peculiarly emergency agents. With the exception, perhaps, of the thyroid gland, they are in general not curative.

The pituitary body will come to the rescue of an exhausted mother, but ergot may prevent the exhaustion.

The dramatic effects which are sometimes obtained through the aid of these hormones has tended to raise unwarranted hopes as to their usefulness and blinded us to their ephemeral character. This is not a depreciation of the value of hormones in medicine; it is rather a new appreciation of old drugs.

Finally, consider the vitamins.

Fifteen years ago dieticians considered that when they understood the calorific value and digestibility of a food that was all that science could tell them about foods. To-day consideration of vitamins has almost obscured that of calories, and foods have become drugs. The leaven has become of more consequence than the bread. Wheat fields are giving way to spinach pastures. The fishes have been changed from brain-food to nose and throat remedies and also supplement the bone-food. The humble are beginning to inherit the earth.

And who knows whether we have yet discovered the real curative powers of vegetable drugs? They still hold a strong place in materia medica. They still baffle us to explain their effects in full. They maintain a mystic value, even with physicians.

I, for one, am not willing to concede that vegetable drugs are as yet threatened with obscurity. They are still worthy of pharmaceutical study.

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DETROIT, MICH.

AN EXPERIMENTAL STUDY OF THE DETERIORATION AND ASSAY OF SPIRIT OF ETHYL NITRITE.

BY MARVIN J. ANDREWS.

(*Continued from page 807, August 1932.*)

STUDY OF THE EFFECT OF ADDED PRESERVATIVES.

The addition of some foreign substance intended to serve as a preservative has been one of the methods most frequently suggested for improving the keeping qualities of this preparation. The different substances which have been recommended for this purpose in the past are given in Table II. Of these, sodium bicarbonate, magnesium carbonate, potassium carbonate and glycerin have been most frequently mentioned and were therefore included among the substances selected for the experiments of this series.

In the first group of tests made, sodium bicarbonate and magnesium carbonate were used. In each case an amount equal to about 1 per cent of the spirit was added. Both salts had been used by other investigators as previously shown, and were used by them to neutralize the acid as rapidly as it was formed, as it was believed that the presence of acid was one of the principle causes of deterioration. A sample of the unaltered spirit was stored along with these two samples for comparison. The rate of deterioration of all three is shown in Tables IX, X and XI.

The second group of substances studied embraced glycerin and castor oil. Here again the amount added was about 1 per cent of the volume of the spirit.

Glycerin was given a trial because, as already stated, it is mentioned as having been used by other investigators. Castor oil, a fixed oil soluble in alcohol, was added with the idea that the effect on the surface tension would tend to retard the loss of ethyl nitrite by evaporation.

A third group of salts, Rochelle salt, disodium phosphate and potassium carbonate, were used in another series of tests. These were added in amounts equal to about 0.1 per cent of the spirit. The first two were tried as it was thought that they might exert a buffer action on the preparation and tend to stabilize it. The effect of the potassium carbonate would naturally be the same as that of the substances used in the first series, and it is only for the purpose of comparison that this salt was used.

The fourth and last series of these tests was carried out to determine the value of 0.1 per cent of saponin as an added preservative. The saponin used was the product of Merck & Co., Inc., a glucoside obtained from *Saponaria officinalis*, L., and existing in other plants, particularly in the bark of Quillaja Saponaria, and labeled pure (9). It was tried because it was thought the effect on the surface tension of the preparation would retard the loss of ethyl nitrite by evaporation.

TABLE X.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 1% SODIUM BICARBONATE.

	Assay Results.		*Loss of Ethyl Nitrite in Per cent.*		
	Direct Sunlight.[7]			Direct Sunlight.[7]	
Date.	White glass, per cent.	Amber glass, per cent.	Date.	White glass, per cent.	Amber glass, per cent.
8/16/29	4.32	4.32	8/16/29	0.00	0.00
8/29/29	0.20*	3.09*	8/29/29	95.45	28.42
3/12/30	0.00	0.15	3/12/30	100.00	96.55

TABLE XI.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 1% MAGNESIUM CARBONATE.

	Assay Results.		*Loss of Ethyl Nitrite in Per Cent.*		
	Direct Sunlight.[1]			Direct Sunlight.[1]	
Date.	White glass, per cent.	Amber glass, per cent.	Date.	White glass, per cent.	Amber glass, per cent.
8/16/29	4.12	4.12	8/16/29	0.00	0.00
8/29/29	0.22*	3.71	8/29/29	94.45	9.95
3/12/30	0.00	0.70*	3/12/30	100.00	83.00

TABLE XII.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

	Assay Results.			*Loss of Ethyl Nitrite in Per Cent.*			
	Refrigerator.[1]	Direct Sunlight.[2]			Refrigerator.[1]	Direct Sunlight.[2]	
Date, 1930	White glass, per cent.	White glass, per cent.	Amber glass, per cent.	Date, 1930.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.
3/17	4.18	4.18	4.18	3/17	0.00	0.00	0.00
3/22	4.17	2.56*	4.03	3/22	0.24	37.48	3.59
3/29	4.10	0.88	3.98	3/29	1.92	77.95	4.79
4/5	4.08	0.08	3.97	4/5	2.39	98.25	5.04
4/18	3.95	0.00	3.76	4/18	5.50	100.00	10.05

TABLE XIII.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 1% GLYCERIN.

	Assay Results.			*Loss of Ethyl Nitrite in Per Cent.*			
	Refrigerator.[1]	Direct Sunlight.[2]			Refrigerator.[1]	Direct Sunlight.[2]	
Date, 1930.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.	Date, 1930.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.
3/30	4.23	4.23	4.23	3/30	0.00	0.00	0.00
3/24	4.09	3.11*	4.19	3/24	3.31	26.49	0.95
3/29	4.04	0.74	4.18	3/29	4.50	82.50	1.16
4/5	3.92	0.08	4.08	4/5	7.34	98.1	3.56
4/18	3.86	0.00	3.78	4/18	8.75	100.00	10.65

TABLE XIV.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 1% CASTOR OIL.

	Assay Results.			*Loss of Ethyl Nitrite in Per Cent.*			
	Refrigerator.[1]	Direct Sunlight.[2]			Refrigerator.[1]	Direct Sunlight.[2]	
Date, 1930.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.	Date, 1930.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.
3/17	4.40	4.40	4.40	3/17	0.00	0.00	0.00
3/22	4.38	3.14*	4.36	3/22	0.45	28.61	0.91
3/29	4.34	1.86	4.34	3/29	1.37	57.75	1.37
4/5	4.28	0.13	4.30	4/5	2.72	97.10	2.28
4/18	4.13	0.00	4.27	4/18	6.15	100.00	2.96

TABLE XV.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

Assay Results.

	Refrigerator.[1]	Direct Sunlight.[2]		Diffused Sunlight.[3]	
Date.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.	White glass, per cent.	Amber glass, per cent.
7/3/30	4.23	4.23	4.23	4.23	4.23
7/7/30	3.93	1.04*	4.04	3.92	3.95
7/10/30	3.90	0.07	3.85	3.91	3.91
7/16/30	3.87	0.00	3.80	3.90	3.90
8/9/30	3.83	...	3.76	3.77	3.78
9/18/30	3.57	...	3.18*	3.58	3.59
12/5/30	3.37*	...	3.05	3.08*	3.30*
2/13/31	3.22	...	2.85	2.83	3.10
3/13/31	3.12	...	2.69	2.59	2.84
		Loss of Ethyl Nitrite in Per Cent.			
7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	8.51	100.00	10.18	7.81	7.81
3/13/31	26.12	...	36.19	38.68	32.84

TABLE XVI.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 0.1% ROCHELLE SALT.

Assay Results.

	Refrigerator.[1]	Direct Sunlight.[2]		Diffused Sunlight.[3]	
Date.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.	White glass, per cent.	Amber glass, per cent.
7/3/30	4.23	4.23	4.23	4.23	4.23
7/7/30	4.02	0.43*	3.94	3.94	4.00
7/10/30	4.02	0.09	3.90	3.94	3.86
7/16/30	3.97	0.00	3.88	3.92	3.83
8/9/30	3.92	...	3.78	3.81	3.82
9/19/30	3.63	...	3.35*	3.58	3.46*.[4]
12/5/30	3.60	...	3.03	3.37*	3.14
2/13/31	3.46*	...	2.64	2.49	2.88[4]
3/13/31	3.30	...	2.32	2.31	2.79
		Loss of Ethyl Nitrite in Per Cent.			
7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	6.16	100.00	8.28	7.34	9.45
3/13/31	22.00	...	45.20	45.45	34.10

TABLE XVII.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 0.1% OF DISODIUM PHOSPHATE.

Assay Results.

	Refrigerator.[1]	Direct Sunlight.[2]		Diffused Sunlight.[3]	
Date.	White glass, per cent.	White glass, per cent.	Amber glass, per cent.	White glass, per cent.	Amber glass, per cent.
7/3/30	4.23	4.23	4.23	4.23	4.23
7/7/30	4.15	0.23*	4.13	4.13	4.09

7/10/30	4.08	0.10	3.84	3.97	3.98
7/16/30	4.01	0.00	3.83	3.94	3.88
8/9/30	3.82	...	3.57	3.92	3.86
9/19/30	3.73	...	3.22*	3.78	3.65
12/5/30	3.48*	...	2.73	3.35*	3.41*
2/13/31	3.32	...	2.46	3.02	3.28
3/13/31	3.29	...	2.24	2.70	2.92
		Loss of Ethyl Nitrite in Per Cent.			
7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	5.21	100.00	9.46	6.86	8.28
3/13/31	22.11	...	47.15	36.20	31.00

TABLE XVIII.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 0.1% OF POTASSIUM CARBONATE.

Assay Results.

Date.	Refrigerator.[1] White glass, per cent.	Direct Sunlight.[2] White glass, per cent.	Direct Sunlight.[2] Amber glass, per cent.	Diffused Sunlight.[2] White glass, per cent.	Diffused Sunlight.[2] Amber glass, per cent.
7/3/30	4.23	4.23	4.23	4.23	4.23
7/7/30	4.03	0.23*	4.00	4.09	3.96
7/10/30	4.03	0.03	4.00	3.99	3.87
7/16/30	3.84	0.00	3.90	3.87	3.84
8/9/30	3.79	...	3.62	3.85	3.67
9/19/30	3.73	...	3.47*	3.68	3.65
12/5/30	3.63	...	3.15	3.40*	3.37*
2/13/31	3.46	...	2.72	3.19	3.17
3/13/31	3.31	...	2.68	2.46	2.87
		Loss of Ethyl Nitrite in Per Cent.			
7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	9.22	100.00	7.81	8.51	9.24
3/13/31	28.80	...	36.62	41.78	32.09

TABLE XIX.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

Date, 1931.	Per Cent.	Diffused Sunlight Amber Glass.[3] Percentage loss.
2/28	4.17	0.00
3/7	4.11	1.44
3/23	4.05	2.88
4/25	3.89	6.72

TABLE XX.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%) WITH 0.5 PER CENT OF SAPONIN.

Date, 1931.	Per Cent.	Diffused Sunlight Amber Glass.[3] Percentage loss.
2/28	4.17	0.00
3/7	4.07	2.39
3/23	4.04	3.11
4/25	3.81	8.62

The results obtained in the first series of experiments, shown in Tables IX, X and XI, indicate that 1 per cent of added sodium bicarbonate or magnesium carbonate fail to retard the rate of decomposition of the ethyl nitrite when the spirit is kept in direct sunlight. Likewise, in the second series of experiments, on comparing the results of Tables XII, XIII and XIV, it appears that glycerin exerts little preservative action, but castor oil on the other hand retards decomposition somewhat over a prolonged period of storage. The principal disadvantage of

castor oil is that it is immiscible with practically all the preparations that are ordinarily prescribed with the spirit. Furthermore, the solution becomes cloudy when stored in a cold place. The results obtained in the third series of tests as shown in Tables XV, XVI, XVII and XVIII, were also negative in so far as preservative effect is concerned.

From the results of the comparatively few assays made in the last series of experiments as shown in Tables XIX and XX, it appears that saponin has no preservative action, and that its presence may even accelerate deterioration.

COMPARISON OF ASSAY METHODS.

In order to follow accurately the rate of deterioration of the spirit of ethyl nitrite it is essential that an assay method be used which can be relied upon. Several methods of assay are in more or less general use to-day. These methods are either of the gasometric type in which the nitric oxide gas is liberated by an oxidizing agent and measured as such, or of the volumetric type in which the reducing power of the ethyl nitrite is measured. The first type is exemplified by the method given in the United States Pharmacopœia (10), which is as follows:

The Method of the United States Pharmacopœia.—"Transfer about 40 cc. of Spirit of Ethyl Nitrite, which has been previously shaken with 0.5 Gm. of powdered potassium bicarbonate, to a tared, 100-cc. measuring flask, and weigh accurately. Add sufficient alcohol to bring the volume to exactly 100 cc., and mix thoroughly. Introduce into a nitrometer, filled with a saturated aqueous solution of sodium chloride, exactly 10 cc. of the alcoholic solution, follow by 10 cc. of potassium iodide T. S. and afterwards by 5 cc. of diluted sulphuric acid. When the volume of gas has become constant (within thirty to sixty minutes), note the volume of gas collected. Multiply this volume in cc. by 0.307, and divide the product by one-tenth of the weight of the Spirit of Ethyl Nitrite taken. At standard temperature and pressure, the quotient represents the percentage of ethyl nitrite in the liquid. The temperature correction is $^1/_{273}$ of the total percentage just found for each degree of temperature, added if the temperature is below, subtracted if above 25° C. The barometric correction is $^1/_{760}$ of the total percentage just found for each mm., added if above, subtracted if below, 760 mm."

A good example of the volumetric type is the following which is a translation of the method of the Netherlands Pharmacopœia (11).

The Method of the Netherlands Pharmacopœia.—Mix 10 cc. of spirit of ethyl nitrite with 15 cc. of a solution of potassium chlorate in water (1:20), and 5 cc. of nitric acid in a closed flask and let stand for 1 hour with occasional shaking. Add 20 cc. of $N/10$ silver nitrate volumetric solution and 10 drops of ferric ammonium sulphate test solution, and titrate the excess of silver nitrate volumetric solution and 10 drops of ferric ammonium sulphate test solution, and titrate the excess of silver nitrate with $N/10$ potassium thiocyanate solution until the red color no longer disappears on shaking. Not less than 8.9 cc. nor more than 11.2 cc. of silver nitrate solution should be consumed. Each cc. of $N/10$ silver nitrate solution corresponds to 22.5 mg. of $C_2H_5NO_2$.

The method of the United States Pharmacopœia given above is not wholly satisfactory, especially when it is desired to compare results obtained by different analysts. The conditions which are most unsatisfactory are the permissibility

to use a nitrometer of only 50 cc. capacity, and the directions to use saturated salt solution for filling the nitrometer and leveling tube.

Experience has demonstrated that if the sample contains over 4% of ethyl nitrite a nitrometer having a capacity of 50 cc. is too small as some of the liberated nitric oxide gas will escape through the equilibrium tube in spite of most careful handling. It is possible that this loss of gas might be avoided by the use of a nitrometer with a globular expansion near the lower end, but nitrometers of this type do not appear to be on the market. A nitrometer having a capacity of not less than 100 cc. should, therefore, be specified.

Our experience has also shown that the use of saturated solution of sodium chloride as the liquid for filling the nitrometer and leveling tube is responsible for low results due primarily to the diffusion of the nitric oxide gas through the liquid and subsequent escape at the open end of the leveling tube. This is especially true when the salt solution is not thoroughly saturated. There is also some great danger of some escape of gas when the tubes are shaken to mix the reagents. Still another cause of error when salt solution is used is the air bubbles which form and remain unnoticed in the rubber tubing connecting the nitrometer and leveling tube.

In view of these conditions, it is suggested that if the United States Pharmacopœial method of assay is employed it be modified as follows: Use a nitrometer of not less than 100 cc. capacity and replace the saturated salt solution with mercury. Shake the tube containing the reacting mixture until the latter becomes colorless, thus insuring complete oxidation of the ethyl nitrite.

The method of the Netherlands Pharmacopœia is also unsatisfactory because there is a considerable loss of ethyl nitrite through evaporation when the spirit is weighed by itself, and because the end-point of the titration is too indefinite where titration is carried on in the presence of silver chloride. This method is more rapid than that of the United States Pharmacopœia and gives more constant results in the hands of different analysts if modified as follows:

Modification of the Netherlands Pharmacopœia Method.—Mix 15 cc. of a solution of potassium chlorate in water (1:20) and 5 cc. of nitric acid (U. S. P.) in a 100-cc. glass-stoppered, volumetric flask and weigh the flask and its contents. Add 10 cc. of spirit of ethyl nitrite from a pipette, stopper the flask, shake and reweigh to ascertain the weight of the spirit added. Set the mixture aside in a moderately warm place for 1 hour and shake occasionally. Add 20 cc. of $N/10$ silver nitrate solution, shake well and fill the flask with distilled water up to the 100-cc. mark. Filter the mixture through a dry filter into a 100-cc. graduated cylinder, rejecting the first 20 cc. of filtrate. Measure off exactly 50 cc. of the remainder by means of a pipette, add 10 drops of ferric ammonium sulphate test solution and titrate the excess of silver nitrate with $N/10$ potassium thiocyanate solution. Each cc. of $N/10$ silver nitrate solution consumed corresponds to 0.0225 Gm. of $C_2H_5NO_2$.

The following tables give comparative data showing the results obtained in the assay of portions of the same sample of spirit by the method of the United States Pharmacopœia, a modification of that method, and in the method of the Netherlands Pharmacopœia.

TABLE XXI.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

	Gasometric Assay (U. S. P. Method). Using sat. NaCl sol., per cent.	Using mercury, per cent.	Volumetric Assay (N. P. Method). Per cent.
1	3.78*	3.97	3.51
2	3.84	3.97	3.53

* Slight diffusion took place with a loss of gas.

All of the above assays were run Feb. 23, 1931, the spirit in each case being taken from the same bottle.

TABLE XXII.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

	Gasometric Assay (U. S. P. Method). Using sat. NaCl sol., per cent.	Using mercury, per cent.	Volumetric Assay (N. P. Method). Per cent.		Gasometric Assay (U. S. P. Method). Using sat. NaCl sol., per cent.	Using mercury, per cent.	Volumetric Assay (N. P. Method). Per cent.
1	4.26*	4.55	4.08	4	4.21	..	4.08
2	4.31*	4.55	4.06	5	3.92*	..	..
3	4.26*	4.54	4.08	6	4.53	..	..

* Diffusion took place with a loss of gas.

All of the above assays were run Feb. 28, 1931, the spirit in each case being taken from the same bottle.

The foregoing data show that the method of the Netherlands Pharmacopœia gives lower results than the original method of the United States Pharmacopœia or its modification. This condition was found to be due entirely to titration of excess of silver nitrate in the presence of silver chloride. If the silver chloride is removed from the reacting mixture by filtration previous to titration, the results obtained are almost identical or but very slightly higher than those obtained by using the modified United States Pharmacopœia method which is shown by the following table.

TABLE XXIII.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

	Gasometric Assay (U. S. P. Method). Using sat. NaCl sol., per cent.	Using mercury, per cent.	Volumetric Assay (Modified N. P. Method). Per cent.		Gasometric Assay (U. S. P. Method). Using sat. NaCl sol., per cent.	Using mercury, per cent.	Volumetric Assay (Modified N. P. Method). Per cent.
1	4.20	4.22	4.28	4	4.11	4.20	4.29
2	3.64	4.24	4.31	5	4.14	4.21	4.31
3	3.77	4.20	4.29	6	4.16	4.22	4.26

All of the above assays were run April 29, 1931, the spirit in each case being taken from the same bottle.

As previously stated, the modified method of the Netherlands Pharmacopœia gives results which are slightly higher than those obtained by the U. S. P. method of assay. If a correction is made for vapor pressure in the U. S. P. method, the difference is still greater than indicated in the preceding tables.

Inasmuch as the foregoing assays by the modified method of the Netherlands Pharmacopœia were carried out on unneutralized samples of the spirit, it was thought that this condition might be responsible in a measure for the comparatively high results obtained. For this reason a series of assays was run on neutralized samples. The results of these assays, which are given in the following table, show, however, that neutralization of the mixture previous to titration with potassium thiocyanate has practically no effect in so far as the final result is concerned.

TABLE XXIV.—EFFECT OF NEUTRALIZATION WITH POTASSIUM BICARBONATE ON ASSAY RESULTS.

	Gasometric Assay (U. S. P. Method).				Volumetric Assay (Modified N. P. Method).	
	Using sat. NaCl sol.		Using mercury.			
Date.	Plain, per cent.	Neutralized, per cent.	Plain, per cent.	Neutralized, per cent.	Plain, per cent.	Neutralized, per cent.
7/7/31	3.95	3.94	4.02	3.96	4.12	4.04
	3.90	3.94	4.01	3.96	4.09	4.08
7/14/31	3.75	3.69	3.88	3.86	3.92	3.89
	3.84	3.81	3.88	3.84	4.00	4.00

The Spirit of Ethyl Nitrite used in these assays was prepared February 10, 1931.

CONCLUSIONS.

From the results obtained in the experiments described above, the following conclusions are drawn:

1. The spirit will deteriorate in strength under any conditions of preparation or storage which have been devised to date.

2. The best solvent to use in its preparation is 99% ethyl alcohol.

3. The addition to the finished product of any of the substances which have been suggested for the prevention or retardation of deterioration is worthless.

4. In the light of our experience the spirit can best be preserved in small, tightly stoppered glass bottles kept in a refrigerator. In fact, the containers should be so small that it will not be necessary to open them more than once, as frequent opening of the container is found to be responsible for a very considerable loss in ethyl nitrite due to evaporation.

5. If the spirit is not kept in a refrigerator, it can best be preserved by storing it in small, tightly stoppered amber bottles.

6. It is recommended that the method of the Netherlands Pharmacopœia as modified above be adopted as the official method of assay for the spirit.

7. In the event that the gasometric assay is retained it is recommended mercury be used in place of saturated sodium chloride solution.

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