SUMMARY.

A survey based upon observation of the growth of three thousand plants of Cannabis sativa in one area has been completed. From this survey the following major conclusions may be drawn:

1. That the alkaline Beam test, as employed and elsewhere described in this report, only gave a "positive" reaction on two-thirds of the plants.
2. That the proportion of male plants reacting "positive" to the alkaline Beam test is the same as the proportion of female plants.
3. That at no time during the growth of the plant was "positive" alkaline or acid Beam test to be obtained from the pith, lower stalk or roots.
4. That plants as small as three inches above ground have the capacity of giving the alkaline Beam test.
5. That the alkaline Beam test and the acid Beam test may result from more than one compound, or may be affected by the presence of other inhibiting compounds which result in a non-uniformity in the degree to which both tests are obtained.
6. That the dried, old fruits give neither test.
7. That neither the alkaline or acid Beam test, either as hitherto proposed or as developed to date, offer any assurance as means of identification from a criminological viewpoint.

PARA-AMINOBENZENESULFONAMIDE.*

NOTES ON THE COLORIMETRIC ASSAY.

BY ASA N. STEVENS AND EDWARD J. HUGHES.[1]

Marshall, Emerson and Cutting (1) have recently published an excellent method for the determination of para-aminobenzenesulfonamide in urine. This is accomplished by using dimethyl-α-naphthylamine to produce a red color which may be estimated quantitatively in a colorimeter after diazotization with sodium nitrite.

Because of the presence of a reddish color in our available supply of dimethyl-α-naphthylamine, and also in a newly obtained sample of this reagent, we found it impossible to make a satisfactory colorimetric determination until this color had been removed by means of fractional distillation.

It is therefore our custom, in all cases where the dimethyl-α-naphthylamine is not of a clear straw color, to provide freshly distilled reagent as an essential part of the colorimetric procedure.

We have found also that it is exceedingly important to use no more than the prescribed 1 cc. of 0.1 per cent sodium nitrite solution for the diazotization, in order to avoid the development of a brown color which gives low results. Furthermore it has been our experience that although the color produced by para-aminobenzenesulfonamide in pure water is stable for several hours, the color produced in urine reaches its maximum intensity in about five minutes and should be compared with a suitable standard within ten minutes. Longer standing tends to produce an orange color which yields lower results.

* Scientific Section, A. PH. A., New York meeting, 1937.

[1] Control Laboratories, Eli Lilly and Company.

In the light of these observations, we have undertaken to slightly modify the Marshall, Emerson and Cutting method as follows.

MODIFIED METHOD.

Place approximately 50 cc. of dimethyl-α-naphthylamine in a 100-cc. distilling flask, which is connected to a suitable condenser, and distil at room temperature, retaining the clear straw-colored fraction that distils between 270° C. and 277° C. Dilute 1 cc. of this distillate to 100 cc. with 95 per cent ethyl alcohol.

Prepare standard solutions of para-aminobenzenesulfonamide containing 1.0 mg., 0.5 mg. and 0.2 mg. in each 100 cc.

Prepare also a 0.1 per cent solution of sodium nitrite in distilled water.

To 10 cc. of a 1 to 100 dilution of the sample of urine under examination, contained in a small flask, add 2 cc. of tenth-normal hydrochloric acid, exactly 1 cc. of a freshly prepared 0.1 per cent solution of sodium nitrite, 5 cc. of 95 per cent alcohol, and 1 cc. of the 1 to 100 solution of dimethyl-α-naphthylamine prepared as directed above. Shake the flask after the addition of each reagent. Ten cc. of each of the standard solutions are similarly treated. Compare the color produced by this solution with those of the standard solutions of para-aminobenzenesulfonamide. From these preliminary observations make a dilution of the sample under examination so that 100 cc. contains from 0.5 mg. to 1.5 mg. of para-aminobenzenesulfonamide.

Then to 10 cc. of this dilution, contained in a small flask, and also to 10 cc. of the standard solution which it most nearly approximates, add 2 cc. of tenth-normal hydrochloric acid, 1 cc. of the freshly prepared solution of 0.1 per cent sodium nitrite, 5 cc. of 95 per cent alcohol and, finally, 1 cc. of the solution of dimethyl-α-naphthylamine prepared as directed. Shake the flasks after the addition of each reagent.

After five minutes, but before ten minutes have elapsed, the solutions are compared in a suitable colorimeter. The results are calculated on the basis of the relative depth of color as indicated below.

$$\frac{\text{Reading of standard} \times \text{concentration of standard} \times 10}{\text{Reading of unknown} \times \text{number of cc. of original material}} =$$

Mg. of para-aminobenzenesulfonamide found in 1 cc. of the unknown specimen.

In order to keep the degree of error within narrow limits, the readings of the standard and the unknown solutions should match within 5 mm., or new dilutions should be made.

REFERENCE.

(1) Marshall, E. K., Kendall, E., and Cutting, W. C., *J. Am. Med. Assoc.*, 108, 953 (1937).

CANCER PRODUCERS.

From the bile acids of the body methylcholanthrene can be obtained, one of half a dozen cancer-producing agents. Last year Professor Louis F. Fieser and his Harvard associates not only synthesized methylcholanthrene but twenty-two related cancer producers. Some of the twenty-two are much simpler in chemical structure than methylcholanthrene and nearly as powerful.