# Non-utilisation of 2-methyladenine and 4:6-diamino-5-formamidopyrimidine for nucleic acid synthesis by the rat

The natural occurrence of 2-methyladenine in the vitamin $B_{12}$ fields[1,2] and the occurrence of 6-methylaminopurine in some bacterial desoxyribonucleic acids[3,4] suggested that the former compound might be incorporated into the nucleic acids of the rat. A similar experiment using 4:6-diamino-5-formamidopyrimidine was designed to see whether the rat could utilise this compound which is closely related to adenine. Although the experiments failed to show incorporation into the polynucleotides or the acid soluble nucleotides, the results are reported.

*Methods.* 4:6-Diamino-5-$^{14}$C-formamidopyrimidine was prepared by the method of CLARK AND KALCKAR[5].

2-Methyl-8-$^{14}$C-adenine was prepared from 2-methyl-4:5:6-tri-aminopyrimidine[6] also by CLARK AND KALCKAR's procedure. In trial runs, consistent yields (80%) of the colourless crystalline 2-methyl-4:6-diamino-5-$^{14}$C-formamidopyrimidine hydrochloride were obtained, m.p. 267° (dec.). The compound was recrystallised from water for analysis. (Found—after drying *in vacuo* at room temperature—C,32.8; H,4.6; N,31.5%. $C_6H_9N_5O.1\frac{1}{2}$ HCl requires C,32.5; H,4.8; N,31.6%). In 0.01 $N$ HCl, maximum absorption was at 265 m$\mu$, $\varepsilon M$ = 12,600. The material ran as a single spot on paper in *iso*propanol/ammonia, and more than 99% of the radioactivity was associated with the ultraviolet absorbing spot. The formamido compound was heated at 200° under nitrogen for 80 minutes in N-formylmorpholine and the solid product which separated on cooling dissolved in hot 6$N$ HCl, charcoaled, filtered and the solution neutralised with ammonia. The colourless precipitate was chilled, filtered off, washed with water, alcohol and dried. This method of preparation is more convenient than that previously described[7]. The chemical yield was quantitative, but as expected[8,9], exchange of the formyl group with formyl morpholine reduced the specific activity of the purine to 60% of that of the formamidopyrimidine. The compound, which absorbed maximally at 265–266 m$\mu$ in 0.01 $N$ HCl, moved as a discrete spot on paper in *iso*propanol/HCl[10] with a slightly greater $R_F$ than adenine. More than 98% of the radioactivity was associated with the ultraviolet absorbing spot.

The compounds were separately injected into white male rats at a level of 0.2 m$M$/kg/day for 3 days. Total urine was collected. 24 hours after the last injection, the rats (2 in each case) were sacrificed and the internal organs investigated. Acid soluble nucleotides were extracted with cold 0.6 $N$ perchloric acid[11] and barium ribonucleotides and desoxyribonucleic acid[12] isolated from the defatted tissue residue. Counting procedures have been described previously[9].

*Results.* In 2 experiments with 2-methyl-8-$^{14}$C-adenine, 66% and 72% of the injected activity was excreted in the urine. Only traces in one experiment and none in the other were associated with the acid-soluble nucleotides and with the nucleic acids. From the urine, 2 ultraviolet absorbing radioactive components could be detected as follows. The diluted urine, brought to pH 7, was loaded on to a column of Dowex-50 in the hydrogen form. After washing with 5 $M$ formic acid, activity was eluted with 2 $N$ ammonia, the solution evaporated and run on paper in *iso*propanol–HCl. Two ultraviolet absorbing spots separated with no detectable radioactivity on the rest of the paper. One spot had the same $R_F$ and absorption maximum as 2-methyladenine while the other moved faster and absorbed non-specifically. No further investigations were carried out.

When 4:5-diamino-5-$^{14}$C-formamidopyrimidine was injected, 43% of the activity was excreted in the urine. Traces of activity were associated with the desoxyribonucleic acid guanine and with the total acid soluble extract, but no more than could reasonably be expected if hydrolysis of the formamido group to free formate had occurred. The activity in the urine was not associated with allantoin, since pure allantoin, recrystallised from water containing some of the urine, was recovered free of radioactivity.

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*Department of Radiotherapeutics, University of Cambridge (England)* D. H. MARRIAN

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