ANTIPYRIN IN TOXICOLOGICAL ANALYSIS.

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SUCCESSFUL toxicological analysis depends very greatly on the existence of good characteristic tests. The absence of such tests proves a most serious difficulty in the cases of veronal and sulphonal and its allies. Fortunately an excellent characteristic test for antipyrin is afforded by F. A. Steensma's reagent (*Pharm. Zeit.*, 1907, 52, 851; *ANALYST*, 1908, 33, 14), which is prepared by dissolving 1 grm. of *p*-dimethylaminobenzaldehyde in 100 c.c. of a solution of 5 c.c. of 25 per cent. hydrochloric acid in 100 c.c. of absolute alcohol. When a liquid containing a trace of antipyrin is evaporated to dryness, together with 5 c.c. of this reagent, a beautiful rose-red colour is developed. The colour is fairly permanent; it is dissipated by warming with sulphuric acid, but reappears on dilution. No reaction is given by commonly occurring substances, such as methyl alcohol, acetone, acetic acid, light petroleum, aniline, ethyl acetate, aldehyde, or formalin. Commercial amyl alcohol, however, yields a faint pink colour, as also does undenatured spirit, and this fact ought to be borne in mind when testing for antipyrin.

Extraction of Antipyrin.—The best solvent for extracting antipyrin is chloroform. A solution of 10 mgrms. antipyrin in 20 c.c. of water yielded, on extraction with 15 c.c. of chloroform, 8 mgrms. from the neutral or ammoniacal solution, and 5 mgrms. from the solution acidified by hydrochloric acid.

Similar experiments showed that light petroleum does not extract antipyrin, and that ether, ethyl acetate, and benzene, do so only in small proportions as compared with chloroform.

Extraction from Urine.—The following experiments illustrate the nature of the results obtained on extraction from urine without submitting the liquid to any process of purification, such as by means of basic lead acetate.

Five mgrms. of antipyrin were dissolved in 50 c.c. of human urine, rendered acid with tartaric acid, and extracted by the solvent mentioned in the tables.

ACID SOLUTION, 50 c.c.

Solvent.	Nature of Residue.	Reaction with Steensma's Reagent.
Light petroleum, 25 c.c. ...	nil	nil
Ether, 25 c.c. ...	dirty red	yellow
Ethyl acetate, 25 c.c. ...	dirty brown	yellow
Chloroform, 15 c.c. ...	fairly clean	yellow, red at edge

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AMMONIACAL SOLUTION, 50 c.c.

Solvent.	Nature of Residue.	Reaction with Steensma's Reagent.
Light petroleum, 25 c.c. ...	colourless	nil
Ether, 25 c.c. ...	nearly colourless, crystalline	yellow, red at edge
Ethyl acetate, 25 c.c. ...	rather dirty, crystalline	yellow, red at edge
Chloroform, 15 c.c. ...	slightly green	distinctly red

Parallel experiments with normal urine, carried out in precisely the same way, showed that the nature of the various residues is very similar to that indicated above. On testing with the reagent, the colours obtained vary from yellow to dark brown when hot, fading in most cases to yellow when cold. The ether and ethyl acetate residues from the ammoniacal extraction sometimes give a pale red colour at the edges, which can be distinguished from the antipyrin coloration which forms in the liquid during evaporation. In a case of doubt it is advisable to make a parallel test with a portion of the residue to which a trace of antipyrin has been added.

Extraction from Viscera.—Four mgrms. of antipyrin were added to 4 ounces of putrid intestine of a dog and extracted with 300 c.c. of pure spirit and 3 grms. of tartaric acid for twelve hours in the cold. After removal of the alcohol in a vacuum the residue was taken up with 20 c.c. of water, filtered, and extracted twice with 20 c.c. of ether. The solution was then made ammoniacal and extracted with 20 c.c. of ethyl acetate, and then with 20 c.c. of benzene.

The combined residues from ether were very dirty, but gave the red coloration distinctly. The residues from ethyl acetate and benzene were in both cases fairly clean, and gave a good positive colour reaction.

This experiment shows that in the proportion given no difficulty need be experienced as regards the recovery of unaltered antipyrin in a routine Stas-Otto separation.

We then made a similar experiment, adding 1 mgrm. of antipyrin to 4 ounces of fresh intestine. The final solution was, however, extracted successively with light petroleum, ether, ethyl acetate, and chloroform, both from acid and ammoniacal solution. Ether and ethyl acetate remove most of the dirt, and the residues from chloroform extraction each gave a positive antipyrin test, that from ammoniacal being more intense than that from acid extraction.

The observations were repeated with 0.2 mgrm. of antipyrin to 4 ounces of intestine. In this case the results were negative.

Our observations show that Steensma's reagent serves to recognise antipyrin extracted from viscera according to the Stas-Otto process with certainty when as little as 1 mgrm. in 4 ounces is present; below this amount the results are doubtful or negative.