CHROM. 3954

Metabolism of xanthine alkaloids in man

Despite the world-wide occurrence of xanthine alkaloids (caffeine, theobromine and theophylline) and the consumption of relatively enormous quantities in a variety of beverages, remarkably little is known about their metabolism in man.

Urinary excretion products have been studied following the ingestion of a large dose of one particular xanthine. CORNISH AND CHRISTMAN[1] found methylated uric acids as well as 1-methyl-, 7-methyl-, and 1,7-dimethylxanthine (paraxanthine) after caffeine ingestion. SCHMIDT AND SCHOYERER[2] in similar studies detected caffeine, theobromine, paraxanthine and 1-methylxanthine in the urine of subjects treated with 300 mg caffeine orally.

It was decided to attempt the separation and identification of caffeine and its metabolites in human blood as a preliminary to further studies of caffeine metabolism in man. After many trials a two-stage system for thin-layer chromatographic separation was devised which achieved an adequate separation of the xanthines after a preliminary removal of blood lipids.

Plasma or red cells, separated from heparinized blood, was extracted twice by shaking with 3 volumes of chloroform (chloroform extracts methylxanthines but not uric acids). The chloroform extracts were clarified by centrifugation and evaporated to dryness. The extracts were then applied to plates prepared by slurrying Kieselgel G according to STAHL with phosphate buffer (pH 6.8) and spreading on glass plates to a thickness of 300 μ. The plates were used after drying and heating to 105° for 30 min. Plasma extracts (20 μl) were applied for runs of 15 cm and 2 μl of appropriate standards were applied at concentrations of 2 μg/ml in 2 N NH_3. Detection was effected by spraying with I_2/KI in ethanol (which coloured the plates brown all over) followed by 95% ethanol containing 25% conc. HCl. By this procedure caffeine was revealed as a brown spot and the dimethylxanthines as purple-blue spots against a yellow background. Spots faded rapidly and required immediate marking. The sensitivity was about 1.0 μg. Since lipids are also stained by this procedure the plates were also sprayed with 5% phosphomolybdic acid in ethanol and heated to 120° for 5 min. Lipids were revealed as blue spots.

Many solvent systems were tried for separation of caffeine and dimethylxanthines. Chloroform–ethanol (9:1) gave no separation of theobromine and paraxanthine and lipids streaked considerably over the xanthine area. Altering the ratio of the two solvents gave no improvement. Butanol–acetic acid–water (4:1:1) was much more successful. Although runs were lengthened to 3 h the separation was greatly improved. R_F values were: caffeine 0.42; theobromine 0.38; paraxanthine 0.47. Butanol–formic acid–water (33:1:7) gave very similar results.

The presence of blood lipids in the chloroform extracts at first complicated the separation of the xanthines since the lipids ran into the same areas in these solvent systems. However, the problem was resolved by the discovery that when the extracts were first run in petroleum ether (b.p. 60–80°) the lipids moved a long way (R_F approx. 0.70) while the xanthines remained on the start line. A general procedure was therefore adopted of pre-running the extracts in petroleum ether and then re-running in butanol–acetic acid–water.

This procedure has now been applied to a series of human volunteers with the following results. (1) Habitual consumers of beverages containing caffeine require about 7 days of abstention from them before caffeine completely disappears from their blood extracts. (2) In such "decaffeinated" subjects administration of 500 mg caffeine orally leads to the appearance of caffeine and paraxanthine in both red cells and plasma within 3 h. (3) Caffeine and paraxanthine then disappear from the blood extracts within 24 h.

From these results it would appear that the first step in the metabolism of caffeine in man is the removal of the 3-methyl group and the formation of 1,7-dimethylxanthine (paraxanthine). Why it takes so long to "decaffeinate" the habitual caffeine consumer as compared with the "decaffeinated" subject treated with an acute dose of caffeine is not known and further work is being undertaken.

I should like to express my gratitude for the enthusiastic collaboration of all the B.Sc. students who have taken part in this work over the past two years.

Department of Biochemistry,
The London Hospital Medical College,
London E.1 (Great Britain)

RUTH N. WARREN

1 H. H. CORNISH AND A. A. CHRISTMAN, *J. Biol. Chem.*, 228 (1957) 315.
2 G. SCHMIDT AND R. SCHOYERER, *Deut. Z. Ges. Gerichtl. Med.*, 57 (1966) 402.

Received January 16th, 1969