Sodium barbital gives quantitative results even with only one extraction. This would be expected on account of the weakly acidic nature of barbital.

The sodium phenobarbital gives low results even when dried at 140° C. This is in accord with the results of the A. D. M. A.[2] who found the salt to form a stable hydrate from which it was impossible to remove all the water even at 140° C. That this method does titrate quantitatively the cation in this salt is demonstrated in the following series of experiments.

As a further check on the method the following experiment was carried out on pure salicylic acid, benzoic acid, phenobarbital and barbital. A suitable weight was titrated directly with standard alkali to a phenolphthalein end-point. The water was removed by evaporating under reduced pressure. The resulting salt was titrated as above and the titration compared directly to that obtained by neutralizing exactly the same volume of sodium hydroxide with standard acid to a methyl orange end-point in the presence of ether. In this manner the effect of possible impurities in the commercial salts was eliminated, the problem being the quantitative recovery of the standard alkali added. Entirely analogous results to those in the above table were obtained. With phenobarbital and barbital quantitative recovery was obtained with one ether extraction; with benzoic acid within 0.2% on the first extraction and quantitative recovery on the second; with salicylic acid at least 0.5% low on the first extraction but quantitative recovery on the second. These experiments act as an independent check on the accuracy of the method.

CONCLUSION.

Henville's method for assay of sodium benzoate and sodium salicylate whereby the salt is titrated directly with standard acid in the presence of diethyl ether to a methyl orange end-point is a general method. It is applicable to water-soluble salts of the type MA where HA is an acid fairly insoluble in water and appreciably soluble in some solvent immiscible with water; where HA is not too strong an acid, apparent dissociation constant less than 2.5×10^{-3}; where MOH is not too weak, apparent dissociation constant greater than 10^{-6}.

The simplified assay method for sodium salicylate suggested by U. S. P. XI in which the end-point is taken after only one ether extraction, gives at least 0.5% low results, two extractions being necessary to give the correct assay.

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DIGITALIS ASSAY ON NORMAL AND EXSANGUINATED CATS.*

BY DAVID I. MACHT.

(WITH THE TECHNICAL ASSISTANCE OF M. B. MACHT.)

Pharmacologists in general recognize the cat method as the most useful means of assaying digitalis preparations. Variations crop up, however, even when this method is employed. To insure the most accurate and reliable data concerning

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assay of digitalis, a knowledge of the different factors affecting or producing such variations is very desirable. A number of these have already been discussed by various authors. Van Wijngaarden has suggested a mathematical formula for calculating the most reliable figures obtained from a given series of cat experiments (1). Macht and Colson have found that there is a marked difference in the killing doses of digitalis for vagotomized and non-vagotomized cats, respectively (2). Furthermore, the present writer has discovered that meteorological conditions, and particularly sudden changes in the barometric pressure, must be taken into account when assaying digitalis on cats (3). In the present communication, another factor markedly influencing the toxicity of digitalis for cats is described.

In this laboratory, fresh arterial blood is often obtained from cats for assaying or determining the activity of heparin, which is manufactured on the premises. Healthy cats are anesthetized with ether for this purpose. One cannula is inserted into the carotid artery and another into the femoral vein. From 30 to 60 cc. of blood are drawn from the artery, and a corresponding volume of physiological saline is then injected into the animal through the vein. After this operation the animal is kept under anesthesia and used for some other physiological or pharmacological experiment. The author and his assistants found that when digitalis preparations were assayed on a cat in which blood (drawn off in the manner described above) had been replaced by physiological saline, the lethal dosage was much smaller than that required to kill a normal cat. The subjoined table exhibits the results obtained with five different lots of digitalis tincture assayed on normal cats, on the one hand, and on cats bled from one-half hour to one hour previously, on the other. In every case the animals were kept under light ether anesthesia;

TABLE INDICATING RESULTS OF ASSAY OF VARIOUS LOTS OF TINCTURE OF DIGITALIS.

	Normal Cats.			Exsanguinated Cats.		
Lot.	Weight of Cat in Kg.	M. L. D.—Cc. per Kilo of 1:10 Dilution.	Lot.	Weight of Cat in Kg.	Cc. of Blood Drawn.	M. L. D.—Cc. per Kilo of 1:10 Dilution.
A	3.0	10.1	*A*	3.0	60	6.2
	3.9	9.2		2.4	50	7.0
	3.0	10.8		2.4	45	7.5
	2.8	10.0		3.2	45	8.1
	3.4	10.0		3.2	60	7.9
B	2.9	14.2	*B*	2.5	25	11.6
	3.0	14.4		2.7	35	13.4
C	3.0	14.0	*C*	2.8	40	10.1
	2.7	14.0		3.0	55	7.3
	3.2	13.8		2.9	45	10.0
D	2.0	13.2	*D*	3.0	50	8.5
	1.9	12.0		3.2	55	9.0
E	3.3	14.9	*E*	3.1	65	12.5
	3.0	13.4		3.2	65	11.5
	2.9	14.4		3.2	60	11.9
Grand average		12.6 cc.				9.5 cc.

and the tincture, diluted 1:10 with physiological saline, was injected through the femoral or saphenous veins according to standard methods at regular intervals. The minimal lethal dose required to kill an animal is expressed in cubic centimeters of the 1:10 dilution. It will be noted that, even when the amount of blood removed

was as little as 25 cc., the killing dose of the digitalis was smaller in the exsanguinated cats than it was in the normal animals. What the explanation of this phenomenon is the writer is not prepared to say. It is certainly not due to any irritation or injury to the vagus and sympathetic nerves in the neck because control experiments were made on animals in which these nerves and the carotid arteries were removed without subsequent bleeding of the animal. The difference in toxicity would seem to indicate that the active principles of digitalis may enter into some loose combination with the proteins of the blood and render them less potent, but this has not been definitely established. The writer has always found that the most reliable figures are obtained when the tests for digitalis are performed on cats weighing not less than 2 Kg. and not more than 3.5 Kg. The difference in the killing doses for exsanguinated and normal cats, respectively, is worthy of notice because the greater our knowledge of the factors responsible for variations in digitalis assay, the more reliable will be the figures obtained by investigators who take these factors into consideration.

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METHODS OF IDENTIFICATION OF THE RHIZOMES OF IRIS VERSICOLOR L. AND IRIS VIRGINICA L.*

BY GEORGE M. HOCKING.[1]

Most of the Blue Flag Root in commerce appears to come from the north- and central-eastern counties of Florida. The whole drug appears on the market in two forms, *viz.*, "with fibre" (*i. e.*, with the roots still attached to the rhizome) and "free of fibre" or "stripped." The species, *Iris versicolor* L., has always been named as the official source of the drug, Iris versicolor. Another species, *I. caroliniana* Watson, was recognized for the first time in the N. F. V, presumably on the basis of Farwell's statement (1) that in his twenty-five years' experience with crude drugs, Blue Flag Root had come almost entirely from this species. By the rule of priority, the name *I. virginica* L. takes preference over the name *I. caroliniana* Watson, and this revised nomenclature is used in this paper.

The statement is sometimes made (2), (3), (4) that Blue Flag Root is adulterated. Thus, Rusby (5), (6) says that this is probably the case to a large extent with Iris versicolor from the south-eastern states. In 1911, he (7) stated that much of the article appeared to come from *I. missouriensis* Nuttall, which is provided with a larger rhizome and was more readily and cheaply collected. The possibility of adulteration from this source now is remote since no Blue Flag seems to be collected in the areas where this species grows. In the south, collectors of and dealers in Blue Flag accept as genuine only the "red root," *i. e.*, the rhizome reddish when

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