5. Practically all of the best grades of sodium chloride on the market in the form of powder or small crystals are unsatisfactory for preparing Locke solution.

6. Only "Reagent" or "Analyzed" sodium chloride in the form of *large crystals* is satisfactory for isolated uterus experiments.

7. The authors are of the opinion that when the above precautions are observed, the Isolated Uterus Method gives better results than any other method so far proposed.

PHARMACODYNAMIC LABORATORY,
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THE PHARMACOLOGY OF PYRETHRI FLORES.*

BY W. H. ZEIGLER.†

Introduction.—The toxicity of the powdered flower heads of the *Chrysanthemum—roseum, carneum* and *cinerariæfolium*—for insects has long been known, experiments having been made by William Carpenter in 1879[1] who said "The toxic action of the powder for insects seems to be directed to the digestive canal and the power of locomotion. The insects, although incapable of moving, give signs of life at least ten hours after the action of the poison." Since these experiments were carried out a great deal of valuable research has been conducted, principally to determine the active principle or principles but up to the present time the results are rather contradictory.[2]

Sato[3] is said to have first isolated the active principle in the form of a clear, light, odorless syrupy resinoid body—at first tasteless, then numbing—which he called "Pyretol."

Recently, Yamamoto[4] found, on chemical analysis, this substance to be a mixture readily altered by heat and air. Thoms reported a glucoside; another, an alkaloid.

No attempt has been made in this research to determine the active principle. That it is volatile is very evident from the fact that when the extract or powder is heated in a closed chamber the vapor is toxic to insects; this also proves that heat does not destroy the toxic principle.

In my opinion the principle is a weak fatty acid—this belief is based upon the fact that alkaline solutions of the extract lose their activity on standing, a white precipitate being deposited. This precipitation occurs very rapidly, especially if the temperature of the room is high. Alkaline solutions of the extract were distilled and both the distillate and residue were found to be inactive. This, in my opinion, is due to the fact that the extract is a weak fatty acid, and, in solution with sodium hydroxide, saponification occurs more rapidly with direct heat; the residue contains the saponified produce which is non-volatile, and the distillate is, of course, inactive.

Whatever the principle is, certainly it presents an interesting study for our chemists—the author would like to see the confusion cleared up.

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This investigation, which began over two years ago, has to do principally with the physiologic effects of the drug on cold- and warm-blooded animals. The statements regarding the toxicity of the drug for warm-blooded animals are somewhat contradictory and the bibliography is obscure.

In the "National Dispensatory"[5] (I would have consulted the original paper had the date been recorded), we find the following statement: "It is very active in cold-blooded animals, but practically inert in those with warm blood."

The "United States Dispensatory"[6] states: "It does not appear to be actively poisonous to man, though it is said to cause some confusion of the head in those who sleep in close apartments where much of it is used."

McCord and Kilker[7] reported what they termed an occupational dermatitis which they found occurring among workers engaged in grinding the pyrethri flores, and in the filling, weighing and sealing of the containers. The author has found only one case recorded where the drug produced symptoms of poisoning by contact on the human subject.

Bosredon[8] reports the following case:

"The case is that of a child of 11 months who was lying in its cradle and playing with a pasteboard box; the nurse had not opened it, but it contained pyrethri flores.

"While playing with it the child pulled off the lid and the powder covered his mouth, nostrils and entire face. At his cries the nurse rushed up and, instead of at once wiping off the child, goes in search of the mother. On their return they find the baby pale, hollow-eyed, shaking with convulsions and seized with nausea. Called in at this juncture, I found the child's vitality low, scarcely reddening when pulled by the hands, refusing the breast. The heart beats were feeble and slowing down, the breathing slightly quick. After washing him carefully to cleanse his nasal cavities, ears and eyelids, I prescribed 20 grams of syrup of ipecac in 2 doses 5 minutes apart. Very free vomitings ensue. The baby cries, brandishes his arms, his face recovers its color, his eyes regain their brilliancy. Visited an hour and a half later a slight inflammation of the conjunctiva (mucous membrane of eyelids) and a carmine redness of lips and tongue are observed, the vomitings have ceased and the child takes the breast. From that time on no special symptoms were observed."

EXTRACTS AND SOLUTIONS.

Extracts.—The "National Dispensatory"[5] gives the following method of assay:

Macerate 8 Gm. of the powder for an hour with 80 Gm. of ether, shaking the mixture frequently. Then decant or draw off 50 cc, add 1 cc of water, and shake thoroughly. Filter and wash the filter well with ether, and distil. Finally weigh the soft residue. Assayed according to this method, it is said that half-open buds yield 6 to 7 per cent. of extract, and unexpanded buds from 7.5 to 9.5 per cent.; that a good sample should yield 7 to 8 per cent. and may run up to 12 per cent.

In order to test this method the author assayed several samples, including well-known brands, with the following results:

A, 4.26 per cent.
B, 6.85 per cent.
C, 3.81 per cent., 46.6% inert matter
D, 3.30 per cent., 25% inert matter
E, 6.44 per cent.
F, 5.63 per cent.

In order to ascertain which of the common solvents removed the largest percentage of extractive matter the following agents were used. The sample was purchased in the open market as the closed-leaf Dalmation variety. The drug was allowed to macerate with the solvent for 24 hours.

Water, 13 per cent.	Acetone, 7.34 per cent.
Alcohol, 28 per cent.	Ether, 3.89 per cent.
Benzol, 2.8 per cent.	Chloroform, 5.1 per cent.

It is of interest to note that the amount of extractive matter is a great deal less than that called for when assayed by the method as outlined. Although alcohol extracted more than any of the other solvents, it required twice the amount of this extractive to produce the effects. The extracts varied in color from a golden yellow to a dirty green. The aqueous extract was found to be inactive.

Solutions.—The solutions used throughout these experiments were made by dissolving the extracts by the aid of sodium hydroxide, 5 cc of the 5% solution being necessary to dissolve 1 Gm. of the extract. It was found convenient to use a 1% solution. All solutions were made up with 0.4 per cent. of sodium chloride for cold-blooded animals, and with 0.9 per cent. for warm-blooded animals.

Extracts used in these experiments were made by the following process:

Forty grams of the drug were macerated with 400 cc of ether for one hour, shaking well, filtering and washing the filter with 50 cc of ether. The filtrate was allowed to evaporate without heat.

The remarkable effects produced by the injection of measured amounts of the extracts of the pyrethri flores into the lymph sac of the frog, and the contradictory statements regarding the toxicity of this drug for warm-blooded animals led the author to conduct the following series of experiments. The experiments were planned with several ideas in mind: to determine (1) the toxicity of the drug for certain insects by mouth; (2) its toxicity for cold-blooded animals; (3) the toxicity for warm-blooded animals; (4) the effect of heat upon the active principle; (5) the effect of temperature on the physiologic activity; (6) a bio-assay method for standardizing the drug.

TOXICITY FOR CERTAIN INSECTS.

Ants.—In order to ascertain if the principle was toxic to insects by way of the digestive canals, a solution of the extract was sweetened by the addition of a small amount of syrup and fed to ants. Under a magnifying glass the ants were seen to feed readily upon the drug and, after eating a small amount, to back away and attempt to remove the cause of the irritation. This was followed closely by convulsions and, when the solution was concentrated enough, by death; it was also toxic when sprayed.

Boll-Weevils.—A solution of the extract was both sprayed and fed to the boll-weevils. It was found to be highly toxic—the insect loses the power of its legs, lies on its back and finally dies. This occurs even when the insect happens to come in contact with the solution.

TOXICITY FOR COLD-BLOODED ANIMALS.

Frogs.—The frogs used in all of the experiments were the common grass frog or leopard frog (*Rana pipiens*). This species of frog was found by Weiss and Hatcher[9] to react with a near approach to uniformity to small doses of strychnine so that it can be used for the quantitative estimation of strychnine. We purchased all of these frogs from the same dealer in Indiana, and, upon arrival, kept them at a temperature of 30° C. After carefully weighing, the frogs were injected with a measured amount of the extract, which had been made into a solution by the aid

of sodium hydroxide. All injections were made into the abdominal lymph sac. Several tests were made to ascertain whether the poison was more rapidly absorbed from the dorsal or ventral sac. No difference in the time could be determined. The solutions were made q. s. with 0.4 per cent. of sodium chloride. About 500 frogs were used in these experiments.

It was found after a number of experiments, that when 0.1 mg. per Gm. body weight is injected by the method outlined, in less than five minutes (see Table No. I) the animal shows signs of stimulation, passing into a peculiar type of convulsions. (See Record No. A.) The secretions are increased. The convulsions are intermittent, the animal using its fore-legs in an apparent attempt to remove the source of irritation by clawing at its head. These effects are closely followed by paralysis and death, which may occur some time later. A series were also injected into the stomach, by the aid of a glass pipette, and this method was found to be effective, although the symptoms were delayed somewhat. The site of the action was located in the cord by successively destroying the brain, medulla and cord.

Turtles.—Turtles weighing about 300 Gm. were injected with a Luer graduated syringe, under the skin of the neck. In a very short time, there was a marked increase in secretions; the animal showed signs of stimulation, attempted to walk, but found its legs useless. After repeatedly kicking, its legs became paralyzed and the animal died several hours later.

TABLE No. 1.—Showing time of first effect when 0.1 mg. per Gm. body weight of the extract of pyrethri flores is injected into the abdominal lymph sac of the frog.

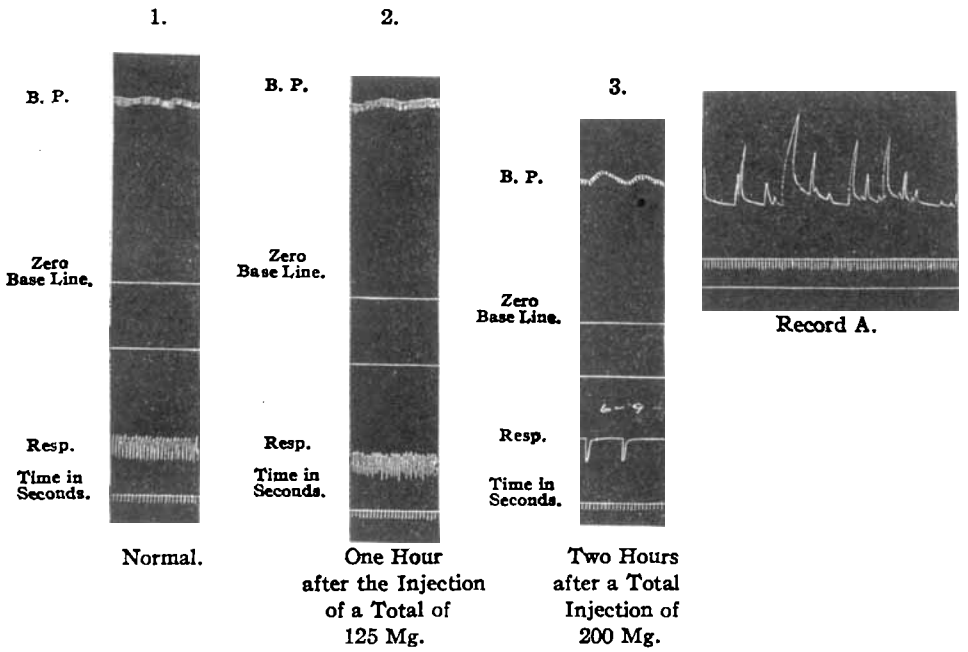
No.	Weight, Gm.	Dose per Gm. B. W., mg.	Time of injection.	First Effects.	Minutes.
1	15	0.1	10:53	10:57	4
2	18	0.1	10:59	10:63	4
3	19	0.1	11:36	11:39	3
4	17	0.1	11:37	11:42	5
5	18	0.1	11:38	11:42	4
6	18	0.1	11:40½	11:45	4½
7	30	0.1	12:32	12:37	5
8	35	0.1	12:33	12:37	4
9	37	0.1	12:40	12:45	5
10	17	0.1	12:42	12:48	6
11	25	0.1	12:42	12:46	4
12	28	0.1	12:45	12:50	5
13	31	0.1	12:46	12:50	4
14	25	0.1	12:47	12:52	5
15	26	0.1	12:50	12:54	4

WARM-BLOODED ANIMALS.

Guinea pigs, rabbits and dogs were used throughout these experiments. The drug was injected both subcutaneously and intravenously. The extract was also administered in capsules and in solution by mouth.

Guinea Pigs.—The guinea pigs were used only for subcutaneous injection and for applying the powder to the mouth and nostrils. Although injected daily with the toxic dose per Gm. body weight for cold-blooded animals there was no evidence of any toxic symptoms; applying the powder to the mouth daily produced no signs of absorption.

Rabbits.—A series of rabbits were carefully weighed and the drug injected into one of the large ear veins. The results were not uniform, due principally to the difficulty of injecting large amounts of the drug by this method. Several of the animals died in convulsions. One, without showing any symptoms of stimulation, died about an hour after the injection of 0.1 mg. per Gm. body weight.



Records 1, 2 and 3 show the effects of a total dose of 200 mg. of the ether extract of pyrethri flores injected into the external jugular vein of a rabbit weighing 2200 Gm. Record A shows contractions of gastrocnemius muscle of the frog, after injection of the pyrethri flores into the lymph sac.

Another group was prepared for blood pressure and respiration tracings; urethane and ether were used as the anesthetic. As seen by the tracings there was at first a slight rise in blood pressure followed by a fall. The respiration was at first quickened, then followed by a decided depression. This animal weighed 2220 Gm. and received a total of 200 mg. of the extract, or about 0.1 mg. per Gm. body weight.

Dogs, Group No. 1.—A series of dogs were carefully weighed, and injected subcutaneously with the toxic dose for cold-blooded animals. All of the solutions used in these experiments were made by dissolving the extract by the aid of sodium hydroxide in 0.9% salt solution. Although the animals were watched for several days no effect was observed.

Dogs, Group No. 2.—Another series were given the active extract in capsules and in solution by mouth. No effect was noticeable, although large amounts were administered.

Dogs, Group No. 3 was arranged for blood pressure and respiration tracings. Both ether and Grehant's solution were used as anesthetics. When ether alone was used no effect was observed, except a slight depression of the respiration. One of

the animals, weighing 11 Kg., under Grehant's anesthesia showed marked convulsions six minutes after the injection into the femoral vein of 50 cc of a 1% solution.

Protocol No. 1.—Blood pressure and respiration. Morphine—Grehant's anesthesia.

Dog—weight 11 Kg.; blood pressure, 120; respiration, 24. At 12:06 P.M. 50 cc of 1% solution were injected into femoral vein; at 12:12 P.M. convulsions, controlled with ether; at 12:19 P.M., blood pressure was 90 and respiration 28; at 12:20 P.M., blood pressure 90, respiration 20.

Second injection of 25 cc of 1% solution was given at 12:22 P.M., convulsions at 12:24 P.M., blood pressure 90, respiration 24; 12:30 P.M., blood pressure 100, respiration, 20; 12:37 P.M., blood pressure 84, respiration 12. At 12:42 P.M. 25 cc more of a 1% solution were injected resulting in convulsions.

As seen by Protocol No. 1, the blood pressure was reduced from 120 to 84, and the respiration from 24 to 12.

Dogs, Group No. 4.—Since the experiments under anesthetics were not uniform, and it was suspected that the ether had masked the convulsions, another series was prepared for injection into the femoral vein, under local anesthesia. Novocaine was used in 2 per cent. solution. When about 35 cc of a 1% solution were injected into a dog weighing 8500 Gm., convulsions of the clonic type occurred. It was of interest to note that the convulsions would subside, but were reproduced after the injection of each successive dose of from 10 to 15 cc of solution. Due to the violent type of convulsions, it was almost impossible to hold the animal and continue the injections, so that only the amount necessary to produce the effect was employed, and only repeated when the convulsions ceased.

By this method it was almost impossible to produce death. The amount necessary to produce convulsions in the dog, not under an anesthetic, was found to be about 0.04 mg. per Gm. body weight.

THE EFFECT OF HEAT ON THE ACTIVE PRINCIPLE.

Solutions of active extracts were evaporated to dryness and redissolved. It was found that although twice the amount was injected no effect was observed. A 1% solution of the extract was also distilled and both the distillate and the residue tested, using frogs; although large amounts were injected no effect was observed.

THE EFFECTS OF TEMPERATURE ON THE PHYSIOLOGIC EFFECTS.

Knowing the susceptibility of the frog to temperature, a series of animals were placed in baths at 32°, 20° and 0° C. It was found that the convulsions occurred in the one at 32 degrees in 4 minutes, the one at 20 degrees in 7 minutes and the one at zero degrees not at all. The frog placed in the 0° bath was changed to the 32° and immediately went into convulsions. This is almost identical with the results obtained by Luchsinger[10] who reported the injection of three frogs with picrotoxin, placing them, respectively, in water at 0°, 15° and 32° C.

DISCUSSION.

From the results obtained in this research, it is very evident that the active principle, or principles, of the *pyrethri flores* is very toxic to both cold- and warm-blooded animals when absorbed into the blood stream. The fact that the drug is toxic to cold-blooded animals by mouth as well as by injection and only toxic to warm-blooded animals when injected into the vein is of interest.

It was found that when the drug is injected into the vein of the dog under local anesthesia, it produces convulsions. These convulsions do not persist and are not necessarily fatal—the animal recovers and convulsions recur with each successive injection of 100 mg. of drug.

It is very evident that the principle is rapidly destroyed by the warm-blooded animal.

The one case recorded in which symptoms of poisoning occurred in the human being by contact is significant. It is possible that the drug was absorbed through the nasal passages. The child probably had an idiosyncrasy for the drug. The cases of dermatitis reported were due undoubtedly to the irritating properties of the principle. It was noted that this occurred during the summer months when the subjects were bathed in perspiration.

The principal physiologic effect, which is produced in both the warm- and cold-blooded animals, is that of extreme stimulation of the central nervous system, the site of action being undoubtedly in the spinal cord.

The toxic effects of aqueous solutions for the boll-weevil both by contact and by mouth is of interest just at this time, when this insect is creating so much discussion.

The author realizes that the supply of this drug could not meet the demands, even if it was proved of greater value than the present much-used calcium arsenate, but the active principle could be analyzed and made synthetically. It would have this advantage over calcium arsenate—it kills by contact and could be used as a spray.

Yamamoto[4] found that in concentrations above 0.077:100 the mixture called by Sato "Pyretol" would check bacterial growth. Is it possible that we have in this active principle not only a valuable insecticide but also a powerful germicide? The author has used the solutions of this drug under the name of "Convulsant" in laboratory exercises, along with picrotoxin and strychnine, to demonstrate the different types of convulsions and the location of same.

CONCLUSIONS.

1—Alkaline solutions of the extract lose their activity on standing.

2—It is toxic to ants when fed to them in 0.5% solution sweetened with syrup.

3—It is highly toxic to the boll-weevil both by mouth and by contact.

4—One-tenth milligram per Gm. body weight of the active extract of pyrethri flores dissolved by the aid of sodium hydroxide in 0.4% sodium chloride solution, injected into the lymph sac of the frog, or subcutaneously into the turtle, produces convulsions within five minutes, followed by paralysis.

5—The location of action is in the spinal cord.

6—The drug is not toxic to rabbits and dogs when injected subcutaneously or administered by mouth.

7—The amount of the extract injected intravenously necessary to produce convulsions in the rabbit or dog under general anesthesia is uncertain; 0.1 mg. per Gm. body weight may prove effective.

8—The dog when injected intravenously, under local anesthesia, with the extract of pyrethri flores dissolved in 0.9% sodium chloride solution by the aid of sodium hydroxide, goes into convulsions, which are not necessarily fatal.

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.*

BY SOMA WEISS AND ROBERT A. HATCHER.†

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

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