but, judging from experience in grinding arsenic trioxide, the particles contained in a single preparation probably vary greatly in size, because of the relatively short grinding to which the materials may have been subjected.

The recommendation herein made is consistent with the policy of raising the standard for drugs. It is important in the medicinal use of undissolved arsenic trioxide and will only slightly increase the work of the manufacturing pharmacist.

This subject is not without some importance to legal medicine and casts an interesting reflection on some medical logic. It has long been believed that the "Arsenic Eaters" of Styria (Austria) become immune to the effects of arsenic. It does not appear, however, that the toxicity of the arsenic which is eaten has ever been tested. Instead of an immunity of the individual to the arsenic, the arsenic is relatively impotent, according to the extent to which it remains undissolved. Since an absolute amount of one preparation representing a fatal dose is not a criterion of the lethal dose of another preparation, as has heretofore been assumed, habituation to arsenic may be said to have never been proved.

DISCUSSION.

Drs. Fantus and Viehoever emphasized the importance of these findings.

Dr. Fantus suggested that the discrepancies in the administration of calomel might also be explained by differences in the fineness of different preparations.

ISOLATED UTERUS ASSAY FOR PITUITARY EXTRACT.*

NOTES ON METHODS OF ELIMINATING SOME DIFFICULTIES ENCOUNTERED WITH THE ABOVE METHOD. (SECOND PAPER.)

BY PAUL S. PITTENGER AND ARNOLD QUICI.

The various laboratories interested in the assay of pituitary extract have used the Isolated Uterus Method with varying degrees of success.

Some workers report this method to be by far the best proposed, while others report that they have found it entirely unsatisfactory.

One of the authors has used this method continuously for the past ten years, and the other for the past six years, and both are of the opinion that it is by far the best which has been proposed for the standardization of pituitary extract as differences of activity which are only just appreciable by other methods are at once obvious in the test on the isolated uterus.

Our experience, however, has proved that satisfactory results cannot be obtained with this method unless all conditions are ideal.

The presence of minute amounts of impurities in the distilled water or chemicals used in preparing the Locke solution will destroy the sensitiveness of the uterus. Bacterial contamination of the Locke solution will poison the uterus and make it impossible to obtain concordant results.

Variations in the temperature of the solution and the amount of muscular tissue present in the uterus are also factors of prime importance.

The many criticisms of the isolated uterus method to the effect that it gave unsatisfactory results, without stating in what way the method was unsatisfactory, or suggesting means of improving it, led us to the opinion that in many cases these

* Read before Scientific Section, A. Ph. A., Cleveland meeting, 1922.

unsatisfactory results were due to the operator's *failure to observe* some of the essential details which we have found to be the key to success or failure.

The literature does not contain reports showing the minute details and care which must be exercised in preparing the Locke solution, or reports attributing the cause of unsatisfactory results to the Locke solution.

When we stop to consider the fact that when the uterus is taken from the animal's body and placed in the Locke solution, this solution is actually replacing the blood of the animal, it is easy to realize how a slight variation in its composition can markedly influence the results obtained.

In a paper read before the Scientific Section of the Penna. State Pharmaceutical Association we presented in the form of notes, some of the difficulties we have encountered, and our methods of eliminating them.

At that time, however, we were not in position to arrive at definite conclusions in reference to the unsatisfactory results obtained with some lots of sodium chloride.

Having solved this problem and the fact that the "Proceedings" of the State Association are not available to most of the members of this Association prompted us to present this "second paper."

DISTILLED WATER.

After obtaining satisfactory results for a period of over two years we had a series of 8 or 10 consecutive assays in which it was practically impossible to obtain concordant results.

The trouble was finally traced to *distilled water*. Our source of supply had been changed and upon testing we found that the water was not quite as pure as that previously employed. On returning to the use of distilled water from the original source the trouble was entirely eliminated.

Therefore absolutely pure distilled water is essential. Glass distilled water is to be preferred.

BACTERIAL CONTAMINATION.

On one other occasion unsatisfactory results were found to be due to excessive contamination of the Locke solution.

A bulk container had been refilled many times without being completely emptied or washed.

After boiling out all containers, tubing, etc., and filling with new Locke solution, satisfactory results were again obtained.

Our experience has since shown that it is not necessary to actually sterilize the apparatus or Locke solution but it is absolutely necessary that all containers, tubing, etc., be thoroughly cleaned with hot water at least once a week.

SELECTION OF TEST MUSCLE.

Uteri differ greatly in their mutual relation as to power and muscular structure. Some specimens are greatly deficient in muscular substance and act feebly, while other specimens show greater muscular development and contract strongly. Some specimens prove absolutely inert and will not respond at all. The normal activity, however, practically runs parallel with the amount of muscular tissue present; the "stringy" uteri are all deficient in normal activity and in response to stimuli, while the thick, more muscular uteri are practically all active and sensitive. This knowledge enables the operator to save considerable amounts of time as it renders

it possible for him to distinguish between active and inactive uteri before connecting them with the apparatus.

PURITY OF CHEMICALS.

When preparing Locke solution only the *highest purity* "Reagent" or "Analyzed Chemicals" should be employed.

By carefully observing the above precautions the authors have for many years obtained highly satisfactory and concordant results.

About eight weeks ago, however, we encountered a new difficulty: the uterus after contracting from an initial dose of the extract would not relax upon replacing the drugged solution with fresh Locke solution.

A series of experiments proved that this condition was produced by some variation in the composition of the Locke solution.

Figure 1 shows a tracing of the contraction produced by pituitary extract when Locke solution of the proper composition is employed. *A* to *B* shows the

Fig. 1.

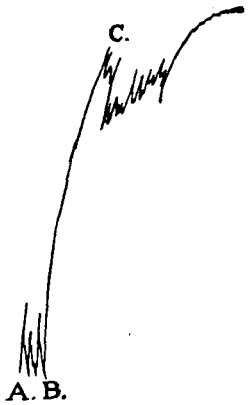

Fig. 2.

Fig. 3.

Fig. 1 shows a tracing of the contraction produced by pituitary ext. when Locke solution of the proper composition is employed. *A* to *B*, normal. At *B*, 0.01 cc pituitary ext. added. At *C*, drug solution replaced by Locke solution.

Figs. 2 and 3 show the contractions produced by pituitary extract when Locke solution of improper composition is employed.

normal contraction. At *B* 0.01 cc of pituitary extract was added. At *C* the drugged solution was replaced by plain Locke solution. You will note that under normal conditions the uterus relaxes immediately.

Figures 2 and 3 show tracings of the contractions produced by pituitary extract when the Locke solution is *not* of the proper composition. *A* to *B* shows the normal contraction. At *B* 0.01 cc of pituitary extract was added. At *C* the drugged solution was replaced by plain Locke solution.

You will note that in both cases the *uterus failed to relax due to the improper composition of the Locke solution.*

By the process of elimination we found that the *trouble was caused by variations in the sodium chloride employed in making the Locke solution.*

We have always used "Analyzed" sodium chloride and never before have experienced any difficulty.

After definitely tracing the above condition to the sodium chloride we purchased several new 5-pound lots of "Analyzed" NaCl, and one lot of "Reagent" NaCl.

Locke solution was prepared from these samples and tested on the isolated uterus. Three of the lots labeled "Analyzed" were found to be satisfactory, while three other lots labeled in the same manner, and the one lot labeled "Reagent" were unsatisfactory.

The statements on the labels as to the purity of the different lots follow:

No. 1.
"Analyzed" Lot No. 52321.

Al_2O_3	0.001%
CaO	0.001%
Fe	0.001%
I	none
K	trace
SO_3	0.001%
MgO	0.001%

No. 2.
"Analyzed" Lot No. 111819.

Al_2O_3	0.001%
CaO	0.001%
Fe	0.001%
I	none
K	trace
SO_3	0.002%
MgO	0.001%
Acidity	0.010%

No. 3.
"Analyzed" Lot No. 42020.

Al_2O_3	0.001%
CaO	0.001%
Fe	0.001%
I	none
K	trace
SO_3	0.001%
MgO	0.001%

No. 4.
"Analyzed" Lot No. 41922.

Al_2O_3	0.001%
CaO	0.001%
Fe	0.001%
I	none
K	trace
SO_3	0.001%
MgO	0.001%
Acidity	neutral

No. 5.
"Analyzed" Lot No. 81621.

Al_2O_3	0.001%
CaO	0.001%
Fe	0.002%
I	none
K	trace
SO_3	0.001%
MgO	0.001%
Acidity	0.003%

No. 6.
"Reagent" Lot No. 21242.

SO_3	0.0020%
I	0.0300%
Ca	0.0100%
Mg	0.0010%
K	0.1800%
NH_3	0.0005%
Fe	0.0005%
Other heavy metals	0.0000%

No. 7.
"Analyzed" Lot No. 41822.

Al_2O_3	0.001%
CaO	0.001%
Fe	0.001%
I	none
K	trace
SO_3	0.001%
MgO	0.001%
Acidity	neutral

Samples Nos. 1, 2 and 3 were satisfactory, and solutions prepared from them gave results as shown in Fig. 1.

Samples Nos. 4, 5, 6 and 7 were unsatisfactory and solutions prepared from these salts produced contractions as shown in Figs. 2 and 3.

A careful study of the statements on the labels will show that it is absolutely impossible to make any deductions from this information as to why some of the samples are satisfactory and others are not.

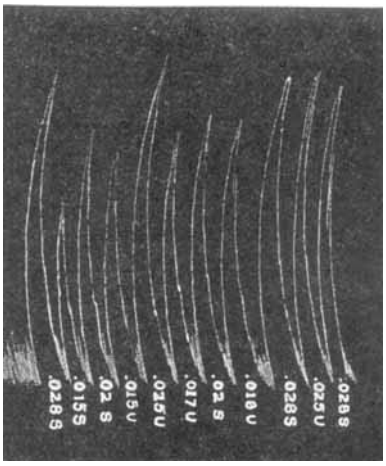

Fig. 4.

Fig. 4 shows tracing of a pituitary assay carried out under the proper conditions. You will note that in the above assay the uterus reacted quantitatively to variations in the dose of 0.001 cc.

We observed, however, that all of the satisfactory lots were in the form of *large* crystals, while all of the unsatisfactory lots were powdered or in small crystals.

We therefore purchased additional lots from different sources and found that in every case the *large* crystals were satisfactory while the salts in the form of powder or small crystals were not.

This led us to believe that perhaps the manufacturers take the sample for assay from the "bulk lot" and that powdering is a subsequent operation during which some form of contamination occurs.

Further investigation, however, proved that this is not the case and that there exists an actual difference in the purity or composition of the two forms of salt. The large crystals are from the "first crop" and usually prepared from sodium bicarbonate while the powder or small crystals are from "subsequent crops" and in some cases prepared from other salts.

These minute differences in composition are negligible when the salt is used for ordinary analytical purposes. When used for preparing Locke solution for isolated uterus experiments, however, they are of the utmost importance and the key to success or failure.

Therefore, only "Analyzed" or "Reagent" sodium chloride in the form of *large* crystals should be used in preparing Locke solution.

The uniformly concordant results which we obtain with the Isolated Uterus Method for testing pituitary extracts led the authors to believe that many of the unsatisfactory results reported have no doubt been due to one or more of the factors mentioned above.

CONCLUSIONS.

In order to obtain satisfactory results with the isolated uterus method of assaying pituitary extracts:

1. Glass distilled water must be used.
2. All chemicals employed in making Locke solution must be of the highest ("Reagent") purity.
3. All apparatus and solution containers must be frequently washed with boiling water, and Locke solution should be freshly prepared.
4. Thin, "stringy" uteri should not be used as they are all deficient in normal activity, and in response to stimuli, while the thick, more muscular uteri are practically all active and sensitive.

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,
H. K. MULFORD COMPANY.
AUGUST 1, 1922.

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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* Scientific Section, A. Ph. A., Cleveland meeting, 1922.

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