The substitution at C-17 in the trimethylsilyl derivative of 6β-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 24h 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 6β-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 1.5ml of urine, from a 24h collection after a 50mg dose of drug, contained 2μg 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 17α-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 (1ml) and incubated under N_2 with [^{14}C]cyclamate (50μg; 0.3μCi) for 60h. 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 48h. The bacteria were then centrifuged, resuspended in broth (1ml) and incubated under N_2 for 60h with [^{14}C]cyclamate (50μg) 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

gut bacteria responsible for the conversion of cyclamate into cyclohexylamine in rat faeces are different from those responsible for the conversion in human or rabbit faeces. It appears that the organisms responsible are clostridia in rat faeces, enterococci in human faeces and enterobacteria in rabbit faeces. Clostridia are uncommon in rabbit faeces (Smith, 1965).

All the colonies of enterococci from the faeces of the human convertor metabolized cyclamate to cyclohexylamine. Relevant to this are the observations that the numbers of enterococci in faeces from Japanese subjects are considerably higher (10^8/g) than in faeces from British subjects (10^5/g) (Hill *et al.* 1971) and that all Japanese subjects examined excreted cyclohexylamine after oral doses of cyclamate (Kojima & Ichibagase, 1966).

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The Metabolism of Methamphetamine in the Rat and Guinea Pig

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Methamphetamine (2-methylamino-1-phenylpropane) is a dangerous drug from the point of view of drug-dependence, but little is known about its metabolism. It is partly excreted as such and as amphetamine in man (Cartoni & di Stefano, 1963; Beckett & Rowland, 1965), and largely demethylated to amphetamine in the dog (Axelrod, 1954).

[^{14}C]Methamphetamine [(±)-2-methylamino-1-phenyl[1-^{14}C]propane] was synthesized and its fate in rats and guinea pigs examined (dose 45mg/kg). A marked species difference was found. In the rat the ^{14}C after oral administration was excreted mainly in the urine (about 80% in 3 days) with little (3%) in the faeces, but in the guinea pig about 70% appeared in the urine and 18% in the faeces after intraperitoneal injection. The main reaction in the rat was aromatic hydroxylation, the metabolites being 4-hydroxy-*N*-methylamphetamine (31%), 4-hydroxynorephedrine (16%) and 4-hydroxyamphetamine (6%), which were excreted as glucuronic acid conjugates. Unchanged methamphetamine (11% of the dose) and amphetamine (3%) were also found in the urine. In the guinea pig no aromatic hydroxylation occurred, for the main metabolite was benzoic acid (32%; mainly conjugated) followed in order by norephedrine (20%), amphetamine (13%) and methamphetamine (4%). These metabolites were found in the urine and faeces except benzoic acid and its conjugates, which were found only in the urine.

The main metabolic reaction of methamphetamine in the rat was thus aromatic hydroxylation and in the guinea pig *N*-demethylation, but both species were able to hydroxylate the α-carbon atom of the side chain since appreciable amounts of norephedrine (2-amino-1-phenylpropan-1-ol) were excreted by the guinea pig and of 4-hydroxynorephedrine by the rat. It has been suggested (Fischer, Horst & Kopin, 1965; Thoenen, Hürliman, Gey & Haefely, 1966) that norephedrine and 4-hydroxynorephedrine may act as false transmitters at nerve endings and may be involved in the undesirable effects of the chronic intake of the amphetamines. An earlier study on amphetamine (Dring, Smith & Williams, 1970) showed that little if any 4-hydroxynorephedrine was formed from amphetamine in the rat and norephedrine was not detected as a metabolite of amphetamine in the guinea pig. Kosman & Unna (1968) have reported that the continuous administration of methamphetamine to the rat and guinea pig causes depression, but with amphetamine given similarly the animals become excited. These latter observations may be correlated with the significant formation of norephedrine or its hydroxy derivative from methamphetamine but not from amphetamine.

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