Add the warm mixture of alcohol and tetrachlorethane to the powdered methylene blue and shake vigorously until dissolved. When cool, add the pyridine and amyl acetate and filter. Keep in tightly stoppered bottle.

The same staining directions are followed in using this last solution as are given under Formula II.

Formula III, which is an alkaline solution, is submitted to meet any possible objection to the use of an acid-methylene blue stain. Pyridine is incorporated as an accessory fixative agent. The function of the amyl acetate is to partially mask the odor of pyridine.

A series of comparative counts made in the laboratory on raw and pasteurized milk of high and low count revealed no apparent variation from the official Breed method.

Although the use of Formula I has been found very satisfactory, it is believed that because of the added advantages possessed by Formulae II and III, they will be found more satisfactory and for this reason are recommended.

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Presence of Physiologically Active Substance in Two California Species of Ephedra.

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Current interest in Ma Huang,[1] the Chinese drug plant *Ephedra,* makes imperative a study of the various species of the genus, especially with respect to the activity and nature of the chemical principles which they yield.

The problem is complicated by the lack of knowledge concerning the effect of species variation and ecological conditions upon the yield of physiologically active substances.

A complete study involves: (1) Determination of the species and varieties which contain physiologically active substances. (2) The isolation and identification of the active principles. (3) The experimental and clinical testing of the pure principles obtained.

In the work reported in this paper our efforts have been confined to a comparative study of the activity of *Ephedra californica*

Wats and *Ephedra nevadensis Wats,* using for comparison *Ephedra* obtained from Chinese drug shops in San Francisco. We have also used an ephedrine sulphate solution prepared by Eli Lilly Company, and a sample of the pure hydrochloride obtained from the Peking Union Medical College.*

Ten per cent infusions of the crude drug have been prepared in a number of ways: water, 0.9 per cent NaCl, 50 per cent ethyl alcohol, 50 per cent ethyl alcohol containing 1 per cent tartaric acid, and water slightly acidified with H_2SO_4. In all cases the solutions were heated on a steam bath 3 to 4 hours. Prior to use, the alcohol extracts were evaporated and original volume restored with 0.9 per cent NaCl. These solutions, kept in tightly stoppered pyrex flasks, retained their original activity up to 19 days, when the experimental work reported here was concluded.

Blood pressure experiments with a number of rabbits indicate that certain specimens of *Ephedra californica Wats* contain a small amount of pressor material. The pressor effects obtained with the extracts of *Ephedra nevadensis Wats* were much more pronounced than those obtained from either the *Ephedra californica* or the material from the Chinese drug shop. In one experiment four separate intravenous injections (1 cc. of the *Ephedra nevadensis* infusion per kilo) gave the following changes in blood pressure† (1) 64 mm., (2) 54 mm., (3) 52 mm., (4) 44 mm. A similar injection of the infusion of the Chinese drug, given between (2) and (3) caused a rise of only 14 mm. Thirty mgm. of the pure hydrochloride (2), given intravenously after (4), caused a rise in blood pressure of 34 mm., which persisted for much longer time. The infusions showing strong pressor effects were also effective in relaxing intestinal strips (rabbit) and in contracting preparations of virgin guinea pig uterus.

While the alkaloid ephedrine has not been isolated from any of our extracts we are led to infer from the above effects that it, or closely related substances, are present.

We failed to obtain uniform results with both the infusions of the crude drug and the solutions of the pure hydrochloride and sulfate. It is of interest to note that the clinical reports to date show similar variations in the human response to this drug.

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[1] Chen, K. K., and Kao, C. H., *J. Am. Pharmacol. Assoc.*, 1926, xv, 625. Miller, T. G., *Am. J. Med. Sci.*, 1925, clxx, No. 2, 157.

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† Expressed in mm. of mercury.