and Marchal (8) state that while they have no actual data concerning the extent to which sensitiveness to the salvarsan group may differ with different strains of mice, "It can only be said that there is no evidence as yet of wide permanent difference in this response between stocks used in different European laboratories."

It is apparent from the foregoing data that it is not possible to make a significant comparison of toxicity among samples of neoarsphenamine when rats from different strains are used. It is equally apparent that the relative non-toxicity of several samples of neoarsphenamine may be quite accurately determined if one strain of animals is used in making the test. Hence, the determination of the toxicity of neoarsphenamine, like most biological assays, is without significance unless a physical standard of reference is used to standardize the resistance of animals from a single colony.

SUMMARY.

1. There is a definite and constant difference in the tolerance to neoarsphenamine of albino rats from different colonies.

2. The relative non-toxicity of several samples of neoarsphenamine remains constant when tested on animals from any single colony.

3. In order to standardize the resistance of animals from different colonies, a physical standard of reference, supplied by some central authority, should be used for comparison in every assay of neoarsphenamine.

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A STUDY OF SOLUTION OF MAGNESIUM CITRATE.*

BY ARTHUR OSOL AND LINWOOD F. TICE.[1]

The purpose of the investigation reported in this paper was to study solution of magnesium citrate U. S. P. XI to definitely establish whether the reduction in citric acid content from 35 to 33 Gm. per bottle constituted a desirable change and assured a stable product.

It has been claimed that 33 Gm. of citric acid provided a sufficient amount to permit the preparation of a stable solution of magnesium citrate, *i. e.*, one which upon standing would not be subject to precipitation. Arny and Schaefer (1) have reported that in their opinion "the increase in citric acid content of the 350-cc. bottle of solution of magnesium citrate from the 33 Gm. of the U. S. P. IX to the 35 Gm. of the U. S. P. X was inadvisable and unnecessary."

* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] From the Laboratories of the Philadelphia College of Pharmacy and Science.

Obviously the best method of investigating this problem was the preparation of a series of samples in which all the variables likely to occur in pharmaceutical practice were introduced and their effects on the product tabulated.

Magnesium carbonate was obtained from two of the largest sources of supply in this country, and these samples assayed for their MgO and CaO content. Sample No. 1 gave results indicating an MgO content of 41.6 per cent and a CaO content of 0.31 per cent, whereas sample No. 2 contained 41.7 per cent MgO and a negligible amount of CaO. The latter was chosen for the experimental work, due to its lower calcium content.

Using a fresh supply of citric acid U. S. P. a series of experimental solutions of magnesium citrate were prepared in which the amount of magnesium carbonate employed was varied so as to give the equivalent of 15 Gm. of a 39.2% MgO carbonate, 15 Gm. of a 40% MgO carbonate, 15 Gm. of a 42% MgO carbonate and 15 Gm. of a 44% MgO carbonate per bottle. This was done in realization of the possible variation in commercial samples of magnesium carbonate. The other two variables introduced into the experimental work were the addition of potassium bicarbonate and sterilization. Thus sixteen experimental batches of solution of magnesium citrate were prepared, kept over a period of three weeks at room temperature and the presence or absence of precipitation observed at weekly intervals. The results of this study are as follows: (Slide I.)

	MgO Content of Carbonate Used.	$KHCO_3$ Used.	Sterile.	Condition on Aging. 1 Week.	2 Weeks.	3 Weeks.
1	39.2%	None	No	Clear	Mold	Mold
2	39.2%	None	Yes	Clear	Clear	Clear
3	39.2%	2.5 Gm.	Yes	Clear	Clear	Slight ppt.
4	39.2%	2.5 Gm.	No	Clear	Slight ppt.	Slight ppt.
5	40.0%	None	No	Mold	Mold	Mold
6	40.0%	None	Yes	Clear	Clear	Clear
7	40.0%	2.5 Gm.	Yes	Clear	Slight ppt.	Slight ppt.
8	40.0%	2.5 Gm.	No	Slight ppt.	Ppt.	Ppt.
9	42.0%	None	No	Clear	Slight ppt.	Slight ppt.
10	42.0%	None	Yes	Clear	Clear	Clear
11	42.0%	2.5 Gm.	Yes	Slight ppt.	Ppt.	Ppt.
12	42.0%	2.5 Gm.	No	Ppt. more than No. 11	Ppt.	Heavy ppt.
13	44.0%	None	No	Mold	Mold	Mold
14	44.0%	None	Yes	Clear	Clear	Clear
15	44.0%	2.5 Gm.	Yes	Ppt.	Ppt.	Heavy ppt.
16	44.0%	2.5 Gm.	No	Ppt. more than No. 15	Ppt.	Heavy ppt.

From the tabulation the following points seem important:

1. With 33 Gm. of citric acid per bottle a 39.2 per cent MgO carbonate or its equivalent must be used to give even fairly satisfactory results.
2. If the potassium bicarbonate is not included, the product is stable (if sterile) regardless of the carbonate used.
3. Using 33 Gm. of citric acid and 15 Gm. of the generally marketed magnesium carbonate, a very unsatisfactory product results if potassium bicarbonate is included according to the directions of the U. S. P.
4. Sterilization in some manner retards to a certain degree the precipitation observed in the product.

The precipitate which formed in the various samples was of the same character as that described by Rees (2) who found such a crystalline deposit to be tribasic

magnesium citrate. It was furthermore noted that those samples in which the magnesium oxide content was higher showed considerably more precipitation. This is in accordance with the findings of Oakley and Krantz (3) who showed that when citric acid in solution is treated with magnesium oxide tribasic magnesium citrate will be formed in solution before all the hydrogen ions arising from the primary and secondary dissociations of citric acid have been neutralized and that, consequently, an excess of citric acid would act in such a manner as to stabilize the solution against precipitation by virtue of its shifting the equilibrium in the direction of forming an acid citrate.

Realizing the importance of the free (titratable) acidity of solution of magnesium citrate the free acidities of the various experimental lots were determined, expressed in terms of cc. of $N/2$ NaOH required to neutralize 10 cc. of the solution in the presence of phenolphthalein indicator. It was found that the actual values obtained corresponded quite closely with those obtained theoretically by calculation, based on the magnesium oxide, potassium bicarbonate and citric acid content. This substantiated the results of Mayer (4) who found such a calculation to give a result very close to that obtained by actual titration.

As an illustration of this close conformity between actual and calculated free acidity values the following are given as examples:

	Actual Value.	Calculated Value.
Sample No. 2	10.13 cc.	10.10 cc.
Sample No. 4	8.76 cc.	8.66 cc.

In no case was the difference between the calculated and actual values greater than 0.3 cc.

Inasmuch as all the samples of solution of magnesium citrate containing potassium bicarbonate prepared in the laboratory showed evidence of precipitation, the authors were led to examine several of the commercial products on the market. Five samples were purchased from retail pharmacies in Philadelphia. Each sample was manufactured by a different large scale producer of this product, and was labeled with the official title.

All five samples were found to be perfectly clear without the slightest trace of precipitate being present. Each was then assayed for magnesium oxide, free acidity and total citric acid. The results were as follows: (Slide II.)

RESULTS OF ANALYSES OF COMMERCIAL SAMPLES OF SOLUTION OF MAGNESIUM CITRATE U. S. P. XI.

Sample No.	MgO Gm./ 100 Cc.	Free Acidity Cc. $N/2$ NaOH per 10-Cc. Solution.	Free Citric Acid (Calculated from Free Acidity Titration) Gm./350 Cc.	Total Citric Acid Gm./350 Cc.
1	1.676	10.92	13.38	33.46
2	1.680	8.89	10.89	31.15
3	1.633	9.18	11.26	30.91
4	1.461	9.58	11.74	29.28
5	1.652	10.92	13.38	33.64

From these figures it will be seen that all were of U. S. P. quality in regard to magnesium oxide with the exception of No. 4 which was 0.139 Gm./100 cc. lower than the U. S. P. XI minimum of 1.6 Gm./100 cc. Samples No. 2, No. 3 and No. 4 were below the U. S. P. XI minimum for total citric acid which may be shown by calculation to be 31.85 Gm. per 350 cc.

In regard to total citric acid deficiency it may be said here that, in the opinion of the authors, the requirement of the U. S. P. XI seems a little too rigid.

The statement at the end of the U. S. P. XI method for total citric acid "Not less than 26 cc. of half-normal hydrochloric acid is consumed," when converted into grams of citric acid per 350 cc. gives 31.85 Gm. This means that only a 3.48 per cent tolerance in citric acid content is allowed which is not in accordance with the tolerance set forth in other pharmacopœial solutions of a similar character.

Furthermore, the method prescribed for total citric acid as a rule yields somewhat low results due to the slight solubility of the calcium citrate precipitated in the process. As a maximum, however, this should not attain a degree resulting in more than 0.5 Gm. of citric acid per 350 cc. escaping detection.

In view of these two points the statement of the Pharmacopœia should, in all fairness, be modified to read "Not less than 25.2 cc. of half-normal hydrochloric acid is consumed." This would compensate for the maximum error of the method, 0.5 Gm./350 cc., plus a tolerance of 5 per cent, namely, 1.65 Gm./350 cc. giving 30.85 Gm./350 cc. This in terms of *N*/2 hydrochloric acid for 10 cc. equals 25.18 cc.

The perfect clarity of all the commercial samples was probably due to their high free acidities but upon close consideration it was seen that such values were impossible if the U. S. P. formula were followed. This was proved by taking the values obtained by assay for magnesium oxide, free citric acid and total citric acid and, assuming that the potassium or sodium bicarbonate was included, calculating the free acidity which the product should possess. The values so obtained were far below those actually indicated by titration. However, making similar calculations assuming the bicarbonate to have been omitted gave a series of figures very close to those actually obtained by titration. This is illustrated in the following tabulation: (Slide III.)

COMPARISON OF FREE ACIDITY VALUES.

Commercial Sample No.	Free Acidity Cc. *N*/2 NaOH 10 Cc. Soln.	Calculated Free Acidity from Determined MgO and Total Citric Acid Content if 2.5 Gm. $KHCO_3$ Were Added.	Calculated Free Acidity from Determined MgO and Total Citric Acid Content without Inclusion of $KHCO_3$.
1	10.92 cc.	9.25 cc.	10.68 cc.
2	8.89 cc.	7.32 cc.	8.75 cc.
3	9.18 cc.	7.60 cc.	9.03 cc.
4	9.58 cc.	7.98 cc.	9.41 cc.
5	10.92 cc.	9.63 cc.	11.06 cc.

Sample No. 5 will be seen to prove an exception, inasmuch as the calculated free acidity omitting the bicarbonate is slightly higher than the figure obtained by titration. This product, in all probability, had some bicarbonate, at least, put in the formula.

Mention has already been made of the somewhat low results given by the U. S. P. XI assay for total citric acid, due to the slight solubility of the calcium citrate precipitated in the process. It is quite unlikely, however, that this error exceeds over 0.5 Gm. citric acid per 350 cc. as a maximum. Assuming that this error were made it would only alter the calculated free acidities by 0.41 cc. The omission of bicarbonate on the other hand is reflected in a 1.43-cc. difference in the calculated free acidity.

These considerations leave little doubt but that the manufacturers whose products were examined are attaining the necessary free acidity to prevent pre-

cipitation by omitting a part, if not all, of the potassium or sodium bicarbonate. This is both illegal and detrimental to the therapeutic properties of the product. The potassium or sodium citrate formed by the added bicarbonate has therapeutic effect not possessed by magnesium citrate and its omission cannot be justified. The Pharmacopœia in permitting the use of CO_2 under pressure definitely states that this may be done in addition to the use of bicarbonate and not as an alternative process.

From our study it would appear that the present U. S. P. formula is unsatisfactory, inasmuch as, when made with many samples of U. S. P. magnesium carbonate on the market, the product is subject to precipitation. The pharmacist cannot be expected to assay his magnesium carbonate before using it, as would be necessary before a stable product could be made. The only means of preventing precipitation with the present formula is to withhold the bicarbonate until the solution is to be dispensed.

CONCLUSIONS.

1. Using 33 Gm. of citric acid per bottle a magnesium carbonate containing not more than 39.2 per cent oxide must be used to produce even a fairly stable solution of magnesium citrate.

2. Precipitation may be prevented using any official magnesium carbonate (39.2–41.5 per cent MgO) if the bicarbonate is withheld until the product is dispensed.

3. Sterilization in some manner retards precipitation observed in the product.

4. Many commercial samples give analytical results indicating that all or at least part of the bicarbonate is omitted from the product.

5. It is recommended that the U. S. P. permit a greater tolerance in the requirement for total citric acid.

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DAPHNIA—THE BIOLOGICAL REAGENT.*

BY ARNO VIEHOEVER.[1]

Daphnia (*magna*), the transparent crustacean, possesses well-developed muscular, nervous and glandular systems (1, 2). It thus may serve as a remarkable test-animal for the detection of the presence or absence of substances, affecting the cells or organs of these systems. Such active agents may either be obtained from the plant, animal and mineral kingdom, or prepared synthetically.

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