(*a*) Increase in body surface area.

(*b*) Exercise. The time in seconds decreases rapidly with increase in physical exercise.

(*c*) E, in seconds, decreases with advance in years.

(*d*) Sex. Time is considerably shorter for women.

(*e*) Fevers. Time in seconds decreases in proportion to the increase in body temperature.

(*f*) The time in seconds for E, is reduced to the maximum degree as basal rate conditions are approached.

(*g*) Reduction in food supply and starvation shortens the time in seconds.

(*h*) Thyroid gland medication shortens the time in seconds.

(*i*) Contrary to expectations, small doses of alcohol shorten the time in seconds considerably. Further tests should be made.

(*j*) High altitudes.

4. Factors and influences which apparently increase the time of E, in seconds:

(*a*) Food. The time in seconds is markedly increased after a meal.

(*b*) Rest, after exercise. (See *b*, above.)

(*c*) Low barometric pressure.

(*d*) Mental depression.

(*e*) Phlegmatic temperament. The indications are that a so-called phlegmatic temperament is the result of a reduction in the rate of tissue oxygen consumption.

(*f*) Time in seconds is apparently increased in those having sub-normal temperatures.

5. The CO_2 content of respiratory air is in proportion to the time that the air is held in the lungs, until a maximum increase is reached. The maximum CO_2 content of expired air in the tests for F and E, as above outlined, is about 12 per cent.

6. Thirty per cent above and thirty per cent below the average may be considered a normal range. These percentage ranges may prove too extreme as additional data are brought into the calculations.

7. There appears to be a correlation between E and the physicomental rating, but the evidence is as yet inconclusive.

THE EARTHWORM METHOD FOR TESTING SANTONIN AND RELATED ANTHELMINTICS.*

BY ALBERT SCHNEIDER, M.D., PH.D.

The author describes a modification of the Trendelenburg method. The rating of the anthelmintics is based on the spasm producing properties of the drug, and not upon its toxic action.

Santonin appears to have a specific action on the common earthworm or rainworm (*Lumbricus terrestris*), causing the muscular tissue to undergo a tonic as well as clonic spasmodic contraction which endures for long periods of time. Other species of earthworms and also intestinal ascarids and the leeches, react in a similar manner. The spasm-inducing action is due to the lactonic nature of the drugs, not being elicited by the sodium salts with santonic acid nor by the oxides, hydrates, or the chlorides of santonin and of related lactone compounds. This action is also produced by lactonic derivatives of santonin, by uncombined cumarin and by oil of chenopodium, and by other substances having anthelmintic prop-

* Scientific Section, A. PH. A., Philadelphia meeting, 1926.

erties. The toxic action of santonin on higher animals (mammalians) is not due to the lactone group, but to the hydrates, oxides and chlorides of santonin. The vermifuge action of santonin is not in proportion to the toxic action, but is dependent upon the spasm-inciting action of the lactone element.

According to Küchenmeister, solutions of santonin in oil will kill ascarids and other vermes within several hours. Other observers apparently failed to get like results. In fact most of the tests of this kind appeared to indicate that the anthelmintic action of the various vermifuges was not due to the toxic action upon the parasites, but was the result of the convulsive effects produced, causing the worms to become detached and to be carried into the lower intestinal tract and finally to the exterior, by the action of the laxatives which are administered simultaneously. It was, however, also demonstrated that stronger solutions or suspensions of the anthelmintics do kill the various parasitic ascarids and other members of the worm group. (Paul Trendelenburg, "Ueber die Wirkung des Santonins und seiner Deribate auf die Wurmmuskulatur, und Bemerkungen zur Wirkung des Oleum Chenopodii." *Archiv für Experimentelle Pathologie und Pharmakologie.* 79, 190–217 (1916).)

Santonin has a direct action on the muscle elements of the earthworm. Curare does not inhibit the tonic nor the clonic effects produced by this drug hence the action is not *via* the end plates, and the contractility is fully elicited after all nerve tissue is eliminated, hence is not of ganglionic nor of central origin. The lactonic nature of pilocarpine is indicated by the fact that it acts much like santonin, while the hydrochloride of pilocarpine is without such action. The Trendelenburg method of testing santonin and other vermifuges, by means of earthworm segments, is briefly as follows:

1. *Test Animals.*—Sound earthworms (*Lumbricus terrestris*) are to be used. Keep in moist rich soil until wanted for the tests.

2. *Preparing the Muscle Strips.*—Do all of the preparing in cool frog saline. By means of a sharp pair of scissors, cut away the anterior portion of the worm, including the clitellum. Cut off segments or strips about 2 to 3 cm. long, at once transfer these to frog saline. Each strip is now cut open on the abdominal side, removing nerve ganglia and other tissues, leaving only the muscular structure which is united to the dermal tissue. These strips may be preserved in frog saline for several days, provided the solution is properly serated. Do not allow the temperature to rise above 18 degrees centigrade.

3. *The Muscle-Lever Preparation.*—Employ the same set-up as for a frog muscle, using smaller containers and lighter weights and levers. Use a slow drum so that the recording may continue for several hours. Allow the set-up to come to rest before beginning the tracing.

4. *Applying the Test Solutions.*—Prepare a concentrated solution of santonin (also of other lactones) in boiling distilled water. Distilled water is to be used to prevent the possible conversion of the lactone into the chloride (the chloride of the frog saline) due to heat action. Pipette off or otherwise remove the frog saline from the muscle strip preparation, and replace it with the test solution, which after cooling may be diluted as desired. Almost at once the muscle strip will show a continuous spasmodic contraction which may endure for hours. Make tracings on a very slow drum. It will be found that weaker solutions of santonin give rise to the same quality of action (tonic contraction followed by a clonic action) as the stronger solutions. The only difference is in degree.

If the santonin solution is replaced by frog saline, the strip again returns to the normal stage. After a time the santonin action can again be elicited. This distinctive reversible action can be developed a number of times in the same muscle strip. Santonin action can be elicited after the action of poisons (curara, nicotin, muscarin), as long as the dose of the poisons is sublethal.

The action on the earthworm muscle preparations may be summarized as follows:

1. The action continues as long as the santonin solution is applied, and may endure without cessation for a day or longer.

2. As soon as the santonin solution is applied, the muscle contracts markedly, and while in contraction shows a spasmodic action which is not rhythmical, from 4 to 10 major contractions per minute. There is therefore a tonic as well as clonic action, both types of spasm continuing without interruption during the entire time that the santonin is applied, and both spasms cease as soon as the drug is replaced by the frog saline.

3. The tonic contraction and the clonic spasm are proportional to the concentration of the lactone solutions applied, and with carefully adjusted dosages it is possible to accurately measure the comparative anthelmintic action of the lactone group.

4. The reversible active and rest state of the muscle preparations is entirely independent of the percentage strength of the test solutions applied.

5. The change in the muscle action of the santonin group is positive, rather than negative; that is, increased concentrations show increased contractility rather than evidence of paralysis or depression. One per cent solutions of santonin in olive oil causes no symptoms of paralysiss

6. The simultaneous application of santonin solutions and small doses of such poison. as nicotine, acrolein, pilocarpine and physostigmine, does not interfere with the characteristic reversible santonin action. Rather, these poisons increase the tonic contraction, with some lessening of the clonic spasms. Acrolein in particular soon results in paralysis when combined with santonin and renders the reversible action irreversible; that is the paralytic action of this poison cannot be overcome by the santonin. Pilocarpine and physostigmine increase the tonic action of santonin, and when applied to muscle strips by themselves show a lesser tonic action than equal strength solutions of santonin, and the clonic action does not develop at all or at most only to a very slight degree.

7. The above action of anthelmintics on worm muscle preparations also substantiates the observations made by investigators; namely, that some vermifuges when given in combination, apparently give rise to a summation action, similar to that following the combination of spices with anthelmintics. Bile also increases the action of anthelmintics.

The Trendelenburg worm segment method as above outlined will be found very satisfactory provided the precautions are taken to preserve the muscular irritability by frog saline, æration of the saline, maintaining a uniformity low temperature, and making the muscle preparations with sharp scalpel and scissors, and the levers are carefully counter-weighted. The method can be greatly simplified with an increase in the accuracy of the results, by using whole sound worms. The use of whole worms, with tissues intact, more nearly simulates the actual conditions in practice. The following is an outline of the proposed method. Sound worms may be gathered in sufficient numbers and kept in fresh moist earth in an earthen jar. They will keep for many days.

1. Place a normal earthworm in a beaker, wash away all dirt by means of hydrant water. Pour off the wash water and replace it with the frog saline containing a trace of calcium chloride (two drops to the half liter). Determine the rate of contraction and relaxation per minute, and the average length of the worm. Continue these observations for five minutes, or until the stationary shortening of the worm has developed.

2. Pour off the saline and replace it with 5 cc. of a saturated aqueous solution of santonin. Note the increase in the rate and in the degree of the contractions. Note the gradual shortening of the worm, and the shortening of the girdle or clitellum. Continue the observations for five minutes. Determine the degree of the shortening as compared with the normal saline worm. Wait until maximum shortening has developed.

3. Pour off the santonin solution, wash in hydrant water, replace in normal saline and note the gradual return to normal in length and in the rate and the degree of the contractions. (2)

and (3) may be repeated three or four times, to demonstrate clearly the reversibility of the santonin action.

4. Again replace the worm in the saline solution, after having washed away the santonin solution. When contractions have reached the maximum in the santonin solution, add two or three cc. of a bile (ox gall) solution. Note the increase in the rate as well as in the degree of the contractions. Bile increases the action of santonin.

5. Extract the crushed pumpkin seeds in water and test the extract on worms as above.

6. The oleo-resin of male fern is to be dissolved in some bland oil and applied to the earthworms. Use varying strengths of the vermifuge and determine that strength solution which will cause a maximum shortening of the worm within five minutes of time, and use this as the standard of comparison. Also try oil of chenopodium, menthol, thymol and turpentine, dissolved in oil, determining the smallest dose which will just cause the maximum shortening of the worm and the clitellum.

The following anthelmintics may be tried out on earthworm muscle preparations and on the whole earthworms:

Aspidium	Cousso	Dichlorbenzol	Pumpkin seeds
Chenopodium oil	Spigelia	Thymol	Squash seeds
Granatum	Dibrombenzol	Menthol	Citrullus seeds

Solutions and suspensions of the anthelmintics may be made in various media, as olive oil, mineral oil, solutions containing bile and bile salts with sodium bicarbonate, water (fresh pumpkin and squash seeds).

The following are suggested strength solutions of the several vermifuges and of other substances to be tried out on the earthworm muscle preparations or on whole earthworms:

1. Oil of chenopodium, 1–500 to 1–16,000.
2. Santonin, 1–1000 to 1–10,000.
3. Artemisin, 1–250 to 1–5000.
4. Oil of chenopodium, 1–5000 to 1–15,000.
5. Cumarin, 1–1000 to 1–10,000.
6. Pumpkin seed (aqueous extract), 1–500 to 1–5000.
7. Acrolein 1–1000 to 1–5000.
8. Pilocarpine, 1–1000.
9. Physostigmine, 1–1000.
10. Salts of santonin, 1–1000 to 1–10,000.
11. Other vermifuges.

Varying amounts of bile, aromatic oils, extracts of spices as of capsicum, pepper and cubeb, may be added to the anthelmintics for the purpose of determining the comparative activating or additive action of these substances. These tests must be carefully checked by controls. If now the amounts of the activators be reduced below the threshold of action when added singly, a number of such activators in sub-threshold doses be added to the anthelmintic, there will again be noted an increase in the action of the drug, suggesting a summation action of the activators.

The two earthworm methods for testing anthelmintics above outlined are simple and can be carried out readily in pharmacologic laboratories of colleges. The microscopic method for testing anthelmintics, as proposed by Trendelenburg, is impracticable. Teased muscle tissue of the earthworm is mounted in normal frog saline and the action of the anthelmintics on the muscle elements

observed under the low power of the compound microscope. It will, however, be found that the procedure invariably kills the muscle tissue even before the preparations are ready for observation.

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SOME COLLOIDAL CHEMICAL ASPECTS OF PHARMACOGNOSY.*

BY ANTON HOGSTAD, JR.

Several years ago there appeared in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION an article by one of the younger generation of American pharmacognosists, namely Prof. E. Wirth, which dealt with the subject of Pharmacognosy, Past, Present and Future.

The author at that time called attention to the fact that the phase of chemical Pharmacognosy was being sadly neglected for that of histological work in our colleges of Pharmacy, and that the chemical side of Pharmacognosy was being riddled by others than pharmacognosists.

Let us ask ourselves the question "Wherein lies the value of pharmacognostical training?" Before same can be answered we must bear the thought in mind that those boys and girls who matriculate in our colleges of Pharmacy do so with the thought in mind that some day they hope to become retail pharmacists. The question would then read as follows "Wherein lies the value of a training in Pharmacognosy for the retail pharmacist?"

Pharmacognosy, if separated from the work in Materia Medica proper, should be considered a preparatory course for that phase of Pharmacy that deals with the action and therapeutics of drugs. Therefore, if our colleges of Pharmacy are devoting some 80 per cent of the time allotted to Pharmacognosy in histological work, I feel that we, as pharmacognosists, have failed in our interpretation of the subject as well as in the fulfilment of the obligations placed upon us as pharmacognosists.

There is a great deal more in chemical Pharmacognosy than the mere definition of a constituent, perhaps with mention of its percentage. Over and beyond this type of Pharmacognosy, to which many attach an overabundance of histological work, lies the application of a pharmacognostical knowledge in later life. It is no easy matter to say how much to leave out in the way of this routine phase of Pharmacognosy, but it certainly is apparent that there is more to Pharmacognosy than this 80 per cent of histological work and a mere mention of the constituents of a drug.

Now what has colloidal chemistry to do with this type of discussion and Pharmacognosy in general? To the one who has followed the trend of thought in the realm of colloidal chemistry in relation to medicine, there is a very close relationship as I shall try and point out in the course of this paper.

Before entering upon the discussion of this relationship, I wish hastily to review a few of the underlying principles in colloidal chemistry, which have materially changed my viewpoint of pharmacognosy.

* Read before Chicago Branch A. PH. A., at the 167th meeting, May 17, 1927.