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The Quantitative Determination of Hypoxanthine and Xanthine ('Oxypurines') in Skeletal Muscle from Two Patients with Congenital Xanthine Oxidase Deficiency (Xanthinuria)

By R. PARKER, W. SNEDDEN and R. W. E. WATTS*
*The Medical Professorial Unit, St Bartholomew's Hospital, London E.C.*1, *U.K.*

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Hypoxanthine and xanthine have been identified in the skeletal muscle of two patients with xanthinuria (congenital deficiency of xanthine–oxygen oxidoreductase, EC 1.2.3.2) by high-resolution mass spectrometry (Parker, Snedden & Watts, 1969). Normal muscle contained traces of hypoxanthine but no xanthine. These qualitative observations agreed with the results of microscopic studies on the same samples of tissue (Chalmers, Watts, Bitensky & Chayen, 1969*b*), It has recently been possible to use the high-resolution mass spectrometer to measure the amounts of these purine bases in the muscle. The method and the results obtained are reported in the present paper.

Materials and methods. The muscle biopsies were the same as those previously studied. Specimens 1 and 3 were obtained from the xanthinuric patients who were previously referred to as patients A and B respectively, and the control tissue specimens were the same as samples 2A and 4 of our previous paper (Parker *et al.* 1969). The tissue was dehydrated as described previously (Parker *et al.* 1969). Weighed (approx. 1 mg) amounts of the pulverized material were admitted to the mass spectrometer (Varian MAT model SM1) by means of the direct-insertion probe inlet. At a resolving power of 10000 the ions derived from the 'oxypurines' were completely separated from background ions having the same nominal mass but differing atomic composition (Parker *et al.* 1969).

It was pointed out previously (Parker *et al.* 1969) that the composition of the vapour in equilibrium with a solid mixture contained in the direct-insertion inlet normally varied during the course of a measurement and also with fluctuations of evaporation temperature. Hence a measured ion abundance was not directly related to the concentration of the corresponding molecular species in the mixture.

To overcome this difficulty the abundances of the ions characteristic of hypoxanthine and xanthine (masses 136.0385 and 152.0334 respectively) were alternately sampled at regular intervals throughout the time taken for complete evaporation of the 'oxypurines' from the dehydrated muscle. The areas under the graphs of ion abundance plotted against time (the evaporation profiles) so obtained were proportional to the absolute concentrations of the hypoxanthine and xanthine in the muscle. This procedure was independent of evaporation temperature. Although temperature fluctuations might alter the shape of the profile, they would not affect the area enclosed by it. Satisfactory profiles were obtained at evaporation temperatures of approx. 250°C. At this temperature 100 ng of hypoxanthine or xanthine was completely evaporated in 2–3 min.

The overall sensitivity of the mass spectrometer was checked at the beginning and end of each profile by recording the abundance of the molecule ion (mass 142) of 1-methylnaphthalene. This internal standard was admitted simultaneously at constant pressure to the mass spectrometer by means of a conventional reservoir inlet. Calibration was performed by obtaining the evaporation profiles for a series of mixtures of hypoxanthine and xanthine of known composition and plotting the corresponding integrated ion abundance against the concentration of each 'oxypurine'.

Results and discussion. The concentrations of hypoxanthine and xanthine in the xanthinuric and control subjects' muscle are shown in Table 1. The quoted errors represent the maximum deviation from the mean of three determinations for each specimen. The experimental precision obtained from a series of calibrations was better than ±10% of the measured concentration over the range 25–5000 ng of 'oxypurine'. Some of the observed error may have been due to inhomogeneity of the muscle specimens.

The smallest amounts of hypoxanthine and xanthine that could be reliably measured in this study were 20 ng and 50 ng respectively. This does not represent the detection limit of the mass spectrometer (which is at least 100 times more sensitive) but is due mainly to the difficulty of handling and evaporating such small quantities of material in a reproducible manner. The higher detection limit of xanthine compared with hypoxanthine is partly due to the lower relative abundance of the xanthine molecule ion ($M_X^+ = 40\%$,

* Present address: The Medical Research Council Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, U.K.

Table 1. *Concentration of hypoxanthine and xanthine in skeletal muscle from xanthinuric and control subjects*

Specimen	Hypoxanthine (ng/mg)	Xanthine (ng/mg)
1 (Xanthinuric)	350±40	315±30
2 (Control)	22± 3	<50
3 (Xanthinuric)	240±30	450±40
4 (Control)	29± 3	<50

M_{Hx}^+ = 60% of the total ion current) (Chalmers, Parker, Simmonds, Snedden & Watts, 1969*a*) and partly due to the greater polarity of xanthine. This could result in adsorption of some of the xanthine on the surfaces of the inlet and ionization chamber with consequent decrease in the proportion reaching the ionizing electron beam. These considerations do not, however, affect the overall accuracy of the measurement, since they are taken into account in the calibration.

It is possible that 'oxypurine' contained within the muscle may be prevented from evaporating to the same extent as an equal amount present in a standard mixture, resulting in an artificially low estimate of the muscle 'oxypurine' concentration. If this were the case, it is possible that the muscle 'oxypurines' would take longer to evaporate and the corresponding profiles would 'tail' excessively compared with those of the standards. However, the shapes of the profiles were essentially similar whether derived from muscle 'oxypurine' or from the standards, and the total evaporation times in both cases were approximately equal. It was concluded therefore that the amount of 'oxypurine' retained by the muscle after evaporation was negligible.

The plasma concentrations of the 'oxypurines' (hypoxanthine + xanthine) are increased to about 0.5mg/100ml in xanthinuria, and it is necessary to consider the possibility that this could account for the present findings. The total water content of the skeletal muscles in the different mammalian species are similar, typical values quoted for human muscle being 70.1 and 79.5g/100g of fresh tissue (Ottoway, 1961). Creese, D'Silva & Hashish (1955) reported that the total water and extracellular water contents of the fresh gastrocnemius muscle of the rat were 760g/kg wet wt. of tissue and 96ml/kg wet wt. of tissue respectively. If the 'oxypurines' were uniformly distributed in the total water of the muscle at the concentrations that prevail in plasma, the concentrations observed in the present study would be about $(0.005 \times 760)/240$mg/g dry wt. of tissue = 16ng/mg dry wt. of tissue. Similarly, if the 'oxypurines' were only present in the extracellular compartment of the muscle water, the observed 'oxypurine' content of the tissue would be about $(0.005 \times 96)/240$mg/g dry wt. of tissue = 2.0ng/mg dry wt. of tissue. These values are much less than those observed in the present work, which therefore lends quantitative support to the view that hypoxanthine and xanthine accumulate locally in the muscle tissue in xanthinuria (Chalmers *et al.* 1969*a*; Parker *et al.* 1969).

The plasma 'oxypurine' concentration in normal subjects is 0.1–0.3mg/100ml (Jørgensen & Poulsen, 1955). Hence the present results also confirm the presence of a small but significant amount of hypoxanthine in the normal muscle tissue. The possibility that hypoxanthine might be formed from adenylic acid via inosinic acid and inosine was discussed previously (Parker *et al.* 1969). It is likely that this sequence of reactions would be favoured during vigorous contractions of the muscle fibres produced by mechanical stimulation during the excision of the tissue, and that the amount of hypoxanthine in normal resting muscle might be much lower.

As with the qualitative observations reported previously (Parker *et al.* 1969), the present method could be extended to the measurement of other substances in different tissues.

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