly lower the resistance of the cats to digitalis poisoning or, on the other hand, do the non-volatile anesthetics mentioned oppose, in some specific manner, the lethal action of this drug? Another possibility, suggested by Dr. Harry Gold (5), is that the influence of the non-volatile anesthetic is indirect; by prevention of struggling, it delays the onset of ventricular fibrillation, so prone to

be precipitated by a struggle in unanesthetized cats after large doses of digitalis. If it is found that other non-volatile anesthetics, chemically unrelated to dial or urethane, affect the resistance of cats to digitalis as do these latter, it would tend to support, but not establish the correctness of Dr. Gold's explanation.

David and Rajaminickam (2) assayed a specimen of the International Powder on five series of cats, using ether, urethane, chlorobutanol, chloralose or paraldehyde in the different series. They report that the chlorobutanol series gave the largest cat unit; the smallest being obtained from the chloralose series; while that from the etherized cats was intermediate in size. Such results would seem to indicate that the various anesthetic agents acted in a specific manner to affect the resistance of the cats to digitalis intoxication, but when it is taken into consideration that the number of animals in each series was only five, except in case of paraldehyde, where four were used, it is obvious to anyone familiar with the practical use of this method that the differences recorded are without significance. It is of interest to note that David and Rajaminickam found the ether cat unit larger than when urethane was used, in contrast to the results obtained by Edmunds, Moyer and Shaw (3) from a much larger number of animals. As already stated (8) a limited number of experiments in our laboratory in which the cat unit for animals anesthetized with chlorobutanol was compared with that obtained with etherized cats, failed to show any material difference, but here, too, the number of experiments was far too small to justify positive conclusions. It seemed desirable, therefore, to make further observations, in the attempt to determine whether the substitution of chlorobutanol for ether anesthesia affects the size of the cat unit.

EXPERIMENTAL

In the first experiments, the procedure was similar to that employed in comparing results under dial-urethane and ether anesthesia. Groups of ten cats were used for each assay; five of the animals received 200 mg. chlorobutanol per Kg. intraperitoneally, a 40% solution in ethylene glycol being used; the other five being lightly etherized. The

digitalis solution, diluted with 0.9% sodium chloride, was injected into the femoral vein at the rate of approximately 1 cc. per 2.5 Kg. body weight every 2½ minutes. The results of these experiments are given in Table I.

Examination of these tabulated results shows that the cat unit for five of the six preparations tested was larger when chlorbutanol anesthesia was used. In the case of Glu. 1036-5313, the difference is small and with Tr. Digitalis S.26, the "ether unit" was actually larger than the "chlorbutanol unit." It should be borne in mind, however, that the cat units given here represent the average from groups of only five animals; as will be shown later, assays of Tr. Digitalis S.26 on forty cats under chlorbutanol anesthesia gave a cat unit of 12.53 cc. of the diluted solution. Even if the supposedly "aberrant" results with Tr. Digitalis S.26 are in-

the others receiving chlorbutanol. Three preparations of digitalis were used; one assay was carried out on two of each of these and eight were carried out on the third; Tr. Digitalis S.26. The results are given in Table II.

With the exception of the third assay of Tr. Digitalis, S.26, the differences in the size of the cat units with the animals under chlorbutanol or dial-urethane anesthesia are slight and lie well within the limit of error for the method when only five cats are used in each assay. In view of the fact that the remaining thirty-five cats anesthetized with chlorbutanol and used for the assay of Tr. Digitalis, S.26 gave a cat unit of 12.81 cc., it is probable that the figure obtained from the average of the individuals in the third group, 10.50 cc., is too low. The separate results in this group were 8.95, 9.67, 11.07, 11.30 and 11.49 cc., so that the use of Van Wijjn-

Table I.—Results of Assays under Chlorbutanol and Ether Anesthesia

Preparation	Cat Unit ^a		Percentage Difference
	Chlorbutanol	Ether	
Glucosides 1036-5313	9.88 cc.	9.56 cc.	3.3
Ampule 5 cc. Sp.	15.25 cc.	10.67 cc.	42.9
Ampule K.	11.97 cc.	8.76 cc.	36.6
Tr. Digitalis W., Conc.	6.15 cc.	3.99 cc.	54.1
Tr. Digitalis, S.26	9.14 cc.	10.16 cc.	-11.2
Tr. Digitalis, S. K.36	10.82 cc.	8.87 cc.	21.9
	Average percentage difference ^b		24.6

^a The Cat Unit is expressed in cc. of the diluted solutions used.

^b The "average percentage difference" is obtained by taking the average of the individual percentage differences.

Table II.—Results of Assays under Chlorbutanol and Dial-Urethane Anesthesia

Preparation	Cat Unit ^a		Percentage Difference
	Chlorbutanol	Dial-Urethane	
Liq. No. 64783	14.72 cc.	15.80 cc.	D- 7.3
Amp. No. 42453	12.01 cc.	12.22 cc.	D- 1.7
Tr. Digitalis, S.26	13.67 cc.	14.05 cc.	D- 2.7
Tr. Digitalis, S.26	11.18 cc.	12.01 cc.	D- 7.4
Tr. Digitalis, S.26	10.50 cc.	13.07 cc.	D-24.5
Tr. Digitalis, S.26	13.70 cc.	12.47 cc.	C- 9.8
Tr. Digitalis, S.26	13.12 cc.	11.84 cc.	C-10.7
Tr. Digitalis, S.26	12.93 cc.	13.33 cc.	D- 3.1
Tr. Digitalis, S.26	13.42 cc.	12.74 cc.	C- 5.3
Tr. Digitalis, S.26	11.70 cc.	13.07 cc.	D-11.7
Average	12.69 cc.	13.06 cc.	...
	Average percentage difference ^b		D- 2.9

^a The "Percentage Difference" is expressed as percentage the difference is of the smaller cat unit; preceded by "D" indicates that the unit under dial-urethane anesthesia was the larger; preceded by "C" that the chlorbutanol unit was the larger.

^b The "Average percentage difference" is obtained by determining the percentage that the difference between 12.69, the chlorbutanol average and 13.06, the dial-urethane average, is of the smaller of these two figures; 12.69.

cluded and the differences expressed as percentages of the smaller cat unit, it is found that the "chlorbutanol unit" is 24.6% larger than the "ether unit." A similar comparison between the results with dial-urethane and ether anesthesia, seven different digitalis preparations being used on a total of ninety-nine cats, showed that the "dial-urethane unit" was consistently larger, the average percentage difference amounting to 30.4 (8).

Because of the greater convenience, dial-urethane solution was substituted for ether in a continuation of the experimentation, 0.6 cc. of this solution per Kg. being given to five of each group of ten cats;

gaarden's formula, in which such implicit confidence seems placed by certain authors, fails to support the view that the cat unit does not represent the true strength of the tincture (12).

Taking the average of all the group assays, the difference between that for the chlorbutanol groups, 12.69 cc., and the dial-urethane groups, 13.06 cc., is only 0.37 cc., or slightly less than 2.9% of the smaller average. From these results, as well as from the direct comparison between chlorbutanol and ether, it seems safe to conclude that the cat unit for a digitalis preparation, as determined on animals under the influence of chlorbutanol anes-

thetia, is definitely higher than that obtained by the use of etherized cats; and further, that no material difference in the size of the cat unit exists whether the determination is made on animals under the influence of chlorbutanol or dial-urethane anesthesia.

The fact that two chemically unrelated non-volatile anesthetics, chlorbutanol and dial-urethane solution produce essentially the same deviation from the cat unit as determined on etherized cats certainly suggests that there is nothing specific in the influence of these drugs in affecting resistance to digitalis poisoning. One must consider the pos-

were allowed to inhale ether until complete cessation of respiration from central action of the drug occurred. In spite of the institution of artificial respiration by means of manual compression, three of the fifty animals succumbed to the ether. The remainder, on their resuming spontaneous respiration, were kept deeply etherized for the operative procedures and until the venous cannulas had been inserted into all members of the group. Five cats of each group received the customary dose of the dial-urethane, 0.6 cc. per Kg. intraperitoneally, with no preliminary etherization. The results of these experiments are given in Table III.

Table III.—Results of Assays under Dial-Urethane Anesthesia Alone or Preceded by Ether

Preparation	Cat Unit		Percentage Difference
	Dial-Urethane Preceded by Ether	Dial-Urethane Alone	
Amp. 65483	10.37 cc. ^b	12.55 cc.	21.0
Glu. 1036-3888	10.42 cc.	11.62 cc.	11.5
Glu. 1036-3888	9.13 cc.	10.33 cc.	13.1
Glu. 1036-3888	10.67 cc.	12.38 cc.	16.0
Tr. Digitalis, S.26	11.62 cc.	12.60 cc.	7.6
Tr. Digitalis, S.26	9.85 cc. ^b	12.75 cc.	33.5
Tr. Digitalis, S.26	10.26 cc.	11.31 cc.	10.2
Tr. Digitalis, S.26	12.10 cc.	11.47 cc.	- 5.5 ^a
Tr. Digitalis, S.26	11.64 cc. ^b	12.36 cc.	10.6
Tr. Digitalis, S.26	11.73 cc.	11.86 cc.	1.1
	Average percentage difference ^c		10.6

^a Preceded by minus sign because the cat unit from the animals under dial-urethane is smaller than that from the animals previously etherized.

^b Average of four cats.

^c The average percentage difference is obtained by determining the percentage that the difference between 10.78 cc., the average for the series in which ether and dial-urethane were used, and 11.92 cc., the average for the dial-urethane series is of 10.78.

sibility that ether lowers the resistance of the animals, but that this is not the case seems probable from experiments carried out in our laboratory by Haag (7) eleven years ago. Twelve cats, under the influence of nitrous oxide-oxygen anesthesia, were decerebrated by the method of Pollock and Davis (11) and the cat unit for a tincture of digitalis as determined on them was found to be 66.2 mg., as compared with a cat unit of 75 mg. obtained by the use of ten etherized cats. MacDonald and Schlapp (10) later made use of spinal cats, being of the opinion that the animals reacted more uniformly than was the case when ether was employed, but from their paper, it is not clear whether they tested the same tincture on spinal and on etherized cats. Of course, the condition of a decerebrated or spinal animal is far from normal; it is quite possible that the operative procedure may render them less resistant to the toxic action of digitalis; indeed, Gold has shown that hemorrhage has such an influence (6). In our next experiments, therefore, the attempt was made to determine whether deep and rather sustained ether anesthesia prior to and at the beginning of the injection of the digitalis solution, followed by administration of dial-urethane would give a cat unit differing from that obtained with the animals under the influence of this latter throughout.

As in all the preceding experiments, groups of ten cats were used in each assay. Five of each group

In Table III, it is shown that, with the exception of a single assay of Tr. Digitalis, S.26, the cat unit was smaller when determined on animals that had been etherized prior to administration of dial-urethane solution. The significance of these differences is hard to appraise; omitting the first, fourth and sixth assays, they are so small as to fall within the limit of error for the method when only five animals are used. The largest differences were encountered in the first and sixth assays; in both these, only four cats were available for the ether-dial-urethane groups, the fifth animal in each group having succumbed to ether poisoning. Another possible contributing factor in bringing about the differences was the method of manual compression employed after respiration had failed in the etherized cats; unfortunately, the condition of the lungs was not examined post mortem. Disregarding these considerations, however, it is found that the average from all of the cats anesthetized with dial-urethane alone is not very much larger than that from the animals that had been etherized before injection of the dial-urethane solution; the former being 11.92 cc. and the latter, 10.78 cc., the difference being 1.14 cc. or 10.6% of the smaller average. It seems safe to conclude, therefore, that if ether does lower resistance of cats to poisoning by digitalis, under the conditions of the above described experiments, it does not do this to any material extent.

SUMMARY

Briefly summarizing, it would appear that: The cat unit for the digitalis preparations examined is, on the average, considerably higher if chlorobutanol anesthesia is substituted for ether in the performance of the assay by the cat method of Hatcher and Brody.

The cat unit, as determined on cats under the influence of chlorobutanol anesthesia, is practically identical with that obtained by the use of animals under dial-urethane anesthesia.

Profound etherization of the animals prior to administration of dial-urethane for anesthesia does not lead to a reduction of the size of the cat unit to any marked extent if a non-volatile anesthetic, dial-urethane, is subsequently given in dose sufficient to prevent struggling.

BIBLIOGRAPHY

- (1) Bauer and Fromherz, *Arch. exp. Path. Pharmacol.*, 172 (1933), 693.
- (2) David and Rajaminickam, *Quart. J. Pharm. and Pharmacol.*, 7 (1934), 36.
- (3) Edmunds, Moyer and Shaw, *Jour. A. Ph. A.*, 26 (1937), 290.
- (4) Epstein, *Quart. J. Pharm. and Pharmacol.*, 6 (1933), 169.
- (5) Gold, Personal Communication.
- (6) Gold, *J. Pharmacol. and Exp. Ther.*, 31 (1927), 291.
- (7) Haag, *Jour. A. Ph. A.*, 16 (1927), 516.
- (8) Haskell, *J. Pharmacol. and Exp. Ther.*, 58 (1936), 111.
- (9) Hatcher and Brody, *Am. J. Pharmacy*, 82 (1910), 360.
- (10) MacDonald and Schlapp, *Quart. J. Pharm. and Pharmacol.*, 111 (1930), 450.
- (11) Pollock and Davis, *Arch. Neurol. Psychiatry*, 10 (1923), 391.
- (12) De Lind van Wijngaarden, *Arch. exp. Path. Pharmacol.*, 113 (1926), 40.

The U. S. P. XI Digitalis Standard

By Orlo F. Swoap and Marvin L. Pabst*

The adoption in the Eleventh Decennial Revision of the United States Pharmacopœia which became official June 1, 1936, of a

* From the Research Laboratories of The Upjohn Company, Kalamazoo, Michigan

standard reference digitalis powder to replace ouabain as a standard for digitalis preparations naturally led to the question of whether any pronounced changes in the activity of official preparations had occurred in the transition from the U. S. P. X.

The growing confusion among physicians and others with regard to the strength of official U. S. P. XI digitalis preparations in terms of the familiar U. S. P. X standard has been recently pointed out by Wright, Fahr, and Lewis (1) in their correspondence to the editor of the *Journal of the American Medical Association*. It therefore, appears timely for us to call attention to conclusions reached in our laboratory on this matter.

We have used the U. S. P. XI Reference Digitalis Powder (No. 3057 X 915921) in this laboratory since it was obtainable. This powder carries the correction factor or relationship that 0.0745 Gm. is equivalent to 1 "U. S. P. Digitalis Unit" or 0.1 Gm. of the International Standard Digitalis Powder. We wish to call particular attention to the fact that this correction factor has always been applied in preparing extracts from the powder so that in all cases we have used a strictly U. S. P. XI Standard Tincture.

Our first tests, when we received this powder in April, 1936, indicated the U. S. P. XI Standard Tincture to be approximately 125 per cent U. S. P. X. Other laboratories (2), (3), (4) had reported that the International Standard was about 125% of the U. S. P. X standard and it was therefore considered that the digitalis standard had been raised 20-30 per cent over the U. S. P. X level. Later parallel runs between the U. S. P. XI standard tincture digitalis and ouabain indicated this value to be somewhat higher. Hence, it was decided to directly compare the U. S. P. XI standard tincture to ouabain at various times and thus by a series of comparative tests, spread over a sufficient time interval, to accurately determine the relationship between these two digitalis standards.

EXPERIMENTAL

Our method of testing these preparations has been that specified in the U. S. P. XI, pages 397-398.