In vitro synthesis of purines by rat intestinal mucosa*

In vitro studies of purine and nucleic acid synthesis from precursors such as glycine, formate and carbon dioxide have been carried out principally with pigeon liver preparations. In the small number of such investigations involving mammalian tissues, regenerating liver[1], bone marrow[2,3] and tumor cells[4] have been utilized. Certain characteristics of rat intestinal mucosa suggested that this tissue would be suitable for studies of purine biosynthesis, e.g., the very high rate of cell division[5], the high uptake of purine precursors in the intact animal[6,7,8] and the high metabolic activity *in vitro*[9]. Further, the mucosa can be prepared with little damage as a suspension in which the tissue particles are sufficiently small so that diffusion is not limiting. This report describes the preparation of such suspensions and some observations of purine biosynthesis in the acid-soluble nucleotides and the mixed nucleic acid fractions of these preparations.

A male Wistar rat was killed by a blow on the head. The small intestine was removed immediately, chilled and cut into 10 cm segments. These were flushed free of contents with the ice-cold medium described below and, after being split open, were applied to a cold glass slab, mucosa upwards. The mucosa was then removed with a microscope slide, as described by DICKENS[9], suspended in 4–5 volumes of ice-cold medium and the strips of mucosa freed by gentle mixing. The tissue was washed several times by centrifuging at low speed followed by resuspension in the cold medium. The volume was made up to approximately 25 ml and the mucosa strips converted to a fairly homogeneous suspension by gently and repeatedly drawing the preparation into and expelling it from a syringe without a needle. The material was then strained through gauze. Throughout all operations the preparation was kept cold. The small intestine from a 200 g rat provided a suspension containing 25–45 mg dry weight of tissue per 3.0 ml portion.

Krebs-Ringer phosphate or bicarbonate solutions containing aureomycin (10 ppm) and glucose (2 mg/ml) were used as the suspending medium. The mucosa suspensions respired actively at a gradually declining rate for 3–4 hours with initial Q_{O_2}'s of 6–8. Bacterial counts**, which did not exceed $4–5 \cdot 10^6$ bacteria per ml at the end of 3 hours incubation, indicated that bacterial contamination in these preparations was not great and would not affect the results significantly.

The suspension of mucosa was added in 3.0 ml portions to Warburg cups which contained 2.25 μM of sodium formate-^{14}C ($8.1 \cdot 10^6$ cpm) and during incubation at 37° C, oxygen consumption was routinely followed. After incubation, the tissue was recovered by centrifugation of the cup contents and the purines of the acid-soluble and nucleic acid fractions obtained by the perchloric acid extraction method described by LE PAGE[4].

The purines of each fraction were separated by paper chromatography using *iso*propanol-HCl[10]. The specific activities of the purines of the nucleic acid fraction were unaltered by re-chromatography in butanol–ethanol–water[11], but it was found necessary to rechromatograph the acid-soluble purines in this solvent.

The purine areas on the chromatograms were located with U.V. light, and for analysis a disc, 26 mm in diameter, was punched out of each area. After determining the radioactivity of the discs, they were extracted with 0.1 M HCl and the purine content of the extracts measured in the usual way by U.V. absorption. The radioactivity determinations were corrected for absorption by the paper discs with empirically determined factors.

The uptake of formate in the purines was considered to be a measure of *de novo* synthesis in this system. That radioactive formate was readily incorporated into the purines of both the acid-soluble nucleotides and nucleic acids is shown in the data of Table I which is typical of a

TABLE I

PURINE SYNTHESIS BY REPLICATE SAMPLES OF AN INTESTINAL MUCOSA SUSPENSION*

Vessel Number	*Specific activity in cpm/mg · 10⁻⁴*			
	*Acid-soluble fraction***		*Nucleic acid fraction****	
	adenine	*guanine*	*adenine*	*guanine*
1	50.7	3.7	0.41	0.16
2	53.7	3.3	0.40	0.15
3	49.9	3.5	0.35	0.14
4	42.0	3.1	0.34	0.13
5	46.5	2.4	0.40	0.15

* Incubated at 37° C for 3 h with 2.25 μM sodium formate-^{14}C ($8.1 \cdot 10^6$ cpm).
** Chromatographed in *iso*propanol-HCl only.
*** Average of duplicate determinations.

number of such experiments. The variation in the replicates indicates that the present method of preparation does not produce a completely homogeneous suspension. Aureomycin was shown to have no effect on the rate of incorporation of formate by the purines.

Glycine-1-^{14}C is also incorporated into the purines of this system but at about half the rate of formate. The addition of glycine, glycinamide and glutamine, singly or in combination, appeared to have no significant effect on the incorporation of formate.

The data of Table I and the rate study shown in Table II (in which a different mucosa preparation was used) indicate that the purines of the acid-soluble fraction are synthesized rapidly. This observation is in accord with reports of the early labelling of the nucleotide pool *in vivo*[12,13,14] and *in vitro* by purine precursors[1] and free purines[15]. Adenine was more active than guanine in both fractions, a finding in agreement with the observations of TOTTER[3] and ABRAMS[8] for nucleic acid purines derived from formate-^{14}C in their *in vitro* experiments with bone marrow. In contrast, LE PAGE[4] has reported that in mouse liver and mouse tumors glycine-2-^{14}C is incorporated to a greater extent in guanine.

TABLE II

RATE OF PURINE SYNTHESIS IN AN INTESTINAL MUCOSA SUSPENSION*

Time of incubation h	*Specific activity in cpm/mg · 10^{-4}*			
	Acid-soluble fraction		*Nucleic acid fraction*	
	adenine	*guanine*	*adenine*	*guanine*
0.5	1.2	0	trace	0
1.0	1.8	trace	trace	trace
2.0	6.5	0.7	0.16	0.07
3.0	13.6	1.7	0.29	0.12
4.0	17.6	1.9	0.33	0.22

* The values presented are averages of duplicate incubations made for each period. Each vessel contained 2.25 μM of sodium formate-^{14}C ($8.1 \cdot 10^6$ cpm).

It has been shown that rat intestinal mucosa, prepared in the form of a suspension, will synthesize acid-soluble and nucleic acid purines and appears to be a useful mammalian system for *in vitro* studies of the synthesis of nucleic acids and their components.

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