9—The convulsant dose of the extract of the pyrethri flores for a dog, injected intravenously under local anesthesia, is about 0.04 mg. per Gm. body weight or about half the dose for the frog.

10—The convulsions are controlled by inhalations of ether.

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STUDY OF A DIGITALIS BODY WHICH IS ELIMINATED RAPIDLY AFTER ITS INTRAVENOUS INJECTION IN THE CAT.*

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I. INTRODUCTION.

After some observations had been made on the behavior of several specimens of chloroform-soluble fractions of digitalis extracts, M. S. Dooley,[1] while working in this laboratory, examined fluidextracts and tinctures of digitalis in order to determine whether they contained a principle which is apparently eliminated within a few hours after its intravenous injection into the cat. Dooley says in the summary of his paper: "... The results are interpreted as indicating the presence in the leaf of a digitalis body having a shorter period of cardiac action in the cat

* A part of the expense of this investigation has been defrayed by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

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than any true digitalis body hitherto studied in this way... There is some clinical evidence in support of the view here expressed."

Objection may be urged against the publication of a paper dealing with a substance which we are unable to describe with enough precision to permit of giving it a name, but it is necessary to learn as much as possible of the constituents of digitalis even though they are unobtainable in pure form. We believe that our results afford a clue to some of the perplexing problems concerning these drugs; and we know that misunderstanding has resulted from naming impure substances and mixtures of principles and describing them as pure principles. This is illustrated by the confusion that has resulted from Keller's use of the term "digitoxin" for an extract that contains a variable percentage of digitoxin, if, indeed, it contains any.[2]

The importance of the presence in digitalis of a rapidly eliminated substance is due in part to its influence on the therapeutic dosage, and in part to its effect on the biologic standardization. It is evident that of two specimens of digitalis of equal activity, one which contains a relatively large amount of an active principle which is absorbed promptly from the gastro-intestinal tract and which is eliminated slowly, thereby producing a prompt and lasting therapeutic effect, is more valuable than another which contains a relatively large amount of active principle which is absorbed poorly and which is eliminated rapidly, and which produces an uncertain effect of short duration.

The absorption and elimination of a drug proceed simultaneously, however the rate of each may vary, and it is obvious that the more rapidly a given digitalis body is absorbed and the more slowly it is eliminated, the less there will be required of it to induce a given effect, such as the arrest of the frog's heart. The ratio of the rate of absorption to elimination varies widely with the different digitalis principles, with any given mode of administration and a given animal, and the rate of absorption is not the same for a single mode of administration in different animals.

The following serves as an illustration of the extraordinary complexity of the problem of biologic standardization of digitalis bodies and its application in determining therapeutic doses. Famulener and Lyons[3] observed that strophanthin is seventeen times as active as digitoxin when they are injected into the lymph sac of the frog; but we know that digitoxin is far more active than strophanthin when they are used daily in therapeutic oral doses, because strophanthin is absorbed poorly and eliminated rapidly, whereas digitoxin is absorbed fairly promptly, and its action is very persistent. Digitalis does not contain strophanthin, it is true, but it does contain unknown principles which render all methods of bioassay unsatisfactory for computing the therapeutic dosage.

The main facts reported in this paper were established during the course of an investigation of a chloroform-soluble substance extracted from digitalis, and the nature of the substance now under discussion was not given careful consideration until several hundreds of the experiments had been performed, and the results of those do not lend themselves for systematic presentation here. This will be understood when we state that the greater number of the specimens of the chloroform-soluble substance were purified by washing with ether, without concern for the fact that some active substance was known to be removed in the process, be-

cause a superficial examination led to the belief that the active substance thus removed did not differ in character from that which remained; this oversight was the more natural since the ether extracted much inert matter.

II. IDENTIFICATION OF THE SUBSTANCE AS A TRUE DIGITALIS BODY.

a. By the Test on the Frog's Heart.—It has happened, not infrequently, that a relatively inert crystalline or amorphous substance has been prepared and marketed under the assumption that it represented the therapeutically valuable constituents of the crude drug from which it was prepared. It is not sufficient, therefore, to know that a substance is active, but we must also know whether its action is qualitatively like that of the drug from which it is obtained, even though it may not be the only active constituent of the drug. All true digitalis bodies have a characteristic action on the frog's heart. It includes partial block, slowing of the rate, irregularity in the beats, and stoppage of the heart with the ventricle in firm systolic contraction, and the auricles widely dilated. It is, therefore, a simple matter to determine whether a soluble substance is a true digitalis body unless this action is feeble, or it is obscured by some other effect. We have applied this characteristic frog's heart test to different specimens of the substance under consideration here, and have always found it to be a true digitalis body.

b. By the Quantitative Synergistic Action with Ouabain.—Another means of identification of a digitalis body is afforded by its quantitative synergistic action with a known digitalis principle, such as crystalline ouabain. It is not inconceivable that one may find a substance capable of increasing the action of a true digitalis body in a manner analogous to the action of small amounts of cocaine in increasing the vascular effects of epinephrin, but it is hardly possible that two substances can be quantitatively synergistic in all of their cardiac effects without possessing a similar action. It has been shown that all of the true digitalis bodies that have been tested in this way are quantitatively synergistic.[4] The procedure is illustrated by the following experiment. A cat received an intravenous injection of 1 milligram of an unknown compound per kilogram of weight dissolved in 1 cc of normal salt solution. After an interval of a few minutes, designed to permit the effects of the drug to develop, and to determine whether it was severely toxic, ouabain, dissolved in 250,000 parts of normal salt solution, was injected intravenously slowly and continuously until severely toxic symptoms, characteristic of digitalis poisoning, were observed. The injection was stopped, and the cat died about two minutes later. It was found that 0.025 milligram of ouabain per kilogram of body weight had been injected in a period of 25 minutes. The activity of the unknown substance is calculated in the following way. The average fatal intravenous dose of ouabain for the cat is 0.1 milligram per kilogram of weight, hence the animal received 25 per cent. of the fatal dose of ouabain. Therefore 75 per cent. of the fatal dose was due to the unknown substance, and since 1.0 milligram of this substance per kilogram equals 75 per cent. of the fatal dose, 1.33 milligrams per kilogram equal the average fatal dose.

III. OCCURRENCE.

a. In Tinctures of Digitalis.—While Dooley[1] presented evidence that digitalis contains an active principle that is eliminated rapidly after its intravenous injection in the cat, he made no effort to isolate it, and the nature of the experiments did

not permit of a quantitative estimation of it. We have been unable to isolate this principle in pure form but we have separated it quantitatively from other digitalis principles and have prepared a specimen of it in a degree of purity (if one may judge of this by its activity alone) approaching that of commercial digitoxin, and much greater than that of digitoxin—Keller, so-called.

The principle involved in the present study of the rapid elimination of this substance, or, expressed somewhat differently, of its persistence of action, is the same as that employed by Hatcher[5] in studies of the persistence of action of various digitalis bodies in animals, and by Eggleston,[6] and Cohn, Fraser, and Jamieson[7] in clinical studies. The chief difference is that while the action of the known digitalis principles in the cat and in man is measured in periods of days and weeks, the period of persistence of action of the substance now under consideration is measured in hours.*

The maximum toxicity of each of several tinctures was determined by injecting it rapidly without allowing time for more than a small amount to be eliminated, and this maximum toxicity was compared with the minimum toxicity, which was determined by injecting the specimen slowly, thus permitting of the elimination of the greater part of the rapidly eliminated fraction, or by injecting a definite quantity of the specimen, and after an interval of several hours its persistence of action was estimated by determining the amount of ouabain then required to cause death.

We examined a specimen of tincture of digitalis that had been submitted recently for testing biologically, and which appeared to contain an unusually high percentage of this rapidly eliminated substance. We used the method previously employed by Dooley, and we are unable to say whether this specimen actually contained an unusual amount of the rapidly eliminated substance, or it was present in a form which made its detection easy. A relatively high percentage of digitoxin tends to obscure the presence of this rapidly eliminated substance, because the action of small doses of digitoxin develops slowly, hence its action is increasing while that of the rapidly eliminated substance is undergoing elimination, and its action is decreasing.

The maximum toxicity of this specimen was determined by taking the average of the fatal doses in five experiments in which the injections were made rapidly. This average was 0.8 cc of the tincture per kilogram of weight, and the period of the injections averaged 32 minutes.[1]

Each of 3 cats then received an intravenous injection of 0.68 cc per kilogram of weight, or 85 per cent. of the minimum fatal dose, at once, and the animal was released for a period of about three hours, after which ouabain, dissolved in 250,000 parts of normal salt solution, was injected slowly and continuously until the occurrence of toxic symptoms which precede death. The first of these three animals

* Prompt recovery from the effects of a drug is almost universally considered *prima facie* evidence of rapid elimination (including destruction, within the term elimination), and slow recovery is as valid as an argument for slow elimination.

[1] One cat died after the injection of only 0.41 cc per kilogram of weight; this experiment was discarded, because the animal was unusually susceptible, and its inclusion would indicate that the tincture contained a larger proportion of the rapidly eliminated substance than we believe that it actually contained.

received 73 per cent. of the average fatal dose of ouabain (0.073 mg. per Kg. of weight) in a period of 65 minutes, the second 46 per cent. of the average fatal dose of ouabain in 45 minutes, and the third 29 per cent. in 35 minutes.

The calculations show that the three animals received totals of 158 per cent., 131 per cent., and 114 per cent., respectively, of the average fatal dose of tincture of digitalis and ouabain combined, indicating that they eliminated a substance having activity equal to 58 per cent., 31 per cent., and 14 per cent. of the average fatal dose, respectively. The intervals following the injection of the tincture and before the death of the animals were 4 hours and 11 minutes, 3 hours and 54 minutes, and 3 hours and 35 minutes, respectively.

It is almost certain that these figures do not show the exact amounts eliminated in each of the experiments, but the average of the three figures (58, 31 and 14), 34 per cent. of the average fatal dose, does very nearly represent the average percentage eliminated. We have conclusive evidence that different individuals vary widely in their capacity for eliminating this substance. We have no evidence upon which to base an opinion concerning the mechanism of this elimination, but if we may be permitted to hazard a guess, we will suggest that the poison is probably fixed in the liver, and either decomposed or excreted in the bile. We hope to put this matter to the test of experiment.

b. In the Chloroform-Soluble Substance.—One of us[8] has described a chloroform-soluble extract of digitalis which is prepared in various ways. This extract has the advantage of being absorbed from the gastro-intestinal tract of man fairly readily, but it is evidently a mixture of substances which show differences in their behavior after intravenous injection in the cat, and probably in man. During experiments with this chloroform-soluble substance it was observed that a larger amount was required to cause death when the injection was made slowly than when it was injected rapidly. This suggested that the difference is due to a substance that is eliminated during the slow injection.

The protocol in brief of an experiment will serve to illustrate the difference between the amounts of a specimen of chloroform-soluble substance required to cause death after rapid, and after slow, intravenous injection in the cat.*

Protocol in brief showing the amounts of chloroform-soluble substance required to cause death following its rapid, and its slow, intravenous injection in the cat:

A specimen of chloroform-soluble substance was prepared by extracting a dilute infusion of digitalis with chloroform, evaporating the aqueous liquid, precipitating inert matter with alcohol, expelling the alcohol, dissolving the residue in water and shaking it with chloroform, distilling the mixed portions of chloroform, washing the residue with petroleum ether, and drying it.

The dried chloroform-soluble substance was fatal to cats when injected intravenously in doses of 0.74 milligram, and 0.75 milligram, per kilogram of weight in periods of 83 minutes, and 90 minutes, respectively, and in a dose of 1.55 milligrams per kilogram when the injection was made in a period of 318 minutes. It was dissolved in a small amount of alcohol and largely diluted with normal salt solution before being injected. The results of these tests indicate that about half of this specimen of the total chloroform-soluble fraction is eliminated rapidly.

* One must avoid the use of general terms with reference to the absorption and elimination of the digitalis bodies. The cat eliminates this substance very rapidly, the rat eliminates it slowly; the rat absorbs only traces of massive oral doses of ouabain, but eliminates it with extraordinary rapidity following its intravenous injection; the rabbit eliminates a nearly fatal intravenous dose of digitoxin in a few hours, while the cat probably requires as many weeks for its elimination.

The chloroform-soluble fraction is prepared most simply by shaking an infusion of digitalis with chloroform (the first step in the preparation of the preceding specimen), distilling the chloroform and washing the residue with petroleum ether, but in this case the petroleum ether does not remove much impurity unless a large amount of chloroform has been used for extracting. A large amount of water is used for preparing the infusion and the yield of chloroform-soluble substance is small, but it usually contains relatively little of the rapidly eliminated fraction. The protocol in brief of an experiment of this type follows:

Protocol in brief of experiment in which the rate of elimination with rapid and slow injection is compared:

Twenty-five grams of digitalis were infused with 2500 cc of cold water during a period of 24 hours; the filtered liquid, measuring 2300 cc, was shaken with 500 cc of chloroform, and then with 1600 cc; the mixed chloroformic extracts were distilled, and the residue, weighing 72.6 milligrams, was tested by rapid injection in two cats. One required 1.38 milligrams per kilogram in a period of 53 minutes, the other required 1.27 milligrams per kilogram in 39 minutes, an average of 1.33 milligrams per kilogram in an average of 46 minutes.

Each of two cats then received an intravenous injection of 75 per cent. of the calculated fatal dose (1.0 mg. per kilogram) intravenously, and after an interval of three hours ouabain solution was injected slowly. One animal died 267 minutes after the injection of the chloroform-soluble substance and, after the injection of 26 per cent. of the average fatal dose of ouabain, the other died 230 minutes after the injection of the first dose and after 52 per cent. of the average fatal dose of ouabain. The combined dose of ouabain and chloroform-soluble substance indicated that no elimination occurred in the first experiment, the result in the second experiment indicated that 27 per cent. was eliminated. The average eliminated in the two experiments equaled 14 per cent. of the average fatal dose, or 19 per cent. of the dose administered.

The infusion left after extraction with chloroform was then evaporated, inert matter was precipitated with alcohol, the alcohol was expelled, the residue was taken up in water, the solution was shaken with chloroform, and the chloroform was distilled. The chloroform-soluble substance obtained, weighing 48.0 milligrams, was tested in the manner just described. Doses of 1.5 and 1.75 milligrams per kilogram each proved fatal; one in six minutes, the other in eight. In another experiment, in which the injection was made slowly and continuously, a dose of 3.04 milligrams per kilogram was injected in a period of 85 minutes. One animal survived a dose of 2.0 milligrams per kilogram; it was released for a period of 3 hours, after which it required the full average fatal dose of ouabain to cause death, indicating that all the chloroform-soluble substance had been eliminated. This animal was evidently tolerant, since it survived a larger dose by rapid injection than the other two, but it certainly eliminated nearly all of the substance during a period of four hours, including the time during which the ouabain was injected.

These experiments afford evidence that this rapidly eliminated substance is more abundant in that portion of the chloroform-soluble substance which is obtained after the concentration of the infusion than in that which is extracted with a moderate amount of chloroform from dilute infusion, though it is quite possible that all of it is extracted when the shaking with chloroform is repeated so long as any bitter substance is extracted. To recapitulate briefly—little of the rapidly eliminated substance is present in the chloroform-soluble fraction when a dilute infusion is shaken with less than its volume of chloroform; it constitutes about fifty per cent. of the chloroform-soluble substance which is prepared by concentrating the infusion and ridding it of much of the inert matter by precipitation with alcohol before extraction with chloroform; and it constitutes nearly one hundred per cent. of the chloroform-soluble fraction which is obtained by concentration of the infusion after it has been extracted by a small amount of chloroform. We do not know that this applies to all specimens of digitalis.

Anhydrous ether was used in varying amounts to remove fat and coloring matter during the preparation of the greater number of the specimens of the chloroform-soluble substance, and one specimen of chloroform-soluble substance, which had been purified by the use of ether, was nearly free from rapidly eliminated substance as shown by the fact that the amounts required to cause death after intravenous injection were nearly independent of the duration of the injection within the limits of periods of several hours. The specimen contained some inert matter. The doses used and the duration of the injections were as follows:

4.10 milligrams per kilogram in a period of 110 minutes.
4.86 milligrams per kilogram in a period of 146 minutes.
4.34 milligrams per kilogram in a period of 208 minutes.
4.78 milligrams per kilogram in a period of 310 minutes.

It will be observed that there is a slight increase in the dosage with increase in the interval with the exception that the second animal received a larger dose than the third or fourth.

c. In Ethereal Extracts of Chloroform-Soluble Substance.—While trying to purify the chloroform-soluble substance with a minimum of loss we observed that successive portions of ether removed diminishing total amounts, and that the successive ethereal extracts contained diminishing percentages of the rapidly eliminated fraction, and increasing fractions of the slowly eliminated, leaving a residue consisting almost exclusively of the slowly eliminated fraction, though the several fractions and the residue were nearly similar in appearance, except that the first portion contained a little oil and more coloring matter in some cases.

In one experiment of this type we found that practically all of a nearly fatal dose of the first portion of ethereal extract was eliminated within a period of about six hours after its intravenous injection, and, in fact, the elimination may have occurred in less time.

d. In Digitoxin—Keller, So-called.—Digitalis contains an abundance of chlorophyl and other coloring matter from which it is difficult to separate the chloroform-soluble substance completely. One of the most active specimens of the rapidly eliminated substance which we have obtained was made from the so-called digitoxin—Keller, prepared in the following manner: Several specimens of chloroform-soluble substance dissolved in diluted alcohol were evaporated to dryness; 3 grams of the residue were dissolved in 425 cc of 30% alcohol, to which was added an excess of lead subacetate; the liquid was filtered and an excess of sodium sulphate was added; the mixture was allowed to stand for several hours and then filtered; the alcohol was expelled, the residue was dissolved in a small volume of chloroform to which were added about 3 volumes of ether and 30 volumes of petroleum benzin.* A flocculent white precipitate formed, which, on drying, afforded a grayish white powder weighing 1.45 grams. It was practically identical with digitoxin—Keller, so-called.

* The literature relating to the chemistry of digitalis is extensive and there is much confusion resulting from the use of the same name for different substances; of different names for similar substances; from the giving of names to mixtures of principles, and from the association of the names of investigators with processes which they did not originate. We do not claim credit for any process here mentioned, but it is not possible to give the names of the authors of the processes which have been developed by many workers.

Two hundred milligrams of this powder were shaken with two portions of anhydrous ether, each measuring 5 cc; the extract weighed 44.5 milligrams. It was fatal in intravenous doses of 0.8 milligram per kilogram of weight. The results of tests similar to those already described indicated that doses of 50 per cent., 60 per cent. and 85 per cent. of the calculated fatal dose were eliminated completely within 3 hours.

One gram of the digitoxin—Keller, so-called, was treated with successive portions of anhydrous ether; the ethereal extracts were of about the same degree of toxicity as the one just mentioned. About half of a nearly fatal dose of the second of these three ethereal extracts was eliminated within about four hours, while little of the third, and practically none of the residue, were eliminated within periods of three hours. The results are summarized in the table.

TABLE SHOWING FRACTIONAL EXTRACTION AND BEHAVIOR OF THE ETHEREAL EXTRACTS.

Amount of ether used.	Weight of substance extracted.	Eliminated within four hours.
10 cc.	71.4 mg.	Nearly all
20 cc	97.4 mg.	50 per cent. (about)
20 cc	83.0 mg.	
20 cc	80.0 mg.	
30 cc	93.4 mg.	24 per cent. (about)
30 cc	69.0 mg.	
Residue	409.0 mg.	Practically none

Digitoxin—Keller, so-called, is sometimes confused with digitoxin, and Keller's method of determining that substance, variously modified in unessential ways, has been recommended frequently for the quantitative determination of digitoxin in proprietary preparations of digitalis. The manufacturers of digipoten, digipuratum and digitol, or their agents claimed that the amounts of digitoxin present in their several preparations could be determined quantitatively by this method or by unessential modifications of it. (See "New and Nonofficial Remedies," 1921, pp. 104, 105 and 106.) The error of these claims has been pointed out by Warren.[9]

e. In Chloroformic Percolate of Digitalis.—Eggleston[10] found that therapeutic effects followed the oral administration of digitoxin more promptly than those of any other digitalis principle or preparation that he studied; and that the dose required was more nearly uniform. Digitoxin is the only one of the pure principles derived from digitalis that is soluble in chloroform, hence it naturally suggested itself to prepare a digitoxin-like substance from digitalis by percolation with chloroform, and, in fact, this was the first method employed by one of us in the search for a readily absorbable digitalis preparation. The results were disappointing, for while the chloroformic percolate was found to be active, it was not absorbed readily from the gastro-intestinal tract of the cat apparently, and the attempt to prepare such a readily absorbable substance in that way was abandoned.

It seemed possible that the failure of moderate oral doses of the chloroformic percolate to induce typical effects might be attributable to rapid elimination and this substance was examined in order to determine its rate of elimination after intravenous injection in the cat. It was found that the effect of a nearly fatal

dose disappeared almost completely within a few hours. The protocol in brief of an experiment with this substance follows.

Protocol in brief of an experiment with chloroformic percolate of digitalis:

Fifteen grams of finely powdered digitalis were percolated during a period of 5 days with enough chloroform to yield 6500 cc of percolate, which was collected in portions of about 1000 cc. Each portion of percolate was shaken with water; the chloroform was distilled; the residue was shaken with petroleum ether and dried. The mixed residues weighed 83 milligrams; this was dissolved in 52 cc of dilute alcohol, and used for various tests. Intravenous doses of 1.0 milligram per kilogram of weight were nearly fatal to cats, but such doses were eliminated almost completely within periods of about six hours in three experiments, and somewhat less completely in a fourth.

The oral administration of a dose of 1.5 milligrams per kilogram followed by one of 4.5 milligrams per kilogram, in a period of about two hours and a half, was without perceptible effect. The administration of doses of 3 milligrams and 4.5 milligrams per kilogram of weight in a similar period of time was also without perceptible effect in a second experiment, but the administration of two oral doses of 4.5 milligrams per kilogram each, with an interval of about two hours and a half between them, induced vomiting in 22 minutes after the second dose.

These results indicate that this chloroformic percolate of digitalis is poorly absorbed from the gastro-intestinal tract of the cat, since oral doses equal to three times the fatal intravenous dose were absorbed so slowly that elimination very nearly kept pace with absorption.

This specimen of chloroformic percolate caused systolic standstill of the frog's ventricle when doses of 12 milligrams per kilogram of weight were injected into the lymph sac. It may be mentioned, for the sake of comparison of effects, that Worth Hale[11] found that doses of about 8.5 milligrams of Merck's digitoxin were required to cause this effect in the frog.

The chloroform remaining in the marc after the percolation of the powder was expelled, and from the marc an infusion was made in the usual way; this was filtered, and enough water was passed through the marc and the filter to make a total of 1000 cc of filtrate. This was shaken with two portions of chloroform measuring 1000 cc each, and with one of 500 cc. The chloroformic extracts were distilled. The total residue weighed 18.6 milligrams, or only 1.25 milligrams per gram of powdered digitalis used, whereas the residue from another portion of this powder which was treated in the same way except for the percolation with chloroform, was equal to nearly 5 milligrams per gram of powder. In another experiment, in which we compared the amounts of the chloroform-soluble substance obtained from a specimen of digitalis with that from the marc left after percolation of the powder with chloroform, the results were essentially similar to those just described.

These results show that the chloroformic percolate of these specimens of digitalis consists almost exclusively of the rapidly eliminated fraction, but that after percolation of digitalis with chloroform, one cannot obtain from the infusion of the marc nearly so much of the readily absorbable chloroform-soluble substance as he can extract from the infusion of the whole drug. It seems probable that a portion of the rapidly eliminated fraction which is extracted during percolation with chloroform, is capable of being converted into the readily absorbable and slowly eliminated fraction during the process of preparing the infusion, and extracting it with chloroform. The extract of another specimen of digitalis prepared by percolation with chloroform consisted of approximately equal parts of the rapidly eliminated, and the slowly eliminated, fractions.

f. In a Pharmaceutical Preparation Labeled "Digitoxin."—A pharmaceutical preparation was received from a manufacturing firm with a request for any information that could be furnished. The label reads: "Digitoxin Solution for use by mouth, strength same as tincture, alcohol 20 per cent." We made no systematic study of this preparation, but we found that it is eliminated almost completely within a few hours after the intravenous injection of almost fatal doses in cats. The solid residue left on evaporation gives one of the color reactions which is commonly considered characteristic of crystalline digitoxin. It is almost insoluble in ether.

g. In a Specimen of Crude Digitalein.—We have recently examined the protocols of several experiments which were performed in 1910 with the assistance of Dr. J. G. Brody in this laboratory. In three of those experiments the average fatal dose of digitalein was found to be 2.93 milligrams per kilogram of weight in the cat, the three doses showing little variation were injected in periods which averaged 53 minutes. Two animals required an average of 4.5 milligrams in periods which averaged 2 hours and 26 minutes, suggesting that part of the digitalein was eliminated rapidly. In every one of five experiments a dose of 2 milligrams per kilogram was injected at once, after which ouabain was injected slowly and continuously until death resulted. These animals required an average of 0.08 milligram of ouabain per kilogram (80 per cent. of the average fatal dose), in addition to the digitalein, but they showed wide individual variations in the amounts of ouabain required.

We are unable to state whether these results are to be attributed to the rapid elimination of the greater part of the digitalein or to a lack of quantitative synergistic action between that specimen of digitalein and ouabain, but one specimen of digitalein which was used about that time was found to be strictly synergistic with ouabain.

Sluyters[12] maintains that digitalis contains some substance which is not a true digitalis body, but which is toxic to frogs, and Cloetta[13] described a decomposition product of digitoxin which lacks the cardiac action of a true digitalis body but which exerts a convulsant action through its effect on the central nervous system. We have frequently observed this convulsant action in various digitalis preparations[14] and have found that it is especially prominent in some specimens of digalen and in some specimens of the rapidly eliminated substance.*

IV. CHEMICAL AND PHYSICAL PROPERTIES OF THE RAPIDLY ELIMINATED SUBSTANCE.

a. Color Reactions.—Two specimens of the rapidly eliminated fraction which were obtained by extracting the chloroform-soluble substance with ether, were used for observing their color reactions, some of which were compared with those afforded by Merck's crystalline digitoxin.

When a small amount of this rapidly eliminated substance is dissolved in glacial acetic acid which contains a trace of ferric chloride, and the solution is poured onto concentrated sulphuric acid without mixing, there is a brownish color formed in the sulphuric acid at the point of contact, a dark blue color forms between the two acids, and the acetic acid is colored dark olive-green. If a trace of the substance

* Many recent papers dealing with the chemistry of digitalis and methods of evaluating preparations by Straub, Sluyters, Kraft, Joachimoglu, Heffter, and others may be found in the *Archives d. Pharm.*, 1912, and later, and in *Arch. f. Path. u. Pharm.*, 1917, and later.

is dissolved in glacial acetic acid, and the solution is shaken with a drop of concentrated sulphuric acid, a dark olive-green color develops at once and persists for some hours. A trace of the substance added to concentrated sulphuric acid gives a brownish red color, whether a trace of ferric chloride be present or not. A small amount of the substance dissolved in concentrated hydrochloric acid gives a light yellow color. It gives the Baljet reaction[15] with alkaline solution of sodium picrate.

A little of the substance was dissolved in concentrated phosphoric acid, and the solution was poured onto concentrated sulphuric acid without mixing. Several digitalis principles were treated in the same way and the results were compared. The colors appeared at once at the line of contact of the acids and penetrated into the phosphoric acid gradually but not into the sulphuric acid. The results are tabulated.

Table showing the color reactions afforded by each of several digitalis principles when dissolved in concentrated phosphoric acid and the solution is poured onto concentrated sulphuric acid without mixing.

	Immediate.	After twelve hours.
Crystalline digitoxin, Merck's	Brown	Unchanged
Rapidly eliminated substance	Brown	Unchanged
Chloroform-soluble substance after extraction with ether	Brown	Cherry-red
Digitoxin—Keller, so-called	Brownish red	Unchanged
Digitalein, crude	Brownish red	Unchanged
Digitalin, German	Cherry-red	Unchanged
Digitin (so-called crystalline digitalin)	Almost none	Light yellow

A comparison of these color reactions suggests that the rapidly eliminated substance has a molecular composition resembling that of crystalline digitoxin; that the chloroform-soluble substance consists of a chemically related substance with a substance which is also present in German digitalin since the cherry-red color which develops at once in the latter is seen only after the lapse of some time in a good specimen of the chloroform-soluble fraction which has been treated with ether. Too much faith must not be placed in color reactions of impure substances, however.

b. Solubilities.—The solubilities of impure substances are at most suggestive, and they frequently change with startling suddenness dependent on the presence of small amounts of impurities in many cases, but the solubility of some of these extracts remains surprisingly constant qualitatively, and we have frequently gotten rid of much inert matter by dissolving the chloroform-soluble substance in dilute alcohol and allowing it to stand for some days. A specimen of the rapidly eliminated substance which showed a high degree of activity was used in the tests for solubility. It is practically insoluble in water, glycerin, and in petroleum ether; it is readily soluble in alcohol, methyl alcohol, acetone, and in glacial acetic acid; it is somewhat less soluble in amylic alcohol, chloroform, and in acetic ether; it dissolves only partially in a very small amount of ether, requiring far more for complete solution than it does of alcohol; it is soluble in about 60 parts of diluted alcohol. The solubility in ether is no index of its activity, though this solvent does serve to separate the rapidly eliminated substance from that fraction of the chloroform-soluble which is eliminated slowly by the cat. On the other hand, the commercial specimen which was submitted under the name of digitoxin, and which is

eliminated completely and rapidly after its intravenous injection into the cat, yields very little to treatment with moderate amounts of ether.

V. DISCUSSION.

It is probable that the chemistry of no drug has been studied more extensively and less successfully than has that of digitalis, but it is becoming increasingly evident that digitalis yields a number of products of the digitoxin type which exhibit many close similarities and many wide differences in behavior, and one cannot say that because one of these preparations exhibits certain characteristic actions of digitoxin in one or two species of animals it will behave like digitoxin in any other animal or in man.

It seems probable that digitalis contains a substance of the digitoxin type which undergoes chemical alteration with great facility and in a variety of ways, because there are so many types of action induced by closely related bodies, or that there are many digitoxin-like substances every one of which is capable of chemical change. The former seems the more probable, but the latter alternative finds support in the fact that only a very minute amount of any pure digitalis principle can be obtained from the leaf.

The substance that forms the subject of the present paper behaves unlike any constituent of digitalis that has been described (digitalein is not considered an entity, but a mixture), and we are unable to say whether it exists in the leaf in the form in which we extract it or is split off during the process of extraction, but it is probable that the active principles of digitalis exist in the leaf in forms unlike anything with which we are acquainted.

We have obtained widely varying percentages of this rapidly eliminated substance from different specimens of digitalis, and from a single specimen with different methods of preparation. Only negligible amounts were obtained from some specimens of crude drug. A quantitative estimation of this fraction in various specimens in connection with clinical studies would probably throw light on one of the greatest of the difficulties encountered in the therapeutic use of the drug, that is, in the regulation of the dosage so as to obtain the maximum of therapeutic effects with a minimum of the toxic.

Specimens of tincture of digitalis have been submitted for biologic testing because they failed to induce the characteristic digitalis action even when used in enormous total amounts during considerable periods of time. In every such case the biologic test indicated a greater degree of activity than the clinical observations would lead one to expect.

It seems probable to us that the therapeutic value of digitalis depends less on the total active constituents, than on the percentage of the readily absorbable fraction that has a fairly persistent action. This problem is now under investigation in this laboratory and in the clinic, but the extraordinary number of disturbing factors make a final solution extremely difficult.

Joachimoglu[16] maintains that the cat is unsuited for the bio-assay of the digitalic bodies because much more of the chloroform-soluble substance is required to cause death when the injection is made very slowly than when it is made rapidly. Our results show, however, that the cat method is essential for the quantitative estimation of the rapidly eliminated substance present in preparations of this

general type. We do not claim, however, that other methods of estimating this fraction may not be developed.

VI. SUMMARY AND CONCLUSIONS.

The evidence presented by Dooley that tincture of digitalis contains a substance that is eliminated rapidly following its intravenous injection in the cat is supported by our results.

This substance has been obtained from the tincture, from chloroformic extracts of the infusion, from digitoxin—Keller, so-called, and from a commercial preparation. The pharmacological behavior and physical properties have been studied.

This substance can be prepared in an impure state in a degree of activity approximating that of crystalline digitoxin, about half as active as digitoxin for cats by intravenous injection, and about two-thirds as active as digitoxin when tested on frogs.

It has been shown to have a true digitalis action by causing it to induce systolic standstill of the ventricle of the frog's heart, and by showing that it is quantitatively synergistic with ouabain. It gives some of the color reactions supposed to be characteristic of digitoxin.

A nearly fatal dose is eliminated within a few hours following its intravenous injection into the cat, whereas the action of a nearly fatal dose of digitoxin persists for several weeks. Its mode of elimination is unknown.

The solubilities have been studied, but since the substance is impure, the results are only of suggestive value, and probably only qualitative.

It is especially interesting to observe that several chloroformic percolates of digitalis—but not all of them—consist almost wholly of the rapidly eliminated fraction, in contrast to digitoxin. The chloroform-soluble substance extracted from the infusion contains a variable amount of this rapidly eliminated fraction from which it may be freed almost completely by washing it with ether. The amount of the rapidly eliminated fraction present in the chloroform-soluble substance can be estimated fairly accurately by means of the differential test on the cat. This test consists in determining the smallest fatal dose by rapid intravenous injection, and the percentage of activity which persists after an interval of several hours following the injection of a given dose.

It is of clinical importance to determine the percentage of rapidly eliminated substance in digitalis preparations, in addition to estimating the total toxicity.

We believe that many of the difficulties concerned in the investigation of digitalis require the coöperation of the clinician, the chemist, and the pharmacologist.

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THE VIRGINIA EXPERIMENT.*

BY WORTLEY F. RUDD.

The Legislature of Virginia defeated prerequisite legislation in 1914 and again in 1916. In 1918 the bill was again offered in its original form, *i. e.*, requiring college graduation and two years of drug store experience for examination.

Everything went well with the bill until about the middle of the Legislative Session, when the writer, and others, who were working for its passage, received notice that something had happened and that our presence at the Capitol was urgent. We were astonished to find that some of the leading members of the State Senate had determined to vote against the bill unless the experience clause was deleted. In our attempt to argue the question with these gentlemen, we were told frankly that we could take our choice—delete the experience requirement and they would pass the bill, leave it in and they would immediately defeat it. It is needless to say that we deleted it. This was surely an anomalous situation; bound as we were by tradition to the experience feature good sensible laymen had to tell us pharmacists where we were in error in educational policy.

The point of all of this is that now we believe they were right. These laymen have laid the foundation for an experiment in pharmaceutical education, which is called by some the most progressive step yet taken by any state in the Union and by others rank radicalism.

Naturally, it took us a little time to catch our breath—the thing was so new and so different—but our law-making body had spoken and it was our duty to make the plan fit the needs.

There is but one school of pharmacy in Virginia, and this a state-aided institution. The problem then was squarely up to this school. It must provide facilities for training its students in practical dispensing so that they might go out from the school directly and serve the people safely and efficiently. A careful study of our potential facilities for doing this brought out the following facts: first, our close affiliation with the medical school, which conducts an out-patient clinic of approximately thirty thousand patients per year, could be used even more effectively than in the past; second, the ownership by the college of three hospitals, *viz.:* The Memorial, St. Philip and the Dooley, offered a field which had heretofore never been utilized as a pharmaceutical clinic. Here, then, was an opportunity to develop our practical work two- or even three-fold. On July 1st of this year (1922) the dispensing work for all of these institutions was taken over by our school of pharmacy and placed under the direct supervision of our professor of pharmacy, W. G. Crockett, and three other thoroughly trained men.

Our senior class, rarely numbering more than 25 or 30, will be divided into very small sections—probably not more than three or four students in each.

* Read before Section on Education and Legislation, A. Ph. A., Cleveland meeting, 1922.