the phosphate in the presence of water, act on the easily decomposable salt and liberate nitric acid. This theoretic action has been found a sad reality by manufacturers who incorporated nitrate salts with a wet "super," the loss of plant food being accompanied by a destruction of the fiber of the bagging containing the goods.

FALLACIES OF POST-MORTEM TESTS FOR MORPHINE.[1]

BY DAVID L. DAVOLL, JR.

Received August 9, 1894.

IN the qualitative investigations upon morphine in a former series of experiments, the tissue was allowed to putrefy in the open air, a condition which is not present in the case of a buried body, for in the unopened cadaver, the bacterial products are very different in their deportment toward certain reagents, from those formed by aerobic germs and are the interferences which would be met with in chemico-legal work and which give some of the tests for morphine.

The writer, during the spring of 1893, in a series of determinations to ascertain the minimum of loss entailed in the recovery of a weighed amount of commercial morphine sulphate—the form generally employed in instances of poisoning—from a given amount of putrefied tissue, obtained the following results by the method given in detail below:

A. From sixty grams of stale bread, containing 0.2387 gram of morphine sulphate, 0.0844 gram of anhydrous alkaloid were recovered, the equivalent of 0.2245 gram of morphine sulphate; this shows a loss of 0.0142 gram or 5.94 per cent. By qualitative tests upon $\frac{1}{500}$ part of this recovered amount, very decided affirmative results were obtained.

B. From 100 grams of fresh liver, containing 0.3455 gram of morphine sulphate, 0.1163 gram of anhydrous alkaloid were recovered, the equivalent of 0.3093 gram of morphine sulphate, a loss of 0.0362 gram.

C. From putrid liver (after standing ten days in the open air and in a warm place) charged, when fresh, with 0.442 gram of morphine sulphate, 0.111 gram of anhydrous alkaloid were

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[1] Read at the Brooklyn Meeting of the American Association for the Advancement of Science, August, 1894.

recovered, corresponding to 0.2952 gram of morphine sulphate, a loss of 0.1468 gram.

After much experience with quantitative methods now in vogue, both in opium assay, and toxicological work I have used the method of titration of the alkaloid by standard acid and alkali, isolating the alkaloid by shaking out with primary amyl alcohol.

A careful review of the qualitative tests for morphine was first gone over, working with normal and decinormal solutions of the anhydrous alkaloid, in the form of sulphate. The figures here given, refer to the anhydrous alkaloid evaporated to dryness upon one spot in a porcelain dish.

Fröhde's Reagent.—See discussion below.

Iodic Acid, with starch paste, is sensitive to 0.00001 gram.[1]

Nitric Acid, gives deep orange color with 0.00001 gram. This reagent also produces a red coloration with certain volatile oils and resinous substances.

Ferric Chloride, in neutral solution gives deep blue color, said by Wormley to be sensitive to $\frac{1}{10000}$. Results with putrefactive tissue are given below by the writer.[2]

Ferric Chloride with *Potassium Ferricyanide*, is sensitive to 0.000005 gram.[3]

Tattersall's Test, with sodium arsenate, followed by other reagents, is very satisfactory. (For details, see "New Color Reaction for Morphine, Codeine, and Atropine," *Pharm. J. Trans.*, [3], **12**, 459-60.)

Many other tests were worked upon, but being for the most part confirmatory and quite intricate in their manipulation, they

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[1] Blyth, 1885; Separation of Morphine from Animal Tissue and Fluids; Poisons, their Effects and Detection, 1, 278. Selvin has pointed out that amyl alcohol extracts from putrefied matters, "ptomaines," agreeing with morphine in setting free iodine from iodic acid.

[2] T. G. Wormley, 1885; Morphine, Micro-Chemistry of Poisons, second edition, 485-6, says "that the blue color produced by $FeCl_3$ upon morphine is discharged by free acids, caustic alkalies, and heat. The blue color *may* not make its appearance if foreign substances be present and it is open to fallacy."

[3] O. Hesse, Morphine, *Pharm. J. Trans.*, 18, 801, says, "In the ferric chloride and potassium ferricyanide test for morphine, the action is due to the formation of pseudomorphine and potassium ferrocyanide.

$$8C_{17}H_{19}NO_3HCl + 8K_3Fe(CN)_6 = 4C_{34}H_{36}N_2O_6.2HCl + 6K_4Fe(CN)_6 + 2H_4Fe(CN)_6.$$

Pseudo-morphine is actually deposited if the solution is not too dilute and this substance being somewhat soluble in water, is the cause of the blue coloration.".

were not applied in this work for reasons which the decisive results render obvious. The negative results in testing for morphine have always been regarded as more conclusive than the positive.

The object of this work was to demonstrate, as far as possible, to just what extent cadaveric alkaloids might respond to the generally recognized tests for morphine; and to this end, the ordinary manner of burial of a corpse was followed, and after exhumation the several parts of the body were subjected to exactly the same treatment that would be followed were morphine suspected to be present as a result of poisoning.

A collie (dog) was taken, weighing thirty-four avoirdupois pounds; was shot between the eyes on March 3rd, at 1.30 P. M. and placed in a tight pine-box, when the cover was screwed on. This was buried under five feet of soil, consisting of clay mixed with gravel, on the forenoon of March 5th. When buried, the body had already begun to give off a strong odor. The body was taken up on the morning of April 26th, after forty-five days burial, the usual spring weather intervening, the box being dry inside. Eight hours after disinterment, all the parts—portion of brain, heart, lung, kidney, liver, spleen, bladder and urine, stomach and contents, piece of intestine with contents—were separately macerating in diluted alcohol.

The weights of the several parts in grams were as follows: Brain, 45; heart, 173; lung, 200; kidney, 70; liver, 345; spleen, 28; bladder and urine, 105; stomach and contents, 450; intestine with contents, 130.

The odor from the opened cadaver was overpowering, particularly from the cranial cavity. All organs were soft, but retaining their form. The brain was of a semi-fluid consistence, and when, later, the liquid extract was shaken out with immiscible solvents it emulsified exceedingly.

The method of extraction, given below, includes some few modifications over the older method, none of which, however, were introduced without previous proof of their applicability to the recovery of the alkaloid, morphine. For convenience of reference in the following comments, the manipulation was divided into arbitrary stages, designated by Roman numerals.

I. The finely divided material is placed in a flask of suitable capacity, from which extends upward a glass tube, three and one-half feet long by one-fourth inch internal diameter; the arrangement provides for the constant alcoholic and acidulous strength during extraction, by air condensation. Enough diluted alcohol (by volume) is added to well cover the material and the mass made slightly acid with acetic acid. The digestion is made upon a long iron plate, heated by means of Bunsen burners, so placed, at intervals, that the flame should strike *between* the flasks and not *directly beneath* them. The flasks are placed in a row upon the hot plate, and further protected from direct heat of the iron plate by interposing match-sticks between the flask-bottom and the hot plate, and are virtually heated by hot air and not by the plate, the wood being such a non-conductor of the heat. The digestion is continued for one hour, the temperature, 70° C., being read from a thermometer immersed in the viscera. Then cool, remove to a funnel and filter through paper by means of a filter-pump, or strain through carefully washed muslin, rinsing the flask with successive small portions of diluted alcohol and passing washings through the tissue, previously well drained. Remove the mass to the extraction apparatus and repeat the process of extraction and filtration as before. Three extractions are accounted sufficient.

II. The alcohol is removed by evaporation upon the water-bath, stirring to promote vaporization and prevent the formation of a crust.

III. The measure of the residual aqueous liquid is now ascertained, and being of a sirupy consistence, is placed in a flask of suitable capacity and three volumes of ninety-five per cent. alcohol added; the evaporating dish is rinsed with seventy-five per cent. alcohol and the washings added to the liquid under analysis. Sulfuric acid, approximately fifty per cent., is added to slight acidulation and all is set aside for eight to twelve hours, agitating occasionally. It is then filtered by means of a filter pump, and the precipitate washed with successive small portions of seventy-five per cent. alcohol.

IV. Remove the alcohol from the filtrate by evaporation upon the water-bath, and dilute with just sufficient water to obtain a limpid consistence. The presence of much fatty matter may necessitate filtration; maintain the acid reaction.

V. The acidulous solution is now shaken with successive small portions of water-washed chloroform, until, upon evaporation, the chloroform solution leaves no appreciable residue; the miscibility of the so-called immiscible solvents causes a slight residue to remain upon evaporation, even after the chloroform has performed its work.

VI. Now shake out the acidulous solution with a cold amyl alcohol. These two solvents (V and VI) remove coloring and fatty matter. The chloroform and amyl alcohol washings should be shaken with slightly acidulated water and this latter added to the original liquid under analysis.

VII. The liquid is now made alkaline with ammonia water of sp. gr. 0.962 at 15° C. and shaken out with several successive small portions of chloroform and ether (three parts to one), this to remove cadaveric alkaloids as much as possible; this was suggested by the solubility of the cadaveric alkaloids in these media and the insolubility of the morphine in the same. This is repeated four times, the aqueous liquid being each time acidulated, the immiscible solvent added, shaken, and again rendered alkaline before the solvent has time to separate into a layer; this is done in order to obtain the alkaloids nascent.

VIII. (*a*) The same conditions of acidulating, adding hot solvent and making alkaline were observed here as in "Stage VII." The suspected (acid) is heated to 70° C., placed while hot in a separatory-funnel, and when alkaline, shaken out with successive small portions of hot amyl alcohol, until a small portion, upon evaporation, gives negative results with some delicate test, three to five shakings being generally enough.

(*b*) The amyl alcohol solutions are mixed, filtered, and then shaken with successive very small portions of water until no precipitate is afforded with barium chloride.

IX. It is then evaporated to dryness upon the water-bath and the residue taken up with slightly acidulated water, fiftieth normal sulphuric acid being the strength employed.

X. Filter this liquid, heat to 70° C., and shake with two successive portions of hot amyl alcohol in *acid* solution, mixing the amyl alcohol washings and agitating with a little acidulated water, which is added to the liquid under analysis. This is done to further remove coloring matter. See remarks further on.

XI. Heat again to 70° C., add hot amyl alcohol, shake, render alkaline with ammonia water (0.962), again agitate and remove the amyl alcohol solution ; repeat this extraction with hot amyl alcohol until all the alkaloid has been removed. Now repeat the shaking with water, as mentioned in "Stage VIII (*b*)" in order to remove ammonium sulphate.

XII. Four-fifths of the final purified amyl alcohol solution are evaporated to dryness upon the water-bath and titrated with fiftieth normal potassium hydroxide and sulphuric acid, using cochineal solution as indicator. The remaining one-fifth is tested qualitatively by all reliable methods.

The above is a detailed account of the method of extraction.

Guareschi and Mosso found commercial alcohol almost invariably to contain small quantities of an alkaloidal substance, having an odor similar to that of nicotine and pyridine. In amyl alcohol, Haitinger has found as much as one-half per cent. of pyridine. The ethyl alcohol and primary amyl alcohol used in this investigation were purified by distillation over tartaric acid, using five grams of acid to each 100 cc. of alcohol, the distillates coming over at 78° and 130° respectively. The basic substance which exists in some samples of ether is pyridine. The ether was water-washed, dried over calcium chloride and redistilled. Ether containing alcohol, as is well known, has a tendency to remove morphine from alkaline media. The chloroform was water-washed and redistilled. None of the above enumerated solvents, upon evaporation of fifty cc., yielded any appreciable residue, and upon applying reagents for morphine to the spot upon which the evaporation had been made, entirely negative results were obtained. "Stage VI" was omitted in extraction of all parts, excepting with liver, kidney, and intestines, since it was thought that, as morphine is removed from acid solution by amyl alcohol, it would be advisable to first purify the alkaloid by stages VII and VIII, thus leaving much coloring and extractive matter behind, and then to shake out the *hot* acid solution with successive portions of hot amyl alcohol, where a less amount of the solvent need be employed and at the same time the very objectionable coloring matter would be more thoroughly removed. It was noticed in "Stage VI" that the *cold* amyl alcohol removed very little coloring matter, but that this same solvent, when *hot*, removed very much in "Stage VIII," which is avoided in "Stage XI." The reason why this method was not employed in the cases of the liver, kidney, and intestines, was that the idea did not suggest itself until after the final tests were applied to these. Before making this departure from the usual method the feasibility of it was first to be ascertained by actual experiment. One-half the final purified extractive matter from the liver was taken, in which negative tests for morphine had been obtained by Fröhde's reagent, Le Fort's test, and ferric chloride. Into this extract was introduced 0.0006 gram of morphine and all evaporated to dryness upon the water-bath; taken up with 100 cc. of fiftieth normal sulphuric acid, and this *hot* solution (acid) shaken out with twenty-five cc. of hot amyl alcohol. The solution was now shaken out with successive portions of twenty-five, twenty, and twenty cc. of *hot* amyl alcohol in alkaline media. The final measure was sixty cc. of purified amyl solution, each cubic centimeter of which *could not* contain more than 0.00001 gram of morphine. One cc. of this solution evaporated to dryness upon one spot gave very decided tests for morphine by Fröhde's reagent, which was the only test applied to the only perceptibly yellow residue. This test was as pronounced and strong as from 0.00001 gram of morphine evaporated from aqueous solution.

The stomach and heart yielded an extract of a very dark color, but by subsequent purification, particularly at "Stage IX," it was reduced to a very pale yellow, and, in fact, all extractive matter was freed from almost all color at this point, as the fiftieth normal sulphuric acid failed to appreciably effect the dark residue left at this stage. This residue needs some further mention; it was brownish to black in color, somewhat resinous and plastic in consistence; when not abundant it appeared as a brownish cryptocrystalline residue, taken up to a considerable extent by the fiftieth normal sulphuric acid. By computation it will be seen that 100 cc. of fiftieth normal sulphuric acid is capable of saturating 0.57 gram of anhydrous morphine, $C_{17}H_{19}NO_3$; or 0.606 gram of crystallized alkaloidal morphine, $C_{17}H_{19}NO_3,H_2O$, corresponding to 1.516 grams of morphine sulphate, $(C_{17}H_{19}NO_3)_2H_2SO_4.5H_2O$.

After treating with fiftieth normal sulphuric acid the solution is filtered through paper.

The final purified solution treated as above, is almost colorless at "Stage XII," and the residue left upon evaporation was, in the case of the brain, almost colorless: light yellow with all others, excepting the heart and stomach, which were yellow to light-brown.

The filtered liquid, after performing "Stage IX," was tested for alkaloids by bromine water, Wagner's solution, Mayer's reagent, sodium phosphomolybdate, and picric acid and all parts of the viscera gave strong precipitates with each reagent, excepting picric acid. The cautious addition of ammonia water to them also gave cloudiness.[1]

The reagents for the final identification tests were prepared for the most part after the directions given in "Reactions" by F. A. Flückiger, translated, revised, and enlarged by J. B. Nagelvoort.[2] Fröhde's reagent was made of the strength 0.010 gram of molybdic acid to each cubic centimeter of sulphuric acid, sp. gr. 1.84, and dissolved by digesting below 150° C.

The nitric acid test, ferric chloride with potassium ferricyanide, and the ferric chloride test were of the above recognized strength (Flückiger).

Le Fort's test was applied as follows: Iodic acid in solution is applied to the dry residue and all intimately mixed; chloroform, in successive small portions, is floated over this until it ceases to remove the pink color of iodine, the chloroform solution being removed by absorption with filter paper; one drop of ten per cent. ammonia water (or until alkaline) is now added and morphine will give a deep "mahogany color" not affected

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[1] For a discussion of the production of precipitates with organic matter by such general alkaloidal reagents as Wagner's solution, Mayer's reagent, bromine water and picric acid. See T. G. Wormley, 1885; Morphine, Micro-Chemistry of Poisons, second edition, 492.

[2] Schönn, *Ztschr. anal. Chem.*, 8, (*1869*), 379., in regard to preparing Fröhde's reagent says: "A moderate heat only should be applied, since the molybdic acid is reduced to a blue precipitate by heat alone at the temperature of incipient vaporization of H_2SO_4."

by chloroform, but changing back to yellow with hydrochloric acid. 0.00001 gram of pure morphine will respond to this test but the writer would not claim such extreme delicacy of reaction in presence of foreign matter; 0.00005 gram gives a good decided test, suitable for recognition in recovered matter.

After somewhat extended work upon morphine in the viscera, it seems to me that under the conditions mentioned, Fröhde's reagent, Le Fort's test, and ferric chloride are the least fallacious of all recognized tests applied.

Fröhde's reagent is especially satisfactory, appearing immediately as a deep purple color, fading to violet, then green, when applied to extractive matter *known* to contain morphine, and under these conditions sensitive to 0.00001 gram. In applying this test two samples, each containing extract from two cc. of the purified amyl alcohol solution were evaporated upon one spot in a porcelain dish upon the water-bath; to one of them was first added, and intimately mixed, 0.00001 gram of alkaloid morphine; the test was applied to both samples at the *same time* and in no instance was there obtained any indications whatsoever of morphine in the sample of extract *not* containing that alkaloid, a light green color only, appearing; but, on the other hand, the sample to which 0.00001 gram of morphine *had* been added, never failed to give a decided purple color *immediately*. These parallel tests were made for the purpose of verifying the conclusion, that, had morphine (or an alkaloidal base acting similarly toward Fröhde's reagent) been present in even so small an amount as 0.00001 gram a reliable test would have been obtained.

With ferric chloride, freshly dissolved and free from acid, the same parallel tests were made as with Fröhde's reagent, with the exception that 0.00005 gram of morphine was introduced in the comparative test, since this test is not as delicate as the molybdate in extractive matter. When applied to the extractive to which morphine had been added, bluish rings appeared, a positive test; but with the pure extract, from all parts of the viscera, no color appeared.

Le Fort's test was applied with a parallel test, containing 0.00005 gram of morphine and was very satisfactory as a reagent, and as far as demonstrating the absence of morphine or any interfering bases was concerned, was conclusive. With the extractive to which morphine *had* been added, a final "mahogany color" had been obtained, but no change with the pure extract from the parts of the body.

Iodic acid gave indications of a reducing agency, since iodine could be detected with clear, fresh starch-paste in all instances.

The nitric acid tests were found to be of no value, ferric chloride, with freshly prepared solution of potassium ferricyanide, gave indications of a base having similar reducing action as morphine. Parallel tests were made, the one containing the extractive from two cc. of final amyl alcohol solution, was evaporated to dryness, taken up with four cc. of fiftieth normal sulphuric acid; the other treated in an exactly similar way, *but* to which had been added 0.00001 gram of morphine. To both these solutions were added eight cc. of a solution of potassium ferricyanide and one drop of solution of ferric chloride. With the portion known to contain morphine, a blue color appeared *at once;* with the simple extractive matter from the viscera, only a light green color appeared, not changing at all in three minutes; slightly darker in five minutes; after ten minutes one could easily distinguish the samples one from the other, but after two hours both had deposited a similar blue precipitate.

During the process of extraction of the different organs, it was noticed that from them, and particularly from the liver, brain, spleen, intestines, and kidney, a decided odor resembling that of trimethylamine was given off, continuing all the way through.

CHEMICAL LABORATORY, UNIVERSITY OF MICHIGAN,
June 2, 1894.

FERMENTATION OF GLUCOSE SYRUPS.

BY HORACE E. HORTON.

Received October 23, 1894.

I WOULD like to call attention to the interesting fact that glucose syrup of 41° Bé. is fermentable. The opinion is general that glucose of this gravity is non-fermentable and I am unable to find any published record of such fermentation.

During the summer of 1893 large quantities of the so-called "mixing glucose" sold in southern cities was found to be in a