SCIENTIFIC SECTION

THE PHARMACOLOGIC ACTIVITY OF CHEMICALLY ASSAYED SPIRIT OF ETHYL NITRITE, U. S. P., INCLUDING p_H VALUES.*

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INTRODUCTION.

In a recent communication, one of us (M. J. A. (1)) reported an extensive investigation of the chemical assay methods, and the stability of Spirit of Ethyl Nitrite prepared by various methods and placed under different storage conditions. The results of this work definitely confirmed earlier work that showed this preparation to be subject to loss of ethyl nitrite as shown by chemical assay, the rate of loss being dependent in large measure to the conditions under which the preparation was kept.

Although Spirit of Ethyl Nitrite is an exceedingly well-known preparation, being used therapeutically to a considerable extent, it appears that this preparation has never been subjected to a thorough pharmacological study, and indeed the various texts relating to pharmacologic action and therapeutic use seem hesitant to concede any significant action to this product. At least, we have been unable to find any satisfactory evidence which would recommend or condemn the preparation. A review of ten authoritative texts on pharmacology may be briefly summed up by stating that Spirit of Ethyl Nitrite is in all instances discussed simultaneously with the so-called "nitrite" series, and although other members of the series such as amyl nitrite, glyceryl trinitrate, sodium nitrite, erythrol tetranitrate, and mannitol hexanitrate have been investigated individually and comparatively, the Spirit of Ethyl Nitrite itself appears to have been quite neglected as far as any specific experimental investigation on this particular preparation is concerned. The present experiments were, therefore, undertaken in the hope of finding an answer to the following questions:

1. Is Spirit of Ethyl Nitrite, U. S. P. X, capable of exerting any significant activity whatever in its usual dosage and mode of administration?
2. Do the accepted chemical methods of assay reflect the pharmacologic potency in the deteriorated preparation, as well as when freshly prepared, or, do new products arise during deterioration which might result in pharmacologic activity even after chemical assay showed either partial or complete loss of activity?
3. In case of activity, does the nature and duration justify the existence of this preparation in the Pharmacopœia?
4. Is the p_H factor of any significance in this preparation?

EXPERIMENTAL METHODS.

Because of its position as a member of the "nitrite" series, and because of the therapeutic use as a vaso-dilator, diuretic, febrifuge and diaphoretic, we have in-

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vestigated the activity qualitatively and quantitatively of freshly prepared and also deteriorated samples of U. S. P. X Spirit of Ethyl Nitrite by recording simultaneously upon both anesthetized dogs and cats, the carotid arterial blood pressure, respiration, kidney volume and urine output, in doses ranging from ineffective to lethal amounts, by intravenous, subcutaneous and oral administration.

(*a*) *Animals Used.*—Dogs, male and female, weighing from 6.0 to 13.0 Kg. Cats, male and female, weighing from 2.2 to 4.1 Kg.

(*b*) *Anesthesia.*—To avoid as much as possible the circulatory effects of anesthesia, our anesthesia was carefully maintained only to a depth necessary to abolish interfering voluntary movement, by the very slow intravenous injection of a just sufficient amount of sodium ethyl-methyl-butyl barbiturate (Nembutal).[1] As the anesthesia tended to wear off with the passage of time (single experiments occasionally lasting as long as seven hours), additional small amounts were given. This method, because of the rapidity of onset of anesthesia and the relatively short duration of effect of this hypnotic, makes it possible, with some experience, to control the anesthesia at any depth desired with an unusual degree of accuracy.

(*c*) *Operative Procedures and Methods of Recording.*—1. Carotid Blood Pressure. This was carried out in the usual manner.

2. Respiration. Respiration was recorded through a tracheal cannula to a suitable tambour recorder.

3. Kidney Volume. This was accomplished by very carefully dissecting out the kidney and placing around it an oncometer of proper size, closing the incision and taking the record through a tambour recorder. The sensitivity of our recording device was sufficiently great to record not only the kidney volume, but even the pulsations coming through the renal artery.

4. Urine Output. Urine flow was obtained, in the experiments upon dogs, by inserting a specially constructed Y-shaped cannula into both ureters at a point near the bladder. Because we were unable to insert a cannula of proper shape and size into the ureters of cats, a specially blown bladder cannula was tied into the bladder so as to receive the urine directly from both ureters, thereby avoiding a "lag" which results if the cannula is of improper shape or is not tied in properly. In either case, the urine flow was recorded through a Becker drop-recorder and signal magnet.

All four types of activity were recorded simultaneously upon a slow-moving kymograph.

(*d*) *Method of Administration of the Test Preparations.*—Since it was desired to evaluate different samples quantitatively as well as qualitatively, the preparations were administered intravenously for this phase of the work. To avoid the effects of irritation which would otherwise be caused by the high alcohol content of the spirit, a cannula fashioned on the order of a Hitchins' Syringe was employed, introduced into the femoral vein. This permitted "washing in" the desired dose with saline solution, without a loss of the highly volatile ethyl nitrite. Aqueous dilution of this preparation before administration is not permissible because of an evolution and loss of ethyl nitrite, thereby destroying the quantitative accuracy of the dose.

Since therapeutic use of Spirit of Ethyl Nitrite is permissible by oral administration only, because of the high alcohol content, it was necessary to also study the effects produced by oral administration. This was accomplished by using a small rubber stomach tube in the usual manner, washing the dose into the stomach with a little water.

In both oral and intravenous administration, control doses of alcohol were frequently given, to avoid improper interpretation of results.

The frequency of dosage was found to be very important from the quantitative standpoint. To avoid discordant results it was necessary to wait until the effects of the previous dose had worn off completely. The waiting time depended upon the size of the dose, and was determined simply by waiting until the carotid blood pressure had returned to normal.

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[1] We are indebted to the Abbott Laboratories for a generous supply of this material.

THE TEST SAMPLES OF SPIRIT OF ETHYL NITRITE EMPLOYED.

Since it was desired to ascertain whether the activity of the deteriorated preparations was identical in kind to that of the freshly prepared preparations of U. S. P. ethyl nitrite content, or whether deterioration or changes due to aging resulted in the formation of other active substances, it was necessary to compare the activity of samples having undergone varying degrees of deterioration with the activity of a freshly prepared preparation meeting all U. S. P. requirements.

Being impossible to illustrate our findings by a complete reproduction of all our experiments, we have decided to show in the following Table I the exact character of the test samples employed in producing those tracings reproduced in this article. Owing to the danger of deterioration, each sample was assayed on the morning of the same day of the pharmacologic tests, the assay values given being those found on the day of the tests.

The p_H values of the samples included in the table were determined potentiometrically by a capillary electrode method which we have found to be accurate and reliable, even when applied to highly alcoholic solutions. This method was briefly described in an earlier communication (2). Although the colorimetric method was also used, we were unable to procure satisfactory results by this method, owing to the properties of this preparation.

TABLE I.—DESCRIBING THE TEST SAMPLES EMPLOYED IN PRODUCING THE REPRODUCED KYMOGRAPH TRACINGS OF THIS REPORT.

Prepared by U. S. P. X Method.

Identification No.[1]	Ethyl Nitrite Content at Time of Mfg.,[2] %.	Age at Time of Pharmacologic Test, Months.	How Stored.	Ethyl Nitrite Content at Time of Pharmacologic Test, %.	p_H Value at Time of Pharmacologic Test.
1	4.25	Freshly prepared	Tight amber bottle in refrigerator	4.25	0.82
2	4.23	23	Tight amber bottle in refrigerator	2.59	0.46
3	4.23	23	As above in diffused sunlight, room temperature	1.54	1.00
4	4.23	23	Tight clear bottle, direct sunlight	0.00[3]	5.95
5	4.23	23	Same as No. 4	0.00[3]	4.34

[1] This number corresponds with that appearing on the tracings.

[2] U. S. P. X Method, replacing salt solution in nitrometer with mercury.

[3] Samples Nos. 4 and 5 had deteriorated completely when assayed after 13 days.

EXPERIMENTAL RESULTS.

Owing to the fact that the usual individual variations in the quantitative response of both dogs and cats were observed, and that the showing of the quantitative relationship between the test samples of Table I is a major purpose of this report, the accompanying tracings of experiments on dogs were obtained from one and the same dog, and likewise the tracings of experiments upon cats were obtained from one and the same cat. The results shown in these tracings are typical, and were reproduced on other cats and dogs repeatedly.—(*Continued on page 492.*)

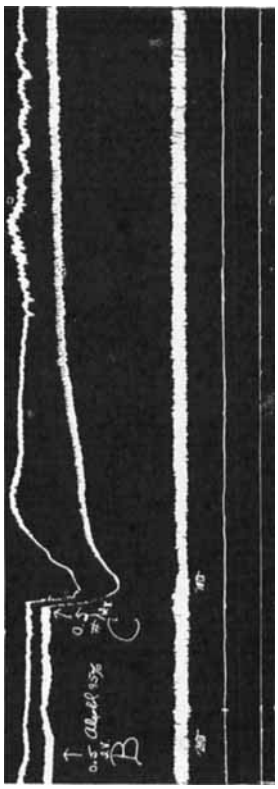

Plate I.

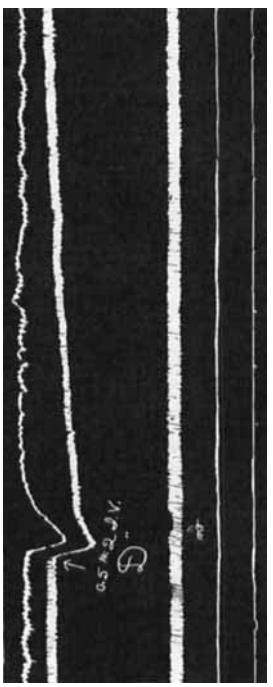

Plate II.

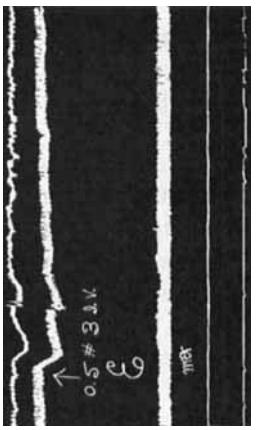

Plate III.

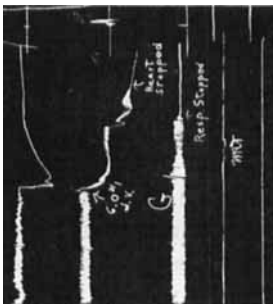

Plate V.

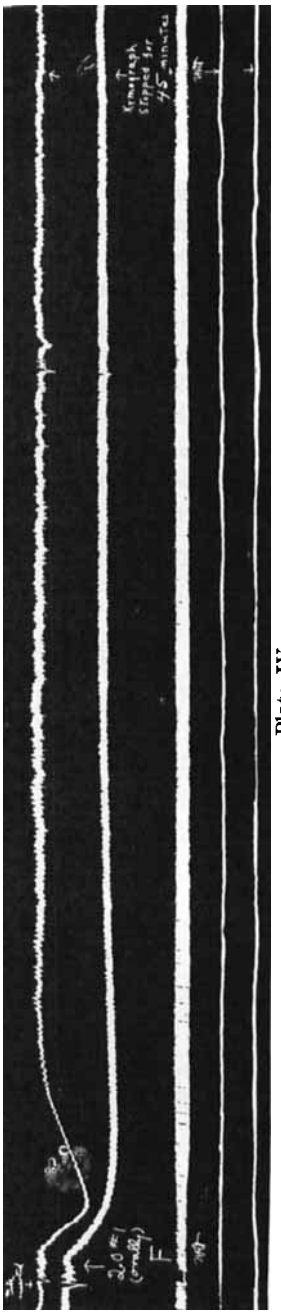

Plate IV.

EXPLANATION OF PLATES.

Plate I.—Dog, male, 12.0 Kg. Recording, from top to bottom, kidney volume, carotid blood pressure, respiration, time in minutes (about 1 cm. per minute) and urine flow in uniform drops. The negligible effect of a control dose of 0.5 cc. of 95% alcohol, washed into the femoral vein at B. At C, the effects of 0.5 cc. of sample No. 1 of Table I, washed into the femoral vein with saline. Note the prompt but transitory decreased kidney volume, prompt and persistent fall in blood pressure, transitory depression then prolonged stimulation of respiration, and a complete retention of urine for 15 minutes, after which the urine again begins to flow, but at a retarded rate. This preparation was of full U. S. P. strength, as shown in Table I, and was used as the quantitative standard of comparison.

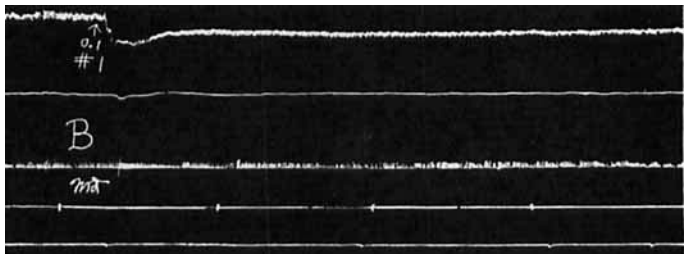

Plate VI.

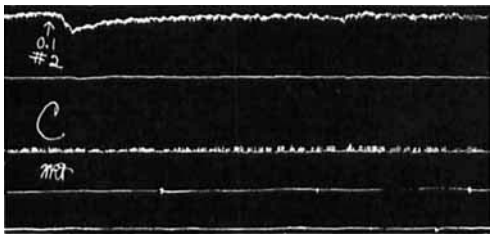

Plate VII.

Plate II.—A continuation of Plate I. At D, the same dose of sample No. 2 of Table I was given, as was given of sample No. 1 in Plate II. Note from Table I that sample No. 2 is approximately two-thirds of the strength of No. 1 in ethyl nitrite content, and that the pharmacologic response of all recorded functions in this figure is approximately two-thirds of that of Plate I.

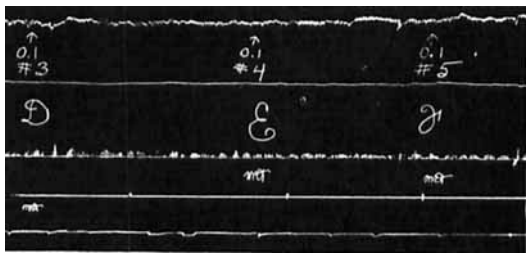

Plate VIII.

Plate III.—A continuation of Plate II. At E, the same dose of sample No. 3 of Table I was given, as was given of sample No. 2 in Plate II, and sample No. 1 in Plate I. Note that the decreased response of all functions is proportional to the diminished ethyl nitrite content as shown by referring to Table I.

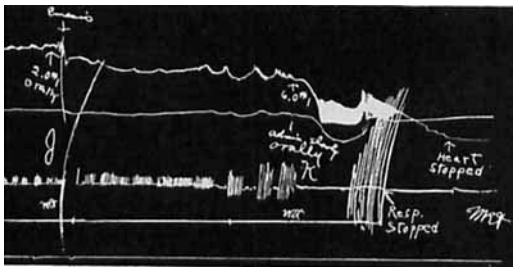

Plate IX.

Plate IV.—A continuation of Plate III. The effects produced by the oral administration at F of 2.0 cc. of sample No. 1 of Table I. This dose caused a fall in blood pressure which had not even started to return to normal in over two and one-half hours, when time necessitated termination of experiment. Note a complete retention of urine during the entire period. Also note the respiratory stimulation, and the prompt return of kidney volume to normal. The temperatures when the experiment was terminated was 4° C. below normal.

Plate V.—A continuation of Plate IV. 6.0 cc. of sample No. 1 was given intravenously at G. Note the continuation of heart beat after cessation of respiration, then death. Also note

return of kidney volume to normal before death, in spite of a continued fall in blood pressure.

Plate VI.—Cat, male, 3.3 Kg. Recording, from top to bottom, carotid blood pressure, kidney volume, respiration, time in 10-minute intervals, and urine output in uniform drops. At B, a dose of 0.1 cc. of sample No. 1 of Table I was washed into the femoral vein. Note the similarity of these effects to those shown in Plate I. They are less in magnitude, however, owing to the smaller dose per Kg.

Plate VII.—A continuation of Plate VI. At C, 0.1 cc. of sample No. 2 of Table I, which was approximately two-thirds of the potency of sample No. 1 given in Plate VII. Note the corresponding decrease in intensity of effects upon all functions recorded.

Plate VIII.—A continuation of Plate VII. Note the proportionately decreased effects produced by sample No. 3 of Table I. Note the absence of effects following the intravenous administration of 0.1 cc. of No. 3 at D, 0.1 cc. of sample No. 4 at E, and the same dose of sample No. 5 at F. As shown in Table I, these preparations were inert by chemical assay.

Plate IX.—A continuation of Plate VIII. The effects of oral doses of 2.0 cc. at J, and an additional 6.0 cc. at K. The heart continued to beat for over two minutes after respiratory failure. Here again, the kidney volume is seen to return to normal even while carotid blood pressure continued to fall. Urine flow interrupted as usual.

(*Continued from page 489.*)

Test preparation No. 1 of Table I was used in these tracings as the quantitative standard of comparison of activity.

The particular dog from which the indicated tracings were obtained was normal male weighing 12.0 Kg. The cat was a normal male weighing 3.3 Kg.

The rectal temperature was noted frequently on both cats and dogs during the course of the experiments.

DISCUSSION OF RESULTS.

There being no method of recording the temperature upon the kymograph at our disposal, a brief description of our observations is important. During the course of the various experiments, the temperature was observed to fall as much as 4° C. This was, of course, the result of several doses upon each animal. Our results showed that the fall in temperature was very roughly related to the size of the dose of ethyl nitrite (by chemical assay), and usually became apparent within a half hour after the dose was given.

Little other explanation of our results is necessary, but we would simply point out the following, which we believe is adequately shown by our results:

The effects observed upon cats and dogs were identical, and will, therefore, be briefly described without reference to either individually. The effects described are for non-toxic doses, unless otherwise stated.

EFFECTS UPON CAROTID BLOOD PRESSURE AND HEART.

This effect was typical of the members of the "nitrite" series. As shown in the tracings, single doses result in a prompt and extensive fall in blood pressure which is of a decidedly lasting nature, persisting for a period varying from a half hour to several hours, the magnitude and persistence depending upon the size of the dose. The effect upon the heart appears to be negligible, being only that secondary influence which is to be expected whenever the arterial blood pressure is materially lowered. A moderate acceleration was generally noted, due to the reflex stimulation caused by the fall in arterial pressure. The fall in blood pressure is due to vaso-dilation, since the heart is accelerated.

EFFECT UPON KIDNEY VOLUME.

As might be expected, the fall in blood pressure was always accompanied by a decrease in kidney volume. It is significant, however, that the kidney volume returned to normal within ten minutes, while the blood pressure remained low for many times this period. In other words, the effect upon kidney volume must be considered quite transitory, and, therefore, negligible in therapeutic employment.

EFFECT UPON RESPIRATION.

The larger intravenous doses produced a transitory respiratory depression, followed by mild, but definite and more prolonged stimulation, but the smaller intravenous doses and even relatively large oral doses produced the prolonged stimulation without the preliminary depression.

EFFECT UPON URINE OUTPUT.

With this effect, we encountered a distinct surprise. Contrary to the statements of several texts, a diuretic action was not observed in a single instance. A very distinct effect was always produced, but as is shown in the tracings, it was an antidiuretic effect, *i. e.*, as long as there was any evidence of effect upon blood pressure, or other recorded functions, there occurred simultaneously a decreased urine-flow in all experiments, and complete urine retention for appreciable periods when the larger doses were given. After the effects of the dose had worn off, it is true that the urine flow became accelerated, but the increased amount did not significantly exceed that which would otherwise have been excreted during the period of urine retention.

EFFECT UPON TEMPERATURE.

Although it is a well-known fact that it is very difficult to significantly lower the temperature of a normal animal, our observations have shown that Spirit of Ethyl Nitrite, U. S. P., is quite active in producing such an effect. A lowering of the rectal temperature of 1° C. was often observed, while the temperature during the course of several experiments fell 3° C. and even 4° C. In the latter case, administration of additional test doses was discontinued and after four hours the temperature had returned to within 1° C. of normal.

This would indicate that the employment of this preparation in febrile conditions (where all antipyretics exert their influence most readily) especially in children, almost invariably accompanied by a somewhat raised arterial blood pressure, is a distinctly rational procedure.

As to the mechanism of the antipyretic effect, we believe it to be due to both decreased heat production (decreased circulation and consequent decreased metabolism), and also increased heat loss (cutaneous capillary dilation and consequent increased radiation).

ACUTE TOXICOLOGICAL EFFECTS.

We have not attempted to accurately determine the M. L. D. of this preparation, but our results show a very safe latitude of therapeutic action. We have had to exceed ten times the minimum effective dose by a considerable amount in order to produce death, by oral administration as this preparation is ordinarily administered therapeutically.

When lethal amounts are given orally or intravenously to either cats or dogs, death is produced by respiratory failure, as shown in Plate V and Plate IX. The heart continues to beat for a short time after respiration has ceased.

THE INFLUENCE OF ROUTE OF ADMINISTRATION.

Decidedly important is the fact that our results show this preparation to be of significant activity when administered orally, as it is employed therapeutically. It is also important that, even when administered orally, the characteristic and significant effects begin almost immediately, or always within five minutes. This fact, together with the persistant character of the action, makes Spirit of Ethyl Nitrite, U. S. P., compare very favorably with the other members of the "nitrite" series.

A COMPARISON OF THE POTENCY OF FRESHLY PREPARED U. S. P. SPIRIT OF ETHYL NITRITE WITH PARTIALLY AND COMPLETELY DETERIORATED SAMPLES, AS INDICATED BY CHEMICAL ASSAY.

By comparing the magnitude and duration of effects upon blood pressure, kidney volume, respiration and urine output, as shown in Plates I, II and III, upon a dog, and Plates VI, VII and VIII, upon a cat, it will be seen that the activity of the freshly prepared sample and the four samples showing varied degrees of deterioration is directly proportional to the ethyl nitrite content as indicated by chemical assay. Deterioration, as indicated by chemical assay, results in the formation of no other pharmacologically active substances of significance in this preparation.

THE SIGNIFICANCE OF p_H.

The p_H of the various preparations studied appears to be of little practical significance as far as influencing the amount of activity is concerned. Generally speaking, the samples showing the highest values in p_H units, showed by chemical assay the lowest ethyl nitrite content and consequently the lowest degree of activity.

SUMMARY.

The quantitative and qualitative pharmacologic activity of freshly prepared and deteriorated samples of U. S. P. Spirit of Ethyl Nitrite of known ethyl nitrite content and p_H has been studied by recording simultaneously the effect upon carotid blood pressure, kidney volume, urine output, respiration and temperature upon anesthetized cats and dogs.

CONCLUSIONS.

1. Spirit of Ethyl Nitrite, U. S. P., has been shown to exert a significant and valuable type of activity when administered by its intended route (orally), by thoroughly reasonable doses.
2. The activity of the preparation diminishes in direct proportion to the loss of ethyl nitrite as determined by chemical assay.
3. The preparation develops no new kind of activity during deterioration which is of any significance when reasonable doses are employed.
4. The general nature of the activity has been shown to be similar to that of the "nitrite" series.

5. Of the effects studied, only those upon circulation and temperature appear to be of therapeutic significance.

6. Our observations do not justify claims of diuretic activity for this preparation.

7. A very safe latitude of therapeutic action has been shown.

8. Lethal doses, intravenously or orally, produce a respiratory death.

9. Since this preparation shows the typical nitrite action, the information provided in authoritative texts regarding methemoglobin production and tolerance development for nitrites as a group, should necessarily be born in mind by the physicians employing this preparation.

10. The p_H values were found to change greatly during deterioration, first showing an early trend toward greater acidity, then changing toward neutrality to a much greater degree. No practical significance is attached to these observations, since it would be impossible to control this factor.

11. Owing to the importance of the high alcohol content relative to the stability of this spirit, prescribing this preparation in any aqueous mixture is to be condemned.

A SUGGESTION.

Since Spirit of Ethyl Nitrite has been shown to possess a therapeutically valuable type of activity, and since it has been definitely established that its lack of stability makes it imperative that it be prepared very near to the time of dispensing, and dispensed in small quantities, we believe that individual small amber-glass ampuls containing the now available ethyl nitrite concentrate, in the amount necessary to make one fluidounce of the spirit, would make it possible to dispense this preparation with its full strength assured.

REFERENCES.

(1) M. J. Andrews, JOUR. A. PH. A., 21 (1932), 799.
(2) M. R. Thompson, *Ibid.*, 20 (1931), 1027.

THE STABILITY OF SOLUTION OF IRON AND AMMONIUM ACETATE U. S. P. X.[1,2]

BY WILLIAM J. HUSA[3] AND GEORGE W. BIRMINGHAM.

INTRODUCTION.

Solution of Iron and Ammonium Acetate, or Basham's Mixture as it is commonly known, though one of the older pharmacopœial preparations, is one which has long been a source of dissatisfaction to physicians and pharmacists. When freshly prepared the solution is a transparent brilliant red liquid and has been referred to as undoubtedly one of the most elegant preparations ever introduced for the administration of iron in dilute solution (1). However, the solution is

[1] Presented before the Scientific Section, A. PH. A., Toronto, Canada, 1932. [2] This paper is based on a thesis presented to the Graduate Council of the University of Florida by George W. Birmingham, in partial fulfilment of the requirments for the degree of Master of Science in Pharmacy.

[3] Head Professor of Pharmacy, University of Florida.