Although the physical constants of the extractives from the different varieties of ginger vary considerably, the addition of appreciable quantities of an adulterant to a fluidextract of ginger may be readily detected by the method here described.

THE ACTIONS AND BIOLOGIC ASSAY OF EPHEDRINE.[1]

BY PAUL S. PITTENGER.

Ephedrine is an active alkaloid originally isolated in an impure form from the Asiatic drug, Ma Huang, by Yamanashi. It was first isolated in the pure form by Nagai.[2]

Ma Huang, identified as *Epheera vulgaris* **var.** *helvetica*, has been used in the practice of medicine in China for more than five thousand years but remained practically unknown until revived by the vast researches and publications of pharmacological and clinical studies by Chen and Schmidt and Chen[3] during 1924 to 1926.

The empirical formula for ephedrine is $C_{10}H_{15}ON$; its chemical structure most probably is 1-phenyl-2-methylaminopropanol-1 $C_6H_3.CHOH.CH(NH-CH_3)CH_3$.

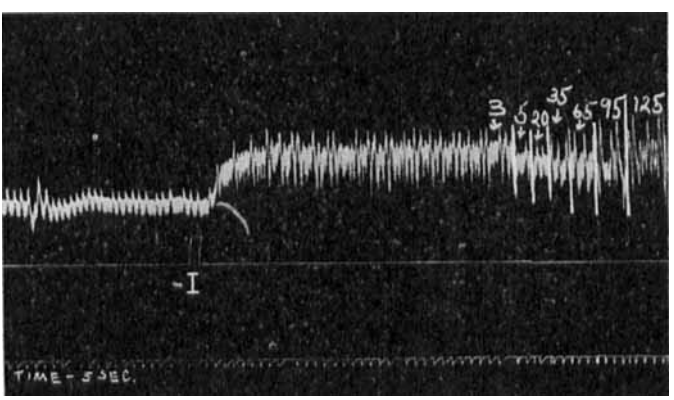

Fig. 1.—Effect of intravenous administration of ephedrine upon the circulation. 0.2 cc. of a 3 per cent solution of ephedrine sulphate intravenously injected at I. Figures above tracing indicate the number of minutes after injection.

It will be noted by the chemical composition of ephedrine that it is allied closely to epinephrine. In many ways it also simulates epinephrine in its physiologic action. Its effects on the circulation, smooth muscle and secretions are due to sympathetic stimulation and resemble qualitatively those of epinephrine. In addition it stimulates the central nervous system and depresses the heart, but these effects are elicited ordinarily only by toxic doses.

It produces a rise in blood pressure due to vasoconstriction and cardiac stimulation. It stimulates uterine muscle and relaxes the bronchial muscle. It also possesses mydriatic action.

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[1] Read before the Scientific Section of the American Drug Manufacturers' Association, New York City, March 30, 1928.

[2] Nagai, *Pharm. Ztg.*, 32 (1887), 700.

[3] Chen and Schmidt, *Proc. Soc. Exptl. Biol. Med.*, 21 (1924), 351; *J. Pharmacol.*, 24 (1924), 339; *China Med. J.*, 39 (1925), 382; Chen, *Proc. Soc. Expti. Biol. Med.*, 22 (1924), 203; 22 (1925), 404; 22 (1925), 568; 22 (1925), 570; JOUR. A. PH. A., 14 (1925), 189; *J. Pharmacol.*, 26 (1925), 83; 27 (1926), 61; 28 (1926), 77; 27 (1926), 87; 27 (1926), 239.

Quantitatively, however, there are important differences in the effects of epinephrine and ephedrine. Ephedrine is much less powerful than epinephrine but its effects are much more persistent. (See Figs. 1 and 2.) These figures show that an intravenous injection of 0.2 cc. of a 1–10,000 solution of epinephrine produced a greater rise in blood pressure than the same size dose of a 3 per cent solution of ephedrine. On the other hand the blood pressure returned to normal within five minutes after the injection of the epinephrine whereas the pressure was considerably above normal one hundred and twenty-five minutes after the injection of the ephedrine.

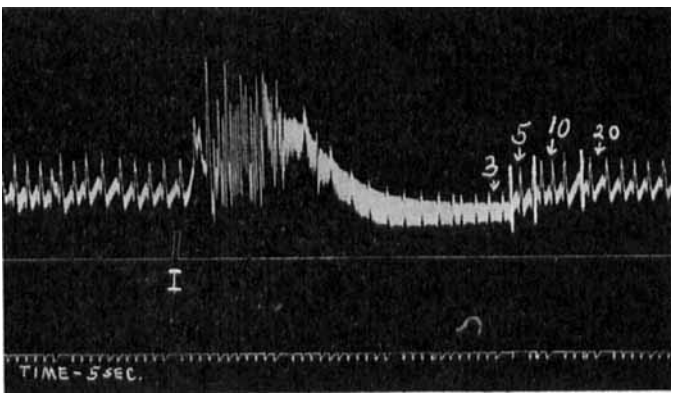

Fig. 2.—Effect of intravenous administration of epinephrine upon the circulation. 0.2 cc. of a 1–10,000 solution of epinephrine hydrochloride injected intravenously at I. Figures above tracing indicate the number of minutes after injection.

Ephedrine also differs from epinephrine in that *it is active when administered orally.* Epinephrine given by stomach does not produce appreciable systemic reactions in normal animals even when administered in large doses. According to Rowe[1]

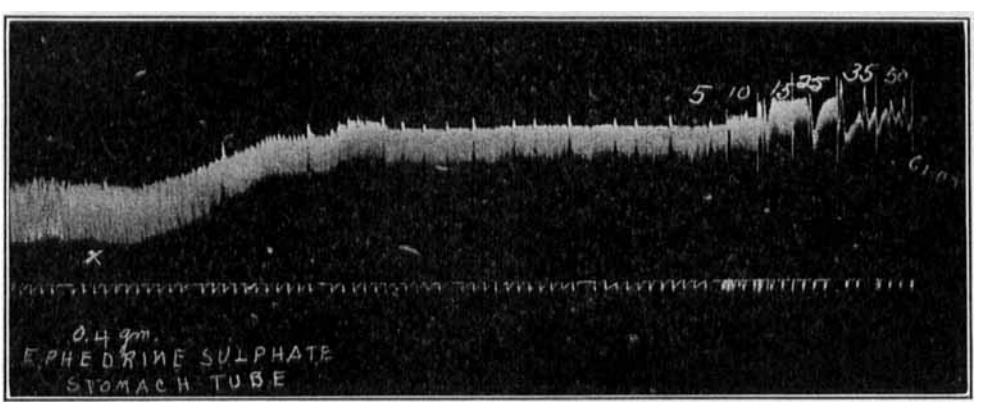

Fig. 3.—Effect of oral administration of ephedrine upon the circulation. 0.4 Gm. ephedrine sulphate administered by stomach tube at X. Figures above tracing indicate the number of minutes after administration.

"This is due partially to the rapid destruction of the adrenalin in the stomach before it can be absorbed and to the prevention of absorption by the powerful vasoconstrictor action on the vessels of the alimentary tract."

[1] Rowe: "The Comparative Pharmacologic Action of Ephedrine and Adrenalin," JOUR. A. PH. A., 10 (1927), 912.

The effect of oral administration of ephedrine upon the blood pressure is shown in Figs. 3, 4 and 5. These tracings show the rises in blood-pressure produced by doses of 0.4, 0.75 and 1.0 Gm., respectively, of ephedrine sulphate administered by stomach tube to anæsthetized dogs, which had been previously starved for 24 hours.

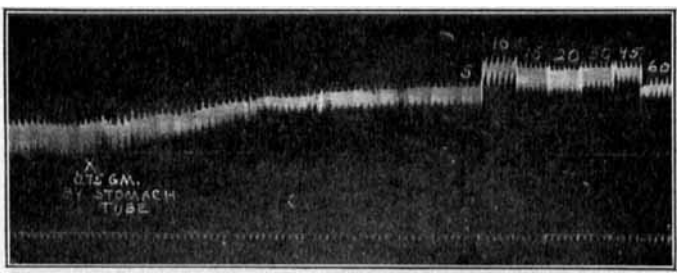

Fig. 4.—Effect of oral administration of ephedrine upon the circulation. 0.75 Gm. ephedrine sulphate administered orally at X. Figures above tracing indicate the number of minutes after administration.

The dose of 0.4 Gm. administered orally produced a rise in pressure of 34 mm. within 5 minutes. The maximum rise of 52 mm. occurred 35 minutes after administration. (See Fig. 3.)

The dose of 0.75 Gm. administered orally produced a rise in pressure of 36 mm. within 5 minutes. The maximum rise of 64 mm. occurred 10 minutes after administration. (See Fig. 4.)

The dose of 1.0 Gm. administered orally produced a rise in pressure of 40 mm. within 5 minutes. The maximum rise of 96 mm. occurred 40 minutes after administration. (See Fig. 5.)

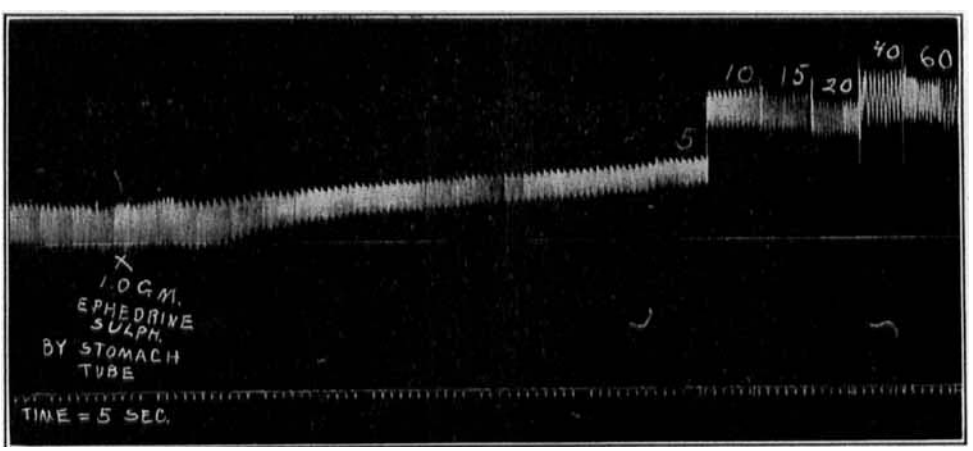

Fig. 5.—Effect of oral administration of ephedrine upon the circulation. 1.0 Gm. ephedrine sulphate administered by stomach tube at X. Figures above tracing indicate the number of minutes after administration.

The above results are typical of those obtained in a series of twenty experiments on the oral administration of ephedrine.

It will be noted, therefore, that the rises in pressure obtained by the author are from 4.5 to 12.7 times as great as Rowe reports having obtained from doses of the same size;[1] also that the author obtained in all cases a rise in pressure of

from 34 mm. to 40 mm. within 5 minutes after administration of the drug whereas Rowe obtained a maximum rise of only 7 to 14 mm. within $1^1/_2$ to 2 hours.

A comparison between the results obtained by the author and those obtained by Rowe from doses of equal size is shown below:

Dose.	Results.
0.4 Gm.	Rowe—Maximum rise of 7 mm. in $1^1/_2$ hours
	Pittenger—Maximum rise of 52 mm. in 35 minutes
0.8 Gm.	Rowe—Maximum rise of 14 mm. in 2 hours
0.75 Gm.	Pittenger—Maximum rise of 64 mm. in 10 minutes
1.0 Gm.	Rowe—Maximum rise of 8 mm. in $1^1/_2$ hours
	Pittenger—Maximum rise of 102 mm. in 17 minutes

Rowe, however, in all cases administered a dose of 10 mg. intravenously from one to two hours previous to the administration of the oral dose. This, he states, is for the purpose of showing the susceptibility of the dog to the drug.

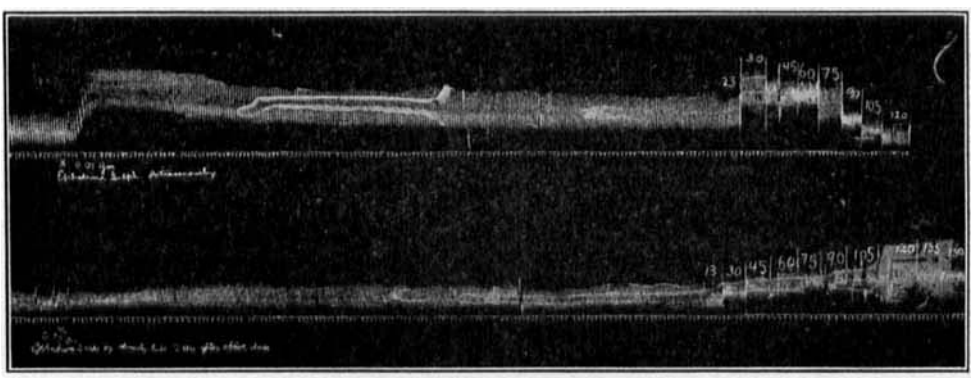

Fig. 6.—Upper row: 0.01 Gm. ephedrine sulphate administered intravenously. Lower row: 0.75 Gm. ephedrine sulphate administered orally to the same dog, two hours after the intravenous injection.

It has been the author's experience and the literature contains references to the fact that in many cases a tolerance is exhibited after the first intravenous injection of ephedrine.

In order to determine if the small preliminary intravenous injection administered by Rowe was sufficient to establish a tolerance or whether the differences in our results were due to variations in the potency of the ephedrine we carried out an experiment according to Rowe's technique using the same ephedrine with which we obtained the above results.

The results of this experiment are shown in Fig. 6. It will be noted that the preliminary intravenous injection of 10 mg. of ephedrine produced a rise in blood pressure of 70 mm. within one minute and kept the pressure above normal for $1^1/_2$ hours. After two hours a dose of 0.75 Gm. administered by stomach tube produced a rise of only 4 mm. within 5 minutes and a maximum rise of only 34 mm. within 2 hours.

It is apparent, therefore, that the preliminary intravenous injection, as employed by Rowe, instead of proving the sensitiveness of the animal actually de-

stroys its sensitiveness to succeeding doses whether administered intravenously or orally.

These results would tend to prove, therefore, that the actual rise in blood-pressure produced by the oral administration of certain doses of ephedrine is from $4^1/_2$ to 12 times as great as previously reported.

Ephedrine further differs from epinephrine in that its solutions are stable indefinitely even when exposed to light and air and are not decomposed on boiling. Solutions of epinephrine decompose rapidly upon exposure to light, air and on boiling. For this reason commercial solutions are protected by amber bottles and are sealed in an atmosphere of carbon dioxide.

Ephedrine combines readily with acids to form the sulphate, chloride, nitrate, acetate, etc. There is no important therapeutic differences between the various salts but in view of the fact that most alkaloids may be purified to a higher degree in the form of their sulphates, most of the manufacturers are marketing ephedrine sulphate in preference to the hydrochloride or other salts.

Ephedrine, therefore, although somewhat similar to epinephrine chemically and in its qualitative actions is quite different in its quantitative action.

Present indications are that its therapeutic usefulness will be more limited and in many cases quite distinctive from those of epinephrine.

BIOLOGIC ASSAY OF EPHEDRA OR EPHEDRINE.

The purity of the various salts of ephedrine may be established by chemical methods. It is, however, advisable to verify these results by biologic tests, due to the fact that when the compound is synthesized the racemic variety is obtained which is not so active as the levo-variety which occurs in the natural product.

A marked variation in the activity is also found in the crude drug. There are many varieties of ephedra[1] some of which are practically inactive. The analysis of a number of samples of Ephedra vulgaris were found to have an alkaloidal content ranging from 0.2 per cent to 0.9 per cent of total alkaloid. (Nielson *et al.*) Later investigations by Feng and Read[2] show that when fully extracted the total yield of alkaloids may exceed 1 per cent.

Of the various physiologic actions of ephedrine there are two which present themselves as possible means of biologic standardization, *i. e.*, the mydriatic action and the action on the blood pressure. The action chosen for testing the drug should be governed largely by the therapeutic effects it is desired to produce.

For example, if it is to be used as suggested by Middleton and Chen[3] in connection with other drugs for the purpose of producing mydriasis, it should perhaps be standardized according to its mydriatic action. At least this procedure should be followed until it has been proven that the mydriatic action and the action upon the blood vessels parallel each other. If it is to be used for its effects on circulation, smooth muscle and secretions due to its sympathetic stimulation it should be tested for its effect upon the blood pressure.

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[1] "The Occurrence and Alkaloidal Content of Various Ephedra Species," by Neilson, McCousland and Spruth, JOUR. A. PH. A., 16 (1927), 288.

[2] "The Ephedrine Assay of Chinese Ephedra," by C. T. Feng and B. E. Read, JOUR. A. PH. A., 16 (1927), 1030.

[3] Middleton and Chen, *Arch. Intern. Med.*, 39 (1927), 385.

It is difficult to devise a quantitative blood-pressure method owing to the peculiarity of the drug in that the size of the dose does not accurately govern the resultant rise in blood pressure, also to the fact that, as previously shown, in many cases a tolerance is exhibited after the first injection of the drug. Feng and Read[2] claim that this variation in response and the tolerance exhibited after the first injection of comparatively large doses may be practically eliminated if the drug is administered in small doses. They state that small doses will produce an appreciable rise in blood-pressure and if repeated at sufficiently long intervals will show like effect accurate enough for assay purposes. They recommend the injection of doses of 0.001 Gm. at intervals of one hour.

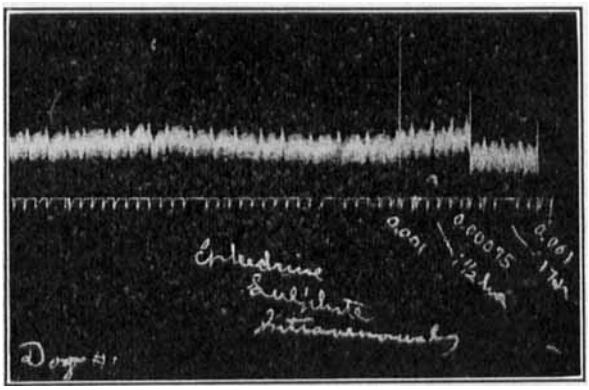

Fig. 7.—Tracing showing tolerance produced by an initial dose of 0.001 Gm. of ephedrine sulphate. Note that the second injection of 0.001 Gm. produced less rise in pressure than the dose of 0.00075 Gm.

We have performed experiments upon ten different dogs in an attempt to check the results obtained by Feng and Read. In every instance, however, the initial injection of 0.001 Gm. produced a tolerance with the result that the rises in pressure produced by succeeding doses were not in proportion to the amounts injected.

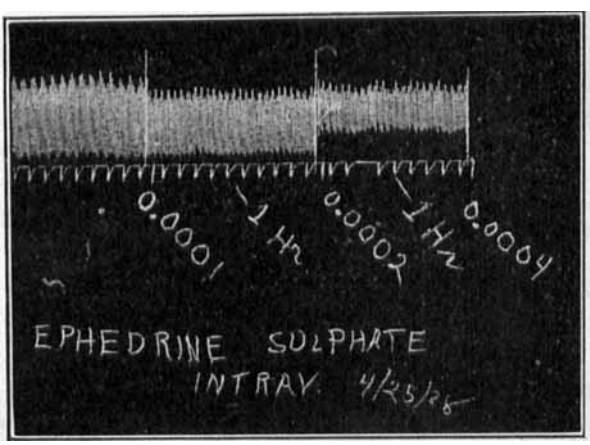

Fig. 8.—Tracing showing tolerance produced by an initial injection of 0.0001 Gm. of ephedrine sulphate. Injections of 0.0001, 0.0002 and 0.0004 Gm., respectively, were administered at one-hour intervals.

Figure 7 is typical of the results obtained. It will be noted that the initial injection of 0.001 Gm. produced a marked rise in pressure. This was followed after one and one-half hours by an injection of 0.00075 Gm. which produced a rise of only 25% of that produced by the initial injection of 0.001 Gm. This was followed after an interval of one hour by a second injection of 0.001 Gm. which produced a rise of less than 25% of that produced by the initial injection.

It has been our experience, therefore, that in every experiment a dose of 0.001 Gm. produces a marked tolerance.

In an endeavor to eliminate, if possible, the tolerance following the first

injection additional experiments were conducted in which the amount of ephedrine sulphate injected was progressively decreased until we determined the approximate minimum-effective-dose. It was found that practically all dogs will respond to intravenous doses of from 0.0001 to 0.0005 or from $^1/_{10}$ to $^1/_2$ the amount employed by Feng and Read.

Experiments were then made to determine whether dogs would respond quantitatively to doses ranging from 0.0001 to 0.0005 Gm.

The results of these experiments were the same as those obtained with the larger amounts. The initial doses of only 0.0001 Gm. in every case but one established a tolerance so that succeeding doses would not produce rises in proportion to the amounts injected.

Figure 8 is typical of the results obtained with these smaller doses. The first injection of 0.0001 Gm. produced a rise in blood pressure of 11 mm. After one hour an injection of 0.0002 Gm. produced a rise of 13 mm. One hour later an injection of 0.0004 Gm. produced a rise of only 6 mm.

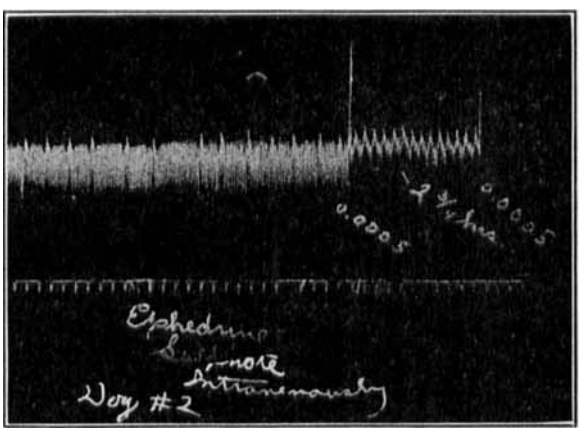

Fig. 9.—Tracing showing tolerance produced by an initial injection of 0.0005 Gm. Note that second injection of the same amount only produced one-half the rise of the first.

Figure 9 shows the results obtained from two injections of 0.0005 Gm. Although an interval of $2^3/_4$ hours was allowed between the injections, the second injection produced a rise only one-half as great as the first.

The only apparent difference in the technique employed by Feng and Read and ourselves is that they state that their animals were luminalized whereas our dogs were anæsthetized with morphine and chlorbutanol.

It is apparent, therefore, that we do not at the present time have a reliable quantitative biologic assay method for ephedrine or uniform methods of measuring the results obtained from the various forms of administration.

In view of the steadily increasing demand and use for this drug I would propose that the Scientific Section of the American Drug Manufacturers' Association appoint a "Committee on Ephedrine" for the purpose of standardizing the technique for measuring its various actions and if possible develop a satisfactory biologic assay method.

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SHARP & DOHME,
BALTIMORE, MD.
April 24, 1928.