Pressor Drugs. I

Chemistry and Pharmacology of an Analogue of Epinephrine*

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The increasing number of clinical reports of the effectiveness of pressor drugs in the relief of certain conditions in the respiratory tract, chiefly asthma and hay fever, led to this coöperative investigation.

The original injection therapy has been supplemented by inhalational therapy with increased benefit to the patients (3, 5, 9). The chemistry of the drugs which increase the blood pressure results from the pioneer investigations of Abel, whose first report was published in 1889 (1) and which has been supplemented by chemical and pharmacological studies by a number of workers throughout the world, notably Aldrich, Barger, Dale, Elliott, von Fürth, Langley, Schäfer and Oliver, and Takamine, whose results have been reviewed by Schultz (10), May (6) and Hartung (4). A detailed discussion is also presented by Cushny (2), and by Munch (7), from the standpoint of isomeric substances. The most widely used product of this group is epinephrine which is officially recognized by U. S. P. XI (8).

In this paper of the series, information is presented on the chemical and pharmacological properties of a synthetic product, racemic methylamino-hydroxy-ethyl-dihydroxy-benzene, and the hydrochloride of this base, the solution of which is known as "Vaponefrin." Comparable studies have been made on U. S. P. Reference Standard Epinephrine and on some of the commercial epinephrine solutions.

EXPERIMENTAL

In these investigations synthetic material was furnished by the Vaponefrin Company from four different lots of the product. These were uniform in behavior. The base is a fine white powder; the hydrochloride somewhat resembles epinephrine hydrochloride, except that it is snowy white. In general, the solubilities of the two products correspond. The method of production of vaponefrin will be considered in a subsequent paper of this series.

A large number of chemical studies have been made in an effort to determine the reactions of vaponefrin and epinephrine, and to develop methods of differentiation. In a number of chemical tests the colors produced by both substances were so nearly identical that the reaction did not appear suitable for this purpose. Nine tests were found in which different types of response were so obvious that they appeared suitable for chemical distinction, qualitatively. The quantitative applications of these color reactions to the pure products and to mixtures of vaponefrin and epinephrine are being studied further.

The different types of responses obtained with U. S. P. Reference Standard Epinephrine and with vaponefrin are given in Table I. The difference in color following the application of concentrated sodium hydroxide to the dry base serves to dis-

Table I.—Chemical Differentiation between U. S. P. Epinephrine and Vaponefrin

Tests Applied	U. S. P. Epinephrine	Vaponefrin
	Powder	
1. Optical rotation	−50° to −53.5°	NO READING
2. Dry base treated with conc. NaOH cold solution	REDDISH BROWN gradually deepening in color	Amber—GREENISH BROWN—brown
	Solution 1: 1000	
3. Oxidation (by passing air through solution)	Pink—VIOLET—brown	Pink—AMBER—brown
4. Iodine T.S.	Pink—pinkish brown to RED upon standing over night	Pink—pinkish brown to GREEN upon standing over night
5. Krull method	CLEAR amber to cherry-red upon standing	MILKY WHITE PPT. CLEARING UP ON SHAKING to amber to cherry-red upon standing
6. Sodium hydroxide solution	Amber—cherry-red upon standing	Amber to BROWN upon standing, after 1 to 2 days to cherry-red
7. Phosphomolybdic acid T.S.	VERY PALE amber	DARK amber or yellowish brown
8. Ferric chloride T.S.	EMERALD-green—cherry-red—brown upon standing	BLUISH green—cherry-red—brown upon standing
9. Potassium ferricyanide T.S.	Red color upon standing HALF hour	Red color upon standing TWO to THREE hours

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tinguish between these products; epinephrine giving a reddish brown color and vaponefrin a greenish brown color. The characteristics in which differences are noted are indicated in capital letters in Table I. In tests on the solutions the iodine test No. 4 and the ferric chloride test No. 8 were the most suitable for differentiation.

A colorless solution was prepared for the pharmacological investigations containing two Gm. of the base in sufficient distilled water to furnish 100 cc. of solution. Before bringing up to final volume, enough $N/10$ HCl was added to complete solution and to produce a p_H of 4.0. This solution was packaged in half-ounce, amber glass bottles. For deterioration studies, solutions were prepared as needed. For other pharmacological investigations 0.5 per cent chloretone was used as a preservative, and some of this material was included in the deterioration studies as a control on the solution without chloretone. Both types of vaponefrin solutions were also used in the inhalational studies.

Toxicity tests were made on vaponefrin and U. S. P. Epinephrine. The results obtained following oral administration to mice, and following intraperitoneal injections to mice and rats are consolidated in Table II. The results presented are stated in terms of mg. of the base per Kg. of body weight. It may be noted that epinephrine is about one and a half times as toxic as vaponefrin base, slightly different ratios being obtained in the different types of tests.

Table II.—Acute Toxicity to Animals

Animal	Method of Administration	Epinephrine, mg./Kg. Survived	Epinephrine, mg./Kg. Killed	Vaponefrin, mg./Kg. Survived	Vaponefrin, mg./Kg. Killed
Mice	Oral	50	60	60	80
Mice	Intraperitoneal	2.5	5	5	10
Rats	Intraperitoneal	0.75	1.0	1.0	1.5

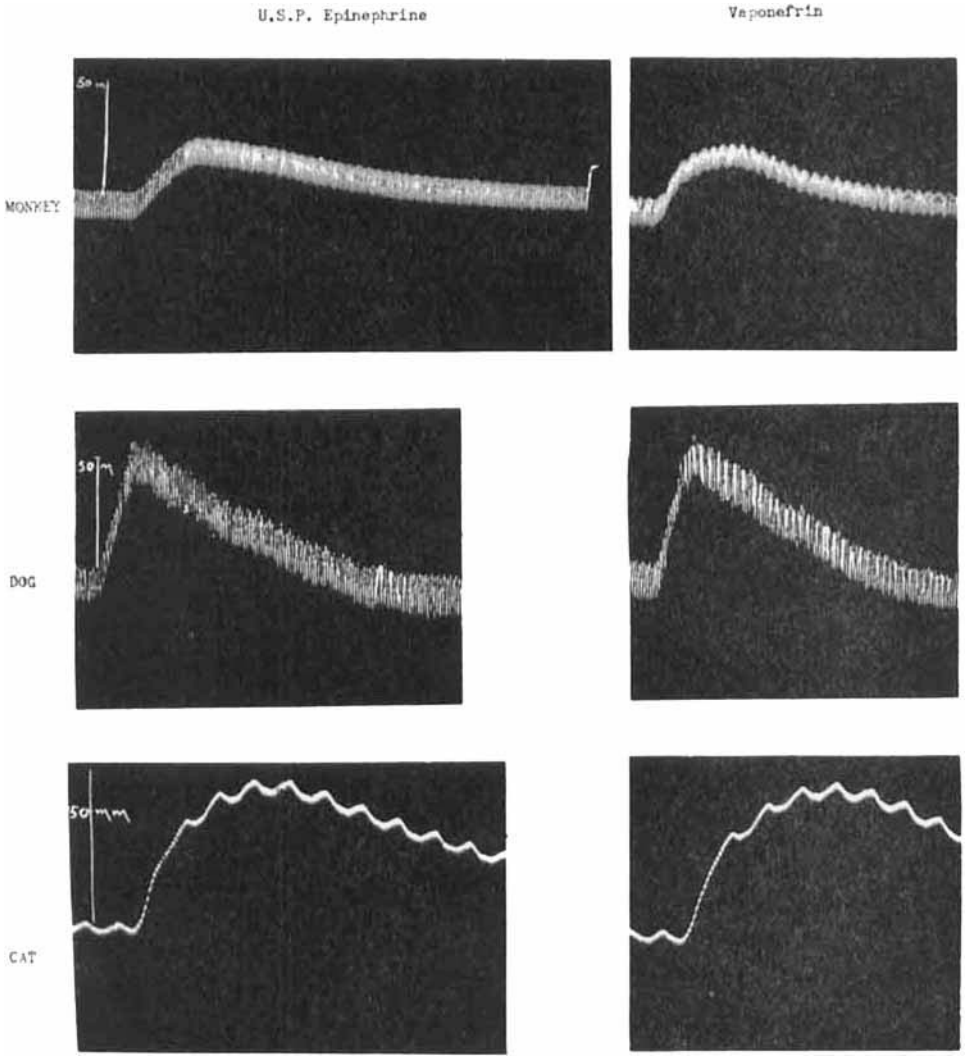

Fig. 1.—Blood Pressure Tracings.

To supplement these types of administration, a series of rabbits were obtained from the same source of supply for inhalational studies. A series of vaponefrin vaporizers were obtained for administration of the vapor, and the orifices reduced by sealing Pyrex tubing to the outlets so that they might be used on rabbits. In groups of animals, 1, 5 or 10 inhalations were given twice daily directly into the mouth as the rabbits inhaled so that the vapor might be carried into the lungs. Animals were treated five days weekly; these studies were started in September, 1939, and will be continued for at least a year. In the period of eight months, a few animals have died from extraneous causes and some have been sacrificed for histopathological study. The other animals of the group are growing at a normal rate and appear to be in healthy condition. Some of these rabbits have received about four thousand inhalations.

As further controls on the inhalational study, additional rabbits from the same source have been given 5 cc. of a 1:200 dilution of vaponefrin solution daily for eight months. A total of about 90 mg. of vaponefrin has been given over a period of eight months without producing any changes in growth or appearance.

A number of studies of the pressor action have been made. Figure 1 shows portions of responses observed following intravenous injections of epinephrine and of vaponefrin to anesthetized cats, dogs and monkeys (*Macacus rhesus*). It will be observed that the rate of increase in blood pressure is similar after the administration of either product to the same species of animal. In general, more rapid elevations were observed in dogs. The pressor potency of vaponefrin base was found to be about two-thirds the pressor potency of epinephrine base. By properly modifying the concentrations of solutions prepared, the same intensities of pressor response were readily obtained.

In Chart 1 the epinephrine and vaponefrin doses intravenously injected into monkeys were 8 and 20 gamma; into dogs 7 and 12 gamma and into cats 10 and 16 gamma, respectively. These tracings are presented to show the qualitative similarity of response, rather than the quantitative pressor values.

Deterioration studies were undertaken on a series of samples of epinephrine and on a lot of vaponefrin (No. 81,839) with a p_H of 4.0. One-half ounce, amber bottles of epinephrine and of vaponefrin under similar conditions were placed in: (1) an incubator at 55° C.; (2) an incubator at 37° C.; (3) at room temperature in the laboratory; and (4) in a refrigerator maintained at a temperature of about 5° C. Samples were removed from time to time for physical examination, p_H determination and measurements of pressor potency by the U. S. P. XI Epinephrine assay on dogs. These samples were prepared and stored on October 12, 1939.

No changes have been observed in the vaponefrin solutions stored under conditions 1, 2 or 4, over a period of five months. A portion of the bottles stored at room temperature (in the dark) showed no change in color or potency. Another portion, which had been stored in direct sunlight but unopened until taken for test, showed no changes in the color or potency. However, a third portion, stored in sunlight and opened daily, showed progressive darkening color to muddy brown after two months, with the appearance of a slight precipitate, in the bottles containing vaponefrin. The contents of these bottles showed a progressive lowering of p_H. No significant changes in physiological activity were detected when the contents of these bottles were tested against freshly prepared vaponefrin solution of the same concentration.

The solutions of epinephrine in bottles, stored in the sunlight and opened daily, showed prompt color changes, reaching a maximum darkening within about two weeks. There was a progressive decrease in pressor potency associated with the development of the darker color. About half of the pressor activity was lost during a period of two to four weeks. The comparative deterioration studies of epinephrine and vaponefrin will be reported in a subsequent paper.

CONCLUSIONS

1. Methods of chemical differentiation between U. S. P. Epinephrine and vaponefrin (racemic methylamino-hydroxy-ethyl-dihydroxy-benzene hydrochloride) have been developed and the qualitative differences presented.

2. In acute toxicity tests on animals, vaponefrin was about two-thirds as toxic as epinephrine, following injections.

3. In chronic inhalational studies on rabbits, no toxic effects have been observed following inhalation of quantities up to four thousand inhalations of vaponefrin.

4. The pressor responses of cats, dogs and monkeys to epinephrine and to vaponefrin were similar. Epinephrine is 50 per cent stronger in pressor potency.

5. Vaponefrin solutions were more stable than epinephrine solutions when stored under similar conditions. Discolored vaponefrin solutions retained their pressor potency.

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The Irritant Effect of Aloin. Preliminary Note

*By Melvin W. Green**

America produced, in 1931, $20,461,000 worth of laxatives (1). But in spite of the economic value of the cathartic drugs, little serious attention has been given to the chemistry, pharmacology or physiology of laxation.

For some time the author has been interested in tools for studying these problems and applying them to the anthraquinone-bearing drugs such as senna, aloes, cascara and rhubarb.

The most obvious study, to begin such a project, is the assay of these drugs for cathartic value. We have already worked out some methods for assaying which, while not sharply quantitative, are very useful as tools (2) (3).

But in addition to assay methods, it is important to find measures of untoward effects which may or may not contribute to catharsis. Alvarez (4) has shown that if rabbits are purged and then killed by a blow on the head, isolated gut strips from such animals are considerably atonic. In addition, the animals appeared to be depressed. Larson and Bargan (5) have also shown in surgical dogs that laxatives upset the normal gut movements so that, in the case of the more drastic purges, it is actually days before the movements become normal.

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EXPERIMENTAL

In order to compare some unofficial aloins with the U. S. P. Aloin, the following technique for determining the irritation of the mucosa was developed. Well-nourished, healthy guinea pigs, weighing around 300 Gm., were subjected to Nembutal anesthesia (30 mg. per Kg.). An incision was made in the abdomen and the ascending colon exposed. A 5-cm. loop of large intestine was tied off with thread and 0.5 cc. of a solution of the aloin in question injected into the loop, the loop returned to the abdomen and the abdomen sewed up loosely. The animal was kept warm, and after an hour the gut loop was again brought to the surface and the isolated loop cut away. The excised loop was carefully washed free of fecal matter, opened and the degree of redness of the interior surface compared with a similar loop from a control animal. The control animal received a solution of aloin, Merck, in a concentration of 1.0–1.5 mg. per cc. The concentration of test aloin and volume of solution injected were always the same as in the control.

Table I indicates the results obtained with some unofficial aloins. These aloins were prepared by the Purdue University School of Pharmacy and submitted to this laboratory for testing. The aloins were extracted from several different aloes and by new and different processes. The standard was considered to have an irritating effect of two plus and the other aloins compared with it. Based on practice determinations, a change of one plus in either direction is of doubtful significance. From these data the unofficial aloins are probably no more irritant than the official product.

Table I.—Effect of Various Aloins on Intestinal Mucosa

Aloin	Inflammation
Standard	++
Socratine aloin	+
Barbadoes aloin	+++
Cape aloin No. 1	++
Cape aloin No. 2	+ ?
Cape aloin No. 3	+

It was thought advisable to check this irritation with actual griping in human subjects. This phase of the problem is in the preliminary stages, but of the aloins tested, none of them is as griping, in 1/4 gr. doses, as is the commercial product.

SUMMARY

In order to compare some unofficial aloins with the U. S. P. Aloin, the author has developed a technique for determining the irritating effect on the intestinal mucosa.

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