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Determination of Small Amounts of Morphine in Blood.

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The fate of morphine in the animal body is a question that has stimulated much excellent work and the development of many methods for the determination of the drug. We do not have space to enumerate, much less to discuss, these methods, 2 of the more recent examples of which are those described by Wolff, Riegel, and Fry[1] and by Abe and Uchida.[2] Most of these methods were unsuited to our purpose since they were not designed for use with blood, and the others required more material or a more elaborate procedure than we wished to employ.

The method herein described has the advantage of simplicity, takes but a few hours to complete, and gives quantitative results with small amounts of blood. It is based on that of Sanchez,[3] which was designed for the determination of morphine in pure solution. We have modified it for use with blood filtrates, deproteinized by the method of Somogyi,[4] introducing scopolamine to bring down the precipitate and permit the removal of the reacting solutions. The method in detail is as follows: A 1-to-10 filtrate is prepared by laking 1 part of blood with 7 parts of water. To this is added with constant stirring 1 part 10% zinc sulphate and 1 part 0.5 N sodium hydroxide.* After standing 10 minutes the precipitate is centrifuged down and the supernatant liquid filtered through No. 2 Whatman paper. Treated in this manner 5 cc. blood yield over 30 cc. filtrate.

To 20 cc. of the filtrate, equivalent to 2 cc. blood, is added 2.5 mg. of scopolamine hydrobromide, from a solution containing 1 mg. per 1 cc. (Proportionally smaller amounts may be used if the concentration of morphine is high.) The morphine is then precipitated by 0.4 cc. of Wavelet's solution.† The material is thoroughly mixed

1 Wolff, W. A., Riegel, C., and Fry, E. G., *J. Pharm. and Exp. Therap.*, 1928, **33**, 329.

2 Abe, K., and Uchida, T., *Jap. J. Med. Sci., Pharm.*, 1934, **8**, 89*.

3 Sanchez, Juan A., *La Semana Medica*, 1930, ii, 333.

4 Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

* The $ZnSO_4$ and the NaOH must be so related that 10 cc. of the former require from 10.8 to 11.2 cc. of the latter to produce a permanent pink color with phenolphthalein.

† Wavelet's solution is made by dissolving 7 gm. sodium carbonate and 1 gm.

by stirring or shaking, and set in a cold place for about an hour. The precipitate is then centrifuged down. The clear liquid is decanted and discarded. After draining and wiping out the tubes to remove all traces of fluid, the precipitate is suspended in 1.5 cc. water and dissolved in 10 drops of a 2% solution of ammonium hydroxide. Solution is promoted by stirring and shaking and is accompanied by the development of a blue color, which persists for at least 20 minutes. This color is compared with that of standards either in conical centrifuge tubes or in a microcolorimeter.

Standards are prepared from blood. Pooled specimens are more easily obtained, are entirely satisfactory, and may give a slightly more uniform standard. The desired amounts of morphine are added to the whole blood before laking. The specimens are then treated exactly like the unknowns, using the same volumes. These protein-free filtrates keep well with refrigeration, and may be used for 2 or 3 weeks, after which there seems to be some loss of strength, as shown by weaker color development. It has been our custom to precipitate the proteins a few minutes after the specimens were drawn. In one case, however, the whole blood was permitted to stand 3 days, with only a slight loss of morphine resulting.

Standards, without morphine, prepared by this method are colorless. With 0.01 mg. of morphine a clear blue color is obtained. Although not directly proportional, the color varies with the concentration of the morphine, 0.005 mg. giving a distinct color, and 0.002 mg. standards being definitely distinguishable from the blanks. It is advisable to prepare a series of standards varying by 0.005 mg. to cover the expected range. Unusual retention of uric acid, over 25 mg. per 100 cc., interferes with the determination, giving a faint blue color with the reagents.

The scopolamine promotes the precipitation of the morphine. It produces no color, since the blanks are water clear; but without it, smaller amounts of morphine are lost. It is apparently most effective in the proportion of 0.7 mg. per 5 cc. of filtrate. If too much is used it does not dissolve readily in the ammonium hydroxide, and tends to color the final solution green. The limit is about 2.5 mg., the amount necessary for 20 cc. of filtrate.

Stronger solutions of ammonium hydroxide, or larger amounts, dissolve the precipitate more rapidly, but fade the blue color. The proportion of 0.1 cc. of the precipitating reagent to 5 cc. of filtrate

disodium acid phosphate in 25 cc. water. To this is added 3.5 gm. freshly calcined molybdic acid, and, following complete solution, 10 cc. concentrated nitric acid. The solution is allowed to stand over night, then filtered. It keeps indefinitely at room temperature.

seems entirely adequate. Larger amounts tend to be retained in the residue and to interfere with the final color. In preparing the standards the morphine must be added to the whole blood, since additions to the filtrate give darker colors. This confirms earlier work[5] that there is some loss of morphine with the proteins, but there is no reason to believe that the loss is not as great with the standards as with the unknowns. This loss is greater with a more concentrated filtrate, as a 1 to 5 dilution, and less with a 1 to 20. The latter is unsatisfactory, however, since it increases the volume of the filtrate, and the determinations as now run are limited to 20 cc.

We have used this method successfully with rabbits, and have found the morphine concentration of the blood to vary with both dosage and time. A 6 kg. rabbit given 64.8 mg. (1 grain) morphine intramuscularly showed 0.014 mg. per 1 cc. blood after 35 min., and the same concentration at the end of 1 hour. Two others, weighing 3.7 and 3.4 kg., were given 16 mg. each. After 45 min. the first had a concentration of 0.004 mg. per 1 cc., and after 1½ hours 0.007 mg. The 2nd had 0.006 mg. after 35 min. These showed a satisfactory uniformity on the basis of the dosage per kilo of body weight. A fourth rabbit, weighing 3.6 kg. and given 16 mg., showed a lower concentration, 0.0035 mg. after 1 hr., and 0.0055 mg. after both 2 and 3¾ hours. The method is, therefore, satisfactory for animal experimentation.

It has not proven sufficiently delicate, as it now stands, for use with humans. The blood following the therapeutic dosage of 10 to 16 mg. does not give any color. That from 2 patients, the first given 3 injections of 16 mg. morphine and 1 of 10 mg., at 3 hr. intervals, and the second a single injection of 32 mg., did show a faint color, using 20 cc. of filtrate, but not sufficient for a quantitative estimation. These results indicate, however, that the blood of addicts would contain sufficient morphine to permit accurate determinations.

We have confirmed the findings of Teruuchi and Kai[5] that morphine is divided between cells and plasma. This necessitates using whole blood for all morphine determinations.

Summary. A simple, inexpensive colorimetric method, adapted from that of Sanchez, is herein described by which morphine may be determined in blood when the concentration is not less than 0.0025 mg. per 1 cc. The method is practical for animal experimentation, but is not sufficiently delicate, as given, for use with human subjects receiving therapeutic doses of morphine. Whole blood is used since morphine can be found in both cells and plasma.

[5] Teruuchi, Y., and Kai, S., *J. Pharm. and Exp. Therap.*, 1927, **31**, 177.