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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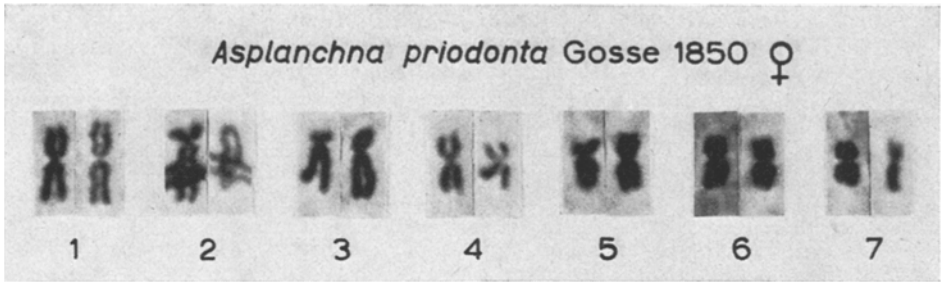

Fig. 1. Karyotype of *Asplanchna priodonta* ♀, $2n = 14$.

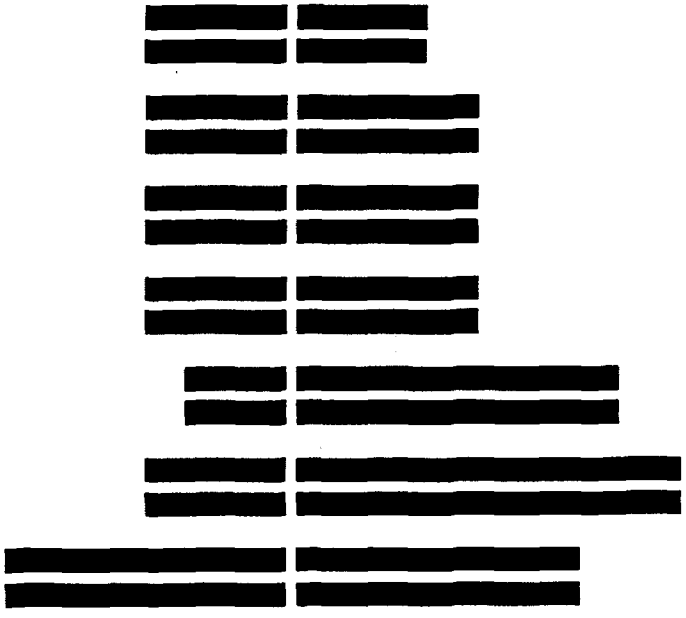

Fig. 2. Idiogram of *Asplanchna priodonta* ♀.

correspond at all to the metacentric and submetacentric chromosomes that can be observed with other techniques.

TAUSON[4,5] asserted in 2 extensive and detailed papers that males are diploid, and such interpretation could not be totally dismissed, although it was not confirmed by other authors.

The chromosome-cycle of heterogonic Rotifers appears therefore to be extremely uncertain on the basis of the existing literature, and the chromosome sets must be reinvestigated with adequate techniques.

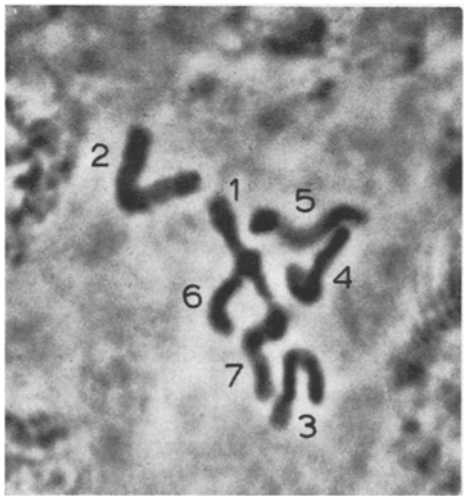

Fig. 3. Metaphase karyotype of *Asplanchna priodonta* ♂, $n = 7$.

Asplanchna priodonta was collected in a small lake near Chiaverano (Turin). Caryological investigations were first carried out in vivo with a phase-contrast microscope and eggs were subsequently extracted from the mother's body, treated 5 min with a hypotonic medium and stained with acetic-orcein overnight. Alternatively, the eggs were fixed and stained under the microscope, with or without a preliminary treatment in a colchicine solution (0.05%, 20 min). Afterwards the eggs were usually broken on a slide, in order to separate the cells which contained mitoses, and then squashed.

Chromosome sets were identified on metaphases, from both male and female embryos. The female diploid karyotype was first established (Figure 1). It consists of 14 chromosomes: 1 pair of large metacentric, 5 pairs of submetacentric and 1 pair of small metacentric ones. If we assume that the length of the largest pair is 100, we obtain the idiogram of Figure 2. In eggs with a high number of cells, there were also some tetraploid metaphases ($4n$–28), possibly pertaining to the cell-lines that will originate polyploid organs such as vitellarium[10] or possibly gastric glands.

The male karyotype, which was examined in cells at various stages of the embryonic development (as far as

[10] C. W. BIRKY, R. ZITO-BIGNAMI, S. M. BENTFELD, Biol. Bull. *133*, 502 (1967).

60 blastomeres), is composed of 7 chromosomes which are clearly recognizable and comparable with the chromosomes which are found in the female embryos. Only in embryos which pertained to very later stages, a few diploid metaphases were observed. It is therefore possible to establish, by using quite simple techniques, that, at least in one heterogonic Rotifer, male somatic cells bear a haploid chromosome set.

The blastomeres of male embryos are all haploid as far as the 5th–6th cleavage-division at least, and only afterwards can one find a few diploid metaphases, which presumably correspond to the tetraploid sets that are found in female embryos.

TAUSON's results concerning diploidy in males of *Asplanchna intermedia* are possibly based on artefacts. As regards the different numbers (n-8, $2n$-16) of STORCH[6], we can either assume that the population observed by STORCH had a different number of chromosomes, or that the technique employed in 1924 led to faulty countings.

Riassunto. Mediante l'impiego di tecniche appropriate è stato possibile definire il corredo cromosomico di *Asplanchna priodonta* Gosse 1850 (Rotatoria) e dimostrare almeno per una specie di Rotiferi, che i maschi sono aploidi.

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Giemsa Banding and Heterochromatin Distribution in *Ornithogalum*

In recent years, linear differentiation of chromosomes, by staining with fluorochrome compounds or Giemsa, have facilitated the qualitative study of heterochromatin distribution in the genome of various organisms[1–4]. Further, the heterochromatic regions have been shown to be the preferential sites for radiation and chemically induced chromosomal aberrations[5–8]. In the present paper, we report the distribution of heterochromatin on the chromosomes of a Liliaceous plant, *Ornithogalum virens*. This plant has fairly large chromosomes but few in number ($2n = 6$); it therefore seems to be ideal material for studying the relationship between heterochromatin distribution and the localization of induced breaks in the chromosomes.

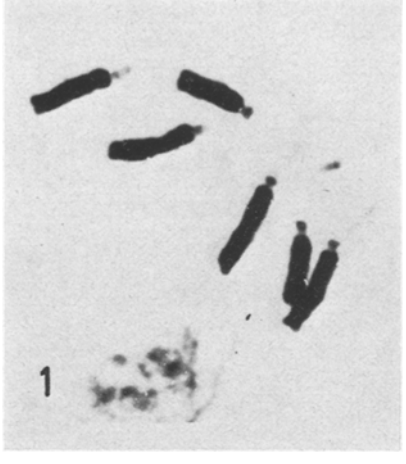

Fig. 1. Karyotype of *Ornithogalum virens* (Feulgen stained). × 2,000.

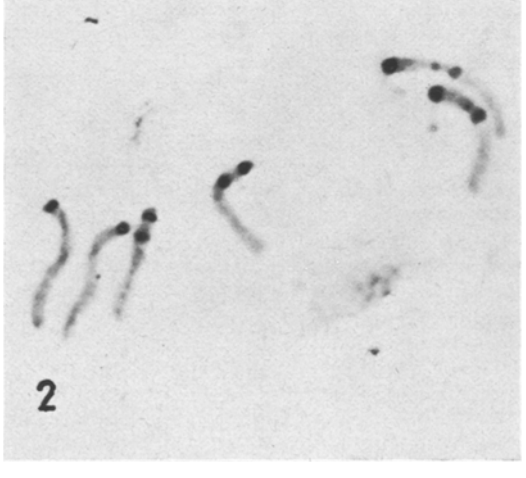

Fig. 2. Giemsa stained mitotic chromosomes showing the banding pattern. × 2,000.

Material and methods. The procedure adopted for Giemsa staining is similar to that described by SARMA and NATARAJAN[9] and SCHWEIZER[10]. Chromosome preparations were made from the root meristem of germinating seeds or sprouting bulbs of *Ornithogalum*. Root tips of about 1–2 cm length were pretreated with saturated aqueous solution of α-bromonaphthelene for 30 min and fixed in 3:1 alcohol-acetic acid mixture. Fixed root tips were hydrolyzed for 2–5 min at 60 °C in 0.1 *N* HCl and squashed in 45% acetic acid on gelatinized slides. The cover-slips were removed by freezing the slide in liquid nitrogen. The slides were washed in 90% and in absolute ethanol, air dried and treated with saturated aqueous solution of barium hydroxide at 40–50 °C for 6 min for alkali denaturation. Alkali treatment was terminated by 2 washes for 2 min each in warm distilled water followed by washing in running water for 30 min. The slides were then incubated in 2 × SSC (0.3 *M* NaCl + 0.03 *M* tri-sodium citrate, pH 7.0) at 60 °C for 2 h. The preparations were stained in Giemsa solution (2 ml of Giemsa stock solution, prepared from E. Merck Giemsa powder, in 100 ml of Sörensens phosphate buffer, pH 6.8) for 1 h at room temperature. Excess stain was removed by repeated washing in distilled water till a clear differential staining of chromosomes was achieved. Preparations were made permanent by mounting the air-dried slides in euparol.

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