Creatinine Metabolism by *Clostridium welchii* Isolated from Human Faeces

For many years it has been assumed that creatinine is metabolically inert in humans[1,2]. However, JONES[3] demonstrated that creatinine degradation could be induced in rat gut flora by feeding creatinine and that several labelled metabolites were produced when methyl- or carbonyl-labelled creatinine was incubated with colonic contents from these animals. Recently, JONES and BURNETT[4] showed that between 16 and 66% of creatinine formed endogenously in persons with decreased renal function is metabolized and isotope studies have confirmed creatinine breakdown to creatine, N-methyl hydantoin, sarcosine, methylamine and glycolate[5] in human and rat gut preparations.

Several strains of bacteria have been reported to metabolize creatinine including *Corynebacterium ureafaciens*[6,7], *Pseudomonas eisenbergii* and *ovalis*[8], and *Pseudomonas stutzeri*[9] isolated from soil and *Clostridium paraputrifactum*[10] isolated from sewage sludge. We have therefore examined faeces to establish that specific organisms can be present in the large bowel of humans and be capable of metabolizing creatinine. A *Clostridium welchii* has been isolated[11] which can deaminate creatinine to N-methyl hydantoin under anaerobic conditions. The enzymic reaction was inducible since prior growth of the organism for 72 h in nutrient broth containing 20 mg 100 ml added creatinine increased the incorporation of carbonyl ^{14}C label into N-methyl hydantoin from 16.4% to 72.8%. No methylamine, methyl guanidine, labelled sarcosine or labelled carbon dioxide was detected.

Techniques included filtration of the incubated broth through 0.22 µm millipore filter and then through an Amicon UM2 Ultrafilter (MW exclusion 1000). Products were identified using cellulose thin-layer chromatography with reference standards and standard staining techniques, (solvent system butanol: glacial acetic acid: acetone: water, 35:10:35:20). Radio isotope incorporation was measured by using standard elution and scintillation counting techniques with 84% of the counts accounted for. Decrease in creatinine concentration and the absence of newly formed methylamine or sarcosine was confirmed by ion exchange chromatography. No label was detected in a hyamine carbon-dioxide trap.

The organism was a Gram positive anaerobic rod showing typical *Clostridium welchii* morphology; no spores were seen. It produced acid from glucose, lactose and sucrose, was indole negative and lecithinase formation was inhibited by *Clostridium welchii* Type A antisera (Wellcome Reagents Ltd.).

Preliminary results from experiments involving the anaerobic incubation of diluted stool from a healthy individual indicate that approximately 10% of the added counts are in N-methyl hydantoin 3–6 h after adding ^{14}C carbonyl labelled creatinine. Most of the remainder probably passes through a pathway generating methylamine within 3 h. The production of N-methyl hydantoin, presumably by *Clostridium* organisms, is therefore probably a significant but minor and relatively slow route for creatinine metabolism in vivo. This metabolism of creatinine can be abolished by high-speed centrifugation of the stool specimen.

These observations indicate that specific organisms are present in the human gut which can influence the metabolism of creatinine and that this substance can no longer be regarded as inert in humans; careful study of its metabolism therefore becomes relevant to the understanding of disordered biochemistry accompanying renal insufficiency.

Summary. A *Clostridium welchii* has been isolated from human faeces which can deaminate creatinine to N-methyl hydantoin. Evidence suggests the reaction is inducible since the rate of conversion is increased by growth of the organism in creatinine-rich media.

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Chromosome Complement and Male Haploidy of *Asplanchna priodonta* Gosse 1850 (Rotatoria)

Information on the numbers of chromosomes and on the chromosome-cycle of Rotifers is very scarce and often conflicting[1,2], both because cells and chromosomes are small and because the methods employed so far for caryological research have been limited to tissue sections and haematoxylin staining, and are scarcely suitable for accurate counting.

Most work on the subject was carried out in the twenties[3–7], and also the more recent research by HSU[8,9] did not employ the present squash-method techniques and, moreover, was restricted to Bdelloid species, which do not produce males.

Asplanchna priodonta, which is more suited to caryological investigation than most other species of Rotifers investigated so far, has been studied by STORCH[6], but data are dubious: 8 chromosomes in the male and 16 in the female embryos, but STORCH's drawings show much higher numbers and dot-like chromosomes, which do not

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