IDENTIFICATION OF p-HYDROXYPHENYLPYRUVIC ACID-O-SULPHATE AS A METABOLITE OF TYROSINE-O-SULPHATE IN THE RAT.

Gillian M. Powell, F. A. Rose and K. S. Dodgson

Department of Biochemistry, University of Wales,
Newport Road, Cardiff, Great Britain.

Received December 26, 1961

Studies (Dodgson, Powell, Rose and Tudball, 1961) on the metabolic fate of tyrosine-O-sulphate, a component of mammalian fibrinogens, showed that desulphation of the ester does not occur to any appreciable extent in the rat. Furthermore, the ester was metabolised to a considerable extent to yield two major radioactive products, one of which was identified as the O-sulphate ester of p-hydroxyphenylacetic acid.

The possibility existed that the second metabolite was an intermediate on the metabolic pathway between tyrosine-O-sulphate and p-hydroxyphenylacetic acid-O-sulphate. Thus it might be expected that deamination of the former would yield the O-sulphate ester of p-hydroxyphenylpyruvic acid which might in turn, give rise to the corresponding p-hydroxyphenylacetate derivative. This possibility has now been examined.

An authentic radioactive sample of the sulphate ester of p-hydroxyphenylpyruvic acid was prepared by sulphation of p-hydroxyphenylpyruvate with S^{35}-labelled chlorosulphonic acid under very strictly controlled experimental conditions which will be described elsewhere. Such conditions were necessary because of the lability of the product which underwent spontaneous conversion to p-hydroxyphenylacetic acid-O-S^{35}-sulphate at neutral pH and room temperature and to the corresponding p-hydroxybenzaldehyde derivative under mildly alkaline conditions.

Mature female hooded rats were injected intraperitoneally with tyrosine-O-S^{35}-sulphate (1.5 mg. / 200 g. body wt.) and the urine was collected over a period of 5 hr. The urine was subjected to paper chromatography and electrophoresis when the unknown metabolite exhibited behaviour identical with that of the authentic sample of p-hydroxyphenylpyruvic acid-O-S^{35}-sulphate. Attempts to isolate the metabolite proved difficult and it became increasingly apparent that the material was subject to spontaneous changes analagous to those undergone by the authentic sample of p-hydroxyphenylpyruvic acid-O-S^{35}-sulphate when the latter compound was subjected to similar procedures.

Free p-hydroxyphenylpyruvic acid has been detected and estimated in urine by measurement of the ultraviolet absorption spectrum in the presence of borate-arsenate buffers, the enol-borate complex giving a characteristic absorption maximum in the region of 300 mμ. (Lin, Pitt, Civen and Knox, 1958). It has now been shown that the corresponding O-sulphate ester behaves in a similar fashion, the enol-borate spectrum showing a maximum at 302 mμ. either in simple aqueous media or in the presence of normal rat urine. The same absorption maximum was obtained with the urine of rats which had received tyrosine-O-S^{35}-sulphate.

A partially-purified preparation of the urinary metabolite was obtained, using rigorously controlled experimental conditions, by passing the urine through a Dowex-50 column (H^+form) and subjecting the first radioactive eluate (see Dodgson et al. 1961) to paper chromatography. The metabolite was eluted from the paper and showed maximum absorption at 302 mμ. in the presence of borate-arsenate buffer.

Further studies showed that the metabolite could undergo spontaneous conversion to the O-S^{35}-sulphate esters of p-hydroxyphenylacetic

acid or p-hydroxybenzaldehyde. Disappearance of the metabolite and appearance of products was followed spectrophotometrically and by paper chromatographic and electrophoresis experiments. Authentic samples of the sulphate esters of p-hydroxyphenylacetic acid and p-hydroxybenzaldehyde were used as reference compounds.

It is now clear that tyrosine-O-sulphate is metabolised in the rat to yield the O-sulphate esters of p-hydroxyphenylpyruvic acid and p-hydroxyphenylacetic acid. It is also clear however, that the latter may arise from the former by non-enzymic means.

We are grateful to the Medical Research Council for financial support.

REFERENCES.

Dodgson, K. S., Powell, G. M., Rose, F. A., and Tudball, N. Biochem. J., 79, 209 (1961).

Lin, E. C. C., Pitt, B. M., Civen, M., and Knox, W. E., J. Biol. Chem., 233, 668 (1958).