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THE ANTHELMINTIC PROPERTIES OF PEPO U. S. P. AND CUCURBITA PEPO.*

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The unsettled question of the activity of pumpkin seed as a vermifuge led us to investigate more fully the official drug and to examine the flesh of ripe pumpkin.

The method of determining anthelmintic activity was by the use of earthworms as directed by Sollman (28). The apparent effect is first, one of stimulation, then paralysis, the killing time being taken when the worm ceased to move and did not recover when placed in fresh water.

1. THE EXAMINATION OF PEPO.

One pound of dried pumpkin seed was extracted with petroleum ether to remove fixed oil. This was followed by 75% alcohol until one liter of percolate was collected. Distillation of the percolate under reduced pressure gave a syrupy residue which was diluted to 75 cc. with distilled water and filtered with the aid of kieselguhr. This solution was mildly acid to litmus but highly active, killing in eight minutes. Treatment of this active solution with lead subacetate produced a voluminous precipitate. This was filtered and the precipitate suspended in water and treated with hydrogen sulphide, again filtered and the filtrate distilled under re-

* Scientific Section, A. PH. A., Rapid City meeting, 1929.

duced pressure. The product was inactive, indicating that the protein isolated by Grübler (8) with lead and barium has no activity.

The filtrate from the first lead precipitate was treated with hydrogen sulphide, filtered and distilled as before, after which the clear syrupy residue was diluted to the original volume. The killing time was the same as before.

Effect of Fermentation.—The above solution, after removal of lead, was next fermented with yeast until it did not reduce Fehling's solution, then filtered with kieselguhr and the filtrate reduced as before to a syrup. When built to the original volume the solution was neutral to litmus and killed in twenty-five minutes.

2. THE EXAMINATION OF CUCURBITA PEPO.

A ripe, twenty-five pound pumpkin was divided into the seeds, the pulp and the rind.

A. The seeds on removal were ground and macerated in alcohol for several days. They were then extracted with alcohol, the solvent evaporated at a low heat and fixed oil removed with petroleum ether. The residue, a syrup, was dissolved in three times its volume of water, giving a killing time of one hour.

B. The pulp was ground and pressed, yielding two liters of juice having a killing time of forty-five minutes. Since the juice was acid to litmus, a portion was heated with calcium carbonate, just short of boiling and filtered while hot. The killing time remained the same.

One hundred cc. of the juice was treated with 0.2 Gm. of barium hydroxide which, like lead, gives a heavy precipitate. The excess barium in the filtrate was removed by treatment with carbon dioxide and heating to boiling. The filtrate when reduced to the original volume showed no loss of activity. The material precipitated by barium was inactive.

All juice not worked the first day was preserved with 15% alcohol, this being subsequently removed by distilling under reduced pressure. The press cake from the pulp was heated to boiling on a water-bath with three pints of alcohol. Expression yielded one gallon of juice which was evaporated to a syrup. Ether and chloroform did not extract any active material. Alcohol removed active material which, when evaporated to a syrup and taken up in three times its volume of water, killed in five minutes.

This pulp from the above expression was dried and extracted with petroleum ether. This yielded the yellow pigment and a small amount of resin. Extraction was continued with alcohol containing 15% ether. Evaporation of the solvent and subsequent solution of the syrupy residue in three times its volume of water, killed in ten minutes.

C. The rind which carried considerable pulp was ground and heated to boiling with 2 quarts of alcohol. The expressed juice when treated as that from the pulp showed somewhat less activity than the corresponding juice from the pulp. After drying the rind and extracting with petroleum ether, it was extracted with alcohol which yielded additional active material.

D. The active material gave no positive tests with any of the alkaloidal reagents. Dialysis showed that the active material was transferred to the diffusate. Hydrolysis of a solution killing in fifteen minutes, by boiling with dilute sulphuric

acid; and removal with barium carbonate, increased the killing time to forty-five minutes.

Five per cent solutions of glucose, sucrose, levulose and lactose had no effect on earthworms, indicating that sugars are not responsible for the activity. Samples of dried active material which were allowed to stand six months in open beakers lost no activity.

E. Action of Pumpkin Pulp.—1000 cc. of expressed juice was evaporated and some of the dried pulp from B added and evaporation continued to dryness, after which pulp was added to a total of 100 Gm. and thoroughly mixed.

We hereby express our thanks to A. L. Brainard, D. V. M., for testing the vermifuge qualities of this product on dogs. The results follow:

In the first series of dogs no laxative was given and negative results were obtained in every case. Dose, 8 Gm. to 15 Gm. each.

In the second series of eight dogs the dose was the same but was followed by castor oil.

First Dog.—Collie; male; weight, 50 lbs.; age, 10 years. Starved 24 hours then 15 Gm. of dried pulp-juice given, followed by castor oil. Result, 2 round worms.

Second Dog.—Mongrel collie; male; weight, 24 lbs.; age, 2 years. Treated as No. 1. Result, several round worms and two tape worms.

Because of lack of material the six remaining dogs were given smaller doses followed by castor oil with negative results.

These results are by no means conclusive but do show an approximate effective dosage and we hope to continue the work on pumpkin pulp in an effort to isolate the active constituent.

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TWO NEW METHODS FOR PHARMACOLOGICAL COMPARISON OF INSOLUBLE PURGATIVES.*

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INTRODUCTORY.

Although the pharmacodynamics of intestinal movements has been studied by many eminent physiologists and pharmacologists such as Magnus (1), Cannon (2) and others, the methods for quantitative comparative evaluation of laxatives and purgatives at the disposal of the pharmacologist are still very inadequate, particularly in respect to those drugs which are not soluble in water or physiological solutions. When dealing with powerful alkaloids and other active principles readily soluble in water, it is a simple matter to follow their effects either on surviving segments of the intestine or isolated strips of muscle or even on the whole intestinal tract *in situ*. When, however, the investigator wishes to ascertain the action of laxative oils, resins and other insoluble substances, the problem is of a very different nature, and the commonest method of approach has hitherto been to feed the various materials to large animals, which had been previously given a dry diet, and then to note the frequency, the quantity and the consistency of the stools. Such experiments have usually been conducted on dogs, cats and rabbits. Sollmann (3) in his comprehensive Laboratory Guide in Pharmacology, can suggest no better method for comparing purgatives than a personal trial of representative specimens from various groups of laxatives by the students themselves. For economical reasons a number of German investigators after the World War attempted to utilize the white mouse for studying intestinal peristalsis. Thus, Laqueur (4) used the whole length of the excised intestine of the white mouse for demonstrating the effects of a number of active principles, which, however, are not used as purgatives. Fühner (5) devised an interesting method of roughly comparing the number of laxatives by feeding them to mice in the form of small pills and, following his work, Loewe and Faure (6) devised a more elaborate method of tracing the passage of ingesta by feeding mice with various laxatives together with small quantities of India ink. The latter method has not been satisfactory in the experience of the present writers but suggested the use of finely divided carbon in the first method to be described.

In connection with a study of the oil of *Ruvettus pretiosus*, or "castor-oil fish," the present authors began an extensive investigation on the comparative laxative

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