OBSERVATIONS ON THE METABOLISM OF TYROSINE-O-SULPHATE

K. S. Dodgson, Gillian M. Powell, and N. Tudball

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

Received January 28, 1960

Since the initial discovery of the presence of tyrosine-O-sulphate in bovine fibrinogen (Bettelheim, 1954) a number of unexpected properties of the ester have been noted. Thus several workers (see Dodgson, 1959) have shown that it cannot be synthesized from free tyrosine by the normal 3'-phosphoadenosine-5'-phosphosulphate (PAPS)-phenolsulphokinase route. Moreover, the ester is not desulphated to any significant extent, in vitro, by any of the three arylsulphatases which are present in mammalian livers (Dodgson, Rose, and Tudball, 1959). Finally, attempts by several workers (e.g., Grimes, 1959) to detect tyrosine-O-sulphate in the urine of rabbits, rats, guinea-pigs, and mice have been unsuccessful, although Tallan et al. (1955) have shown that relatively large amounts of the ester are present in normal human urine.

In order to throw further light on the significance of tyrosine-O-sulphate in the mammalian organism, a study of the metabolic fate of the ester in rats has been undertaken. Tyrosine-O-S^{35}-sulphate was prepared by treating tyrosine with S^{35}-sulphuric acid according to the method of Dodgson, Rose, and Tudball (1959). Several different preparations have been used during the course of the work and these varied in activity from 3,000 counts/min./μ mole to 40,000 counts/min./μ mole, when measured as an infinitely thick plate of $BaS^{35}O_4$, following the hydrolysis of the ester with N HCl.

Male and female mature hooded rats were injected intraperitoneally with the ester. The urines and feces were collected separately for 24 hr. and examined quantitatively for S^{35} by the methods described by Dodgson and Tudball (1960). In a few cases male rats were given terramycin (total dose of 300 mg.) and sulphasuxidine (total dose of 1.5 g.), per os, over a period of 70 hr. prior to the administration of tyrosine-O-S^{35}-sulphate.

Examination of the feces of these animals showed that this treatment resulted in a 900-fold reduction in the number of viable microorganisms. Table 1 summarizes the results obtained.

Table 1

Distribution of S^{35} in Urine and Feces of Rats 24 hr. After Intraperitoneal Injection of Tyrosine-O-S^{35}-Sulphate. [a]

Dose µ moles	Sex	No. of animals	Distribution of S^{35} in urine		S^{35} content of feces
			Inorganic sulphate	Ester sulphate	
15.1	M	2	5.3	79.7	1.4
15.1	F	2	3.7	83.9	1.0
3.7	M	3	3.4	78.9	3.3
14.1	M [b]	3	2.0	88.7	5.6

[a] Results are expressed as percentages of the S^{35} injected.
[b] Animals which had received terramycin and sulphasuxidine.

Clearly tyrosine-O-S^{35}-sulphate is not desulphated to any appreciable extent in vivo, irrespective of the state of the intestinal flora. These findings are in accord with the in vitro enzyme studies previously reported by Dodgson, Rose, and Tudball (1959).

At first sight the results would suggest that tyrosine-O-sulphate is metabolically inert. However, when the urine of rats receiving tyrosine-O-S^{35}-sulphate was subjected to descending paper (Whatman no. 1) chromatography using n-butanol-acetic acid-water (50:12:25), tert-butanol-formic acid-water (8:3:4) or n-propanol-ammonia (25%)-water (6:3:1), radioautograms showed that the bulk of the S^{35} was associated with two distinct spots. The chromatographic mobilities of these spots were different from that of tyrosine-O-S^{35}-sulphate which was present in traces only. The possibility that the two main radioactive spots were chromatographic artifacts was eliminated by various control experiments. Similar results were obtained with the urines of rats which had received terramycin and sulphasuxidine in addition to tyrosine-O-S^{35}-sulphate.

The apparent stability of the ester sulphate linkage of tyrosine-O-sulphate to enzymic hydrolysis, together with the fact that there is nothing in the molecular configuration of this ester to suggest that the sulphate group could be transferred, via 3'-phosphoadenosine-5'-phosphate, to other compounds (cf. the sulphate group of p-nitrophenyl sulphate; Gregory

and Lipmann, 1957), points strongly to the fact that the two main radioactive spots on the chromatograms represent two principal metabolites. Experiments aimed at identifying these two metabolites are at present in progress.

These results probably explain why various workers have previously failed to detect tyrosine-O-sulphate in rate urine.

One of us (G. M. P.) is grateful to the Medical Research Council for a Research Assistantship.

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