

tests (after removal of the dissolved chloroform by gentle evaporation). The fraction supposed to be digitoxin was also tested for activity by the pharmacologic method.

Duplicate assays of the specimens of digitan gave, respectively, 1.104 and 1.064 percent of substance supposed to be digitoxin, or more than $2\frac{1}{2}$ times the quantity permitted by the standards established by the test.

The results of the chemical examination are tabulated herewith.

CHEMICAL ASSAY OF DIGITAN.				
Sample.	Weight of Sample.	Chloroform Soluble Residue.	Chloroform-Ether-Petroleum-Ether Residue.	Digitoxin.
A	10.0098 Gm.	0.1837	0.0682	0.1104
B	10.0037 Gm.	0.1822	0.0678	0.1064

The digitoxin residue was dissolved in dilute alcohol and the solution diluted with normal salt solution before testing its activity on cats. The toxicity when injected continuously into cats by the intravenous method was found to average 1.8 mg. \times Kg. of body weight. (Full details of the method and protocols of the findings will be published in the *A. M. A. Therapeutic Research Reports*.) A dose of this residue of 1.5 mg. \times Kg. of body weight was then injected intravenously into each of three cats, the animals were returned to their cages, after which an interval of three hours was permitted to elapse and the animals were then injected with ouabain solution 1-200,000 until death occurred. The quantity of ouabain required to produce death indicated a toxicity for the residue being tested, of about one-sixth of that of pure digitoxin.

By the same method, the chloroform-insoluble fraction of digitan was found to contain about two-thirds to three-fourths of the total toxicity of the product.

The residue obtained by the evaporation of the chloroform-ether-petroleum-ether solution from which the digitoxin was supposed to have been precipitated was totally inert.

It is evident that the substance obtained from digitan in the digitoxin assay method as described in "New and Nonofficial Remedies" is not pure digitoxin. For the determination of digitoxin the method is, therefore, valueless.

LABORATORY OF THE
AMERICAN MEDICAL ASSOCIATION.

DETECTION OF WOOD SPIRIT IN ALCOHOLIC BEVERAGES.

BY A. B. LYONS.

One of the tests most easily applied for the presence in a liquid of methyl alcohol is that known as Hehner's Test, in which milk plays the part of detective, aided and abetted by a trace of ferric iron. Ordinary milk, however, as a chemical reagent leaves much to be desired. As long ago as 1905 I proposed the substitution for milk of a solution of beef peptone.¹ The suggestion was favorably received, as indicated by a recently published routine method for the detection of methyl alcohol in distilled spirits.² It was possibly a retrograde step, but I ventured to suggest in 1920³ the use in this test of powdered skim milk.

¹ *Proc. Am. Pharm. Assoc.*, 1905, p. 326.

² P. Hasse, *Pharm. Zentr.*, 61, 177, 1920 (*J. Soc. Chem. Ind.*, 345A, 39, 1920).

³ "Practical Standardization of Organic Drugs," p. 84.

Recently I have undertaken a study of this modification of the Hehner test, with rather interesting results. Uniformity in composition of the milk powder, which is now easily obtainable as a food product, is its most important recommendation for use as a reagent. For use it is to be dissolved in distilled water in the proportion of 0.200 Gm. to 10 cc of water. The test for methyl alcohol is made by oxidizing the distilled sample with potassium permanganate in the usual manner, adding to a measured quantity of the filtered solution about half its volume of the "milk" and underlaying the mixture with sulphuric acid containing a trace of ferric sulphate. If the fluids are mixed at once by shaking, a purple color is produced of an intensity corresponding with the quantity of wood spirit potentially present.

It is assumed that some ethyl alcohol is present in the fluid under examination—approximately 10 percent previous to oxidation. In absence of methyl alcohol the color resulting from mixing the fluids as described is at first a dull orange with an amber shade, then gradually darkening towards brown. If the sample contained a little wood spirit the color is more or less deeply tinged with purple, and if the quantity of methyl alcohol is at all large (*e. g.*, 0.2 percent of the fluid previous to oxidation) the color becomes almost at once strongly purple. It is important, however, that the heat produced by mixing the fluid with the reagents be normal in amount, otherwise the color develops only slowly. Indeed if the fluids be chilled by plunging the tube containing them into ice water before mixing them no change of color takes place at once—possibly none on standing several hours at room temperature.

Again, the color results are influenced greatly by the quantity of milk added. If the quantity of milk is too small, the purple color is pale; if the quantity is excessive, the color shows an admixture of brown. In spite of these irregularities it is possible to devise a routine procedure which will lead to reasonably satisfactory conclusions.

A BETTER WAY.

My colleague, Martin Dodsworth, suggested a trial of egg albumen in place of milk powder or peptone. This may be had in dry form, and can be kept indefinitely in the laboratory as a reagent. A convenient strength for the solution is 0.100 Gm. in 10 cc of water. Repetition of the tests above described, substituting dried egg albumen for milk powder, demonstrated at once the immense superiority of the former. In absence of methyl alcohol the color resulting from the test was a pure golden yellow, exhibiting a strong green fluorescence. The same fluorescence is present also when milk powder is used. The effect of a very minute quantity of methyl alcohol is seen in a faint change in the yellow color of the fluid after 60 seconds. Increase in the quantity of methyl alcohol reduces the time before any noticeable color change occurs to 40, 20 and 10 seconds, and gradually it becomes plain that the new tint is purple. The test then makes it possible to demonstrate with certainty the presence of mere traces of methyl alcohol, and to estimate with a fair degree of precision the quantity of this by comparison colorimetrically with standards of known strength. Absence must be postulated of compounds (*e. g.*, some of the higher alcohols) which behave like methyl alcohol under this test.

ROUTINE PROCEDURE FOR DETECTION OF WOOD SPIRIT
IN ALCOHOLIC BEVERAGES.

In case of distilled spirits containing approximately 45 percent (vol.) of alcohol, add to 5 cc of the sample 20 cc of water and distil off 20 cc (or distil slowly 10 cc and add to this distilled water to bring the volume to 20 cc). In case of wines or other beverages containing 10 to 20 percent of alcohol, mix 10 cc of the sample with 20 cc of water, add 1 cc of alcohol U. S. P. (0.5 cc if the alcoholic strength is 15 percent—none at all if the strength is 20%), and distil to obtain 20 cc as above. The distillate is intended to contain all the methyl alcohol present and besides this approximately 10 percent of its volume of ethyl alcohol of U. S. P. strength (or 9.5 percent of absolute alcohol). Provide for the tests:

1. "Ten percent alcohol," *i. e.*, a mixture of 1 volume of alcohol U. S. P. with 9 volumes of water.
2. A solution of potassium permanganate, 1 Gm. in 100 cc of water.
3. Diluted sulphuric acid U. S. P.
4. Ferrated sulphuric acid. Dissolve 0.030 Gm. of ferric-ammonium alum in 1 cc of distilled water and add to 100 cc of sulphuric acid U. S. P.
5. Solution of albumin (0.100 Gm. dried egg albumen dissolved in 10 cc of distilled water).

For a preliminary test, add to 1 cc. of the distillate 4 cc of diluted sulphuric acid and 2 cc of the solution of potassium permanganate. Complete decoloration of the solution will occur in a few minutes. Filter the mixture through a dry filter. (The filtrate should be quite colorless; if it shows any purple tint pass it repeatedly through the filter until this disappears.) To 1 cc of the filtrate add from a pipette, which delivers 30 drops to the cc, 10 drops of the albumin solution. Shake the mixture, add 1 cc of the ferrated sulphuric acid, and shake once more. In absence of methyl alcohol the solution will be golden yellow, the color remaining unchanged at least 10 minutes, and no further tests need be made. If the solution becomes instantly purple, the beverage is to be condemned as containing either methyl alcohol or something probably equally harmful.

The first thing naturally is to apply a confirmatory test, preferably that of Schiff (fuchsin decolorized by sulphurous acid or a sulphite). The test by morphine or apomorphine is available and readily made, since we have already an oxidized solution. Probably the best plan will be to distil in small fractions (say 0.2 cc) what remains of the filtrate, by aid of a simple tube condenser, testing each fraction by adding to it 1 drop of a 2 percent solution of morphine sulphate and underlaying with strong sulphuric acid. The first fractions will contain acetaldehyde, giving a "ring" of brownish color, but some one or more of the subsequent fractions will show the purple-blue ring characteristic of formaldehyde. I have not yet verified the statement that when apomorphine is substituted for morphine the interference of other aldehydes becomes comparatively negligible. I confess that I am skeptical about it.

Let us, however, assume that the color produced is really due to presence in the beverage of wood spirit, any other supposition being highly improbable. It remains to make an approximate estimation of the percentage of the poisonous alcohol in the sample. Prepare a standard which shall show the color produced in case the sample contains 1 percent (volume) of Columbian spirit. Add to 1 cc

of Columbian spirit 9 cc of the "10 percent" alcohol. Mix and add 1 cc of the mixture to 9 cc of the 10% alcohol. Oxidize 1 cc of the latter mixture just as described above, treat 1 cc of the filtrate with 10 drops of albumin solution and add 1 cc of ferrated sulphuric acid, to prepare the 1 percent standard.

Proceed now to match this standard color by increasing or diminishing the quantity of distillate taken, being careful to maintain an ethyl alcohol standard of 10 percent in every instance. For example, suppose the color obtained from 1 cc of the distillate is very much stronger than that of the standard. Mix 2 cc of the distillate with 2 cc of "ten percent alcohol," oxidize 1 cc of the mixture and test 1 cc of the filtrate with the albumin solution and ferrated sulphuric acid. The color may now approach so closely to that of the standard that no new tests need be made, the colors being matched by diluting one or the other of them with sulphuric acid diluted with an equal volume of water.

A preferable plan is that which I have practiced. Given a trial test resulting in a color considerably stronger than that of the standard, but not twice as strong; note the number of drops of the filtrate delivered by a 1 cc pipette—most likely about 35. Make new tests using, *e. g.*, 15 or 25 drops with water enough to make up 1 cc, adding the reagents and making new comparison with the standard.

Enough in the way of details. One important fact is to be kept in mind. In comparative tests let temperature conditions be as nearly as possible identical. The solutions to be mixed and the test-tube in which the mixture is to be made must be at room temperature, there must be no delay in making the mixture, and by no means any chilling of the contents of the tube.

Instead of mixing the fluids as in the foregoing directions we may make the tests as "ring tests;" there is no advantage in this except in cases where only traces of wood spirit are present. When the test made in the usual way shows only a slight change within 60 seconds from the normal golden yellow color, it will be found that 2-5 drops of the oxidized solution with 2 drops of albumin solution, underlaid with ferrated sulphuric acid, develops in a short time a fine violet ring which may persist some hours.

There is now on the market normal propyl alcohol, which is employed as a denaturant in one of the authorized formulas. I find that this alcohol produces with Hehner's test in all its modifications colors closely resembling those characteristic of methyl alcohol, only less intense. I find further that Schiff's reagent produces when propyl alcohol is present in the sample tested, a red color, less intense than that produced by wood spirit, and much less purple. In either case the color reaches a maximum intensity only after several hours, then fades slowly—more quickly when due to propyl alcohol than when caused by wood spirit. I am continuing an experimental study of this branch of the subject, as also differences in color tests with morphine, codeine and apomorphine.

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