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Identification in Urine of a Low-Molecular-Weight Highly Polar Glycopeptide Containing Cysteinyl-galactose

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Several distinct types of glycopeptide linkages are known. These include the β -glycosidic bond of *N*-acetylglucosamine to the amide group of asparagine (Marshall & Neuberger, 1964), the *O*-glycosidic link of serine or threonine (Anderson *et al.* 1964) and the β -glycosidic linkage between galactose and the hydroxyl group of hydroxylysine (Butler & Cunningham, 1966). To date no linkage has been described in which the thiol group of cysteine is involved, although complex glycosides of 1-thio-D-glucose are common in mustard oils (Horton & Hutson, 1963).

We have recently isolated, from normal human urine, an octapeptide in which two galactose residues are *S*-glycosidically linked to cysteine.

Urine was concentrated 300-fold by a method designed to protect labile groups (Weiss & Steven, 1968; C. J. Lote & J. B. Weiss, unpublished work), and applied to a Sephadex G-15 column. The fraction corresponding to a 900–1000 molecular weight was further analysed on a split-stream peptide analyser. Of the 36 peptides separated one appeared to have a covalently bonded sugar. Galactose was the only sugar detected in this peptide by g.l.c. For each 2mol of galactose amino acid analysis revealed 1mol each of aspartic acid, serine, cysteine, glutamic acid, glycine and alanine and 2mol of histidine. Sequential analysis by the dansyl-Edman technique (Beale, 1969) indicated the following structure:

Cys-Glu-His-Ser-His-Asp-Gly-Ala

Alkaline borohydride reduction indicated that serine was not involved in the glycopeptide bond.

Prolonged digestion of the peptide with leucine aminopeptidase (EC 3.4.1.1) at pH 8.4 released a single component, which on mild acid hydrolysis was shown to contain only cysteine and galactose. No galactose was detected in the remaining portion of the peptide.

We conclude that the galactose is linked glycosidically to the sulphur atom of cysteine. A thiazolidine-type structure is excluded by the availability of the amino group of cysteine for dansylation and by the lability of the bond to mild acid hydrolysis. In view of the extreme polarity of the peptide adjacent to the thioglycoside its function as a binding or carrier site cannot be excluded, and we are currently investigating the possibility that this peptide may be part of a membrane glucose-carrier system.

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A Sensitive Method for the Detection of Metabolites of Dianabol in Urine in Man

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The mass-spectrometric technique of single-ion monitoring has been investigated as a rapid, sensitive and quantitative method for the detection of major human metabolites of the anabolic drug Dianabol (methandienone; 17 β -hydroxy-17 α -methylandrostan-1,4-dien-3-one).

Single-ion monitoring is the monitoring of a single preselected mass number by mass spectrometry so that a response is obtained from any compound giving a fragment of that mass value. Detection of a characteristic ion at the correct retention time for a particular compound affords a good basis for identification.

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The substitution at C-17 in the trimethylsilyl derivative of 6 β -hydroxy-Dianabol, the major metabolite (Rongone & Segaloff, 1963), is reflected in the formation of an intense ion at m/e 143 originating from a cleavage of the C-13-C-17 and C-14-C-15 bonds accompanied by hydrogen rearrangement.

A sample of urine from a 24 h collection after oral administration of the drug was extracted with chloroform-ethyl acetate, and the extract, after solvent removal, was treated with bis(trimethylsilyl)acetamide and chlorotrimethylsilane. The trimethylsilylated mixture was subjected to g.l.c. and the effluent passed into the mass spectrometer tuned to detect m/e 143. The presence of 6 β -hydroxy-Dianabol (which is excreted almost solely in the free form) was indicated by a response at the correct retention value.

By this method 1-2ng of the metabolite was easily detected. The amount of the metabolite present in the extract was measured by comparison of the m/e 143 response from a sample of the trimethylsilylated extract and from a weighed standard of authentic material similarly trimethylsilylated. The equivalent of 1.5 ml of urine, from a 24 h collection after a 50 mg dose of drug, contained 2 μ g of the major metabolite.

The single-ion monitoring technique was extended to other metabolites of Dianabol. These included in the 'free' fraction a compound with an additional double bond, one with a reduced double bond (cf. Gardi & Galletti, 1968), two isomers of the major metabolite and an isomer of Dianabol. The last may be the 17 α -hydroxy compound reported by Macdonald, Sykes, Adhikary & Harkness (1971). Of the two principal metabolites in the 'conjugated' fraction one appears to contain a 6-hydroxy- Δ^4 -3-one system and the other a 3-hydroxy analogue of this.

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The Conversion of Cyclamate into Cyclohexylamine by Gut Bacteria

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There is evidence to suggest that the gut bacteria in man and other species acquire the ability to convert cyclamate (*N*-cyclohexylsulphamate) into cyclohexylamine when cyclamate is administered daily (Renwick & Williams, 1969, 1970). Clostridia isolated from dog gut contents can carry out this conversion to a slight degree (Golberg, Parekh, Patti & Soike, 1969).

The numbers of bacteria of the various genera occurring in human faeces were not altered by daily cyclamate administration, but in the rat the numbers of clostridia in the faeces were significantly increased (from $10-10^3$ /g to 10^5-10^9 /g) by cyclamate feeding.

Strains of bacteria from the faeces of a human convertor of cyclamate into cyclohexylamine and from the faeces of rat and rabbit convertors were isolated by the procedure of Drasar (1967) (except enterococci, which were isolated by the procedure of Schaedler, Dubos & Costello, 1965). The isolated colonies (five of each) of enterobacteria, enterococci, clostridia, bacteroides and bifidobacteria were transferred to tryptone-yeast extract broth (1 ml) and incubated under N_2 with [^{14}C]cyclamate (50 μ g; 0.3 μ Ci) for 60 h. The [^{14}C]cyclohexylamine formed was determined by solvent extraction combined with isotope dilution, a method that could detect a conversion of 0.05%. Five colonies of enterococci from human faeces, two colonies of clostridia from rat faeces and two colonies of clostridia and one of enterobacteria from rabbit faeces showed significant metabolism of cyclamate to cyclohexylamine (0.1-0.5% of the added cyclamate).

Faecal homogenates (10%) in tryptone-yeast extract broth were incubated under N_2 with 0-1% cyclamate for 48 h. The bacteria were then centrifuged, resuspended in broth (1 ml) and incubated under N_2 for 60 h with [^{14}C]cyclamate (50 μ g) as before. The bacterial preparation from rat faeces after preincubation with cyclamate (1%) converted more than 5 times as much cyclamate into cyclohexylamine as did the preparation preincubated without cyclamate, whereas the activity of preparations from human or rabbit faeces was decreased by about 90% by such treatment. On the other hand preincubation with cyclohexylamine (0.5%) in a similar manner suppressed the activity of the rat faecal preparation, but not that from human faeces. These observations suggest that the