

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

MONITORING OF COLUMN EFFLUENTS FOR RADIOACTIVITY
BY CONTINUOUS LIQUID SCINTILLATION COUNTING

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I. INTRODUCTION

The molecular causes of the inborn errors of purine metabolism in man can be established by measurement of cellular levels of purine bases, purine nucleosides and purine nucleotides. Such measurements are conveniently done either by slow elution of a single ion exchange column (Anderson *et al.*, 1963; Horvath and Lipsky, 1967; Khym, 1975), high pressure ion exchange chromatography (Brown, 1970; Green *et al.*, 1966), reverse phase chromatography (Brown *et al.*, 1977; Hartwick and Brown, 1976) or gel filtration chromatography (Khym, 1976; Sweetman and Nyhan, 1969). The compounds eluted from the column are measured by highly sensitive UV detectors which can measure microgram quantities of compounds at relatively fast rates. The diagnosis of a molecular abnormality is, however, generally facilitated by using compounds labeled with radioactive tracers. In order to facilitate detection and quantitation of nonradioactive and radioactive compounds we have employed highly sensitive UV detection and liquid scintillation counting. In the case of slow elution this combination of detection worked quite well. However, in HPLC, measurement of radioactivity posed a difficult problem. Small amounts of radioactivity were eluted in very small volumes at a very fast rate. Thus measurement of isotope by collecting fractions for manual analysis or by passing the effluent through a flow cell utilizing low efficiency solid scintillators produced poor results.

We solved the problem of isotope measurement in the effluent for HPLC by mixing column effluent with liquid scintillation fluid and passing it through a hollow flow cell (Bakay, 1975a; Bakay, 1975b; Bakay, 1977). The quantitation of UV compounds and radioactivity was carried out simultaneously and was completed in 120 minutes.

II. MATERIALS AND METHODS

The major apparatus components were a gradient mixer which generates a linear gradient in which the concentration of tetraborate and pH decreased and concentrations of NH_4Cl increased (Fig. 1). The gradient was filtered through a 15 micron filter and was pumped by a 5000 psi Mini Pump (28 Rpm) through a 50 cm long, 2 mm i.d. stainless steel column packed with Aminex A25 ion exchange resin. The column was wrapped with electric heating tape and heated to 60°C . The effluent of the column was immediately passed through a UV detector which was connected to a Spectrophysics Integrator and connected

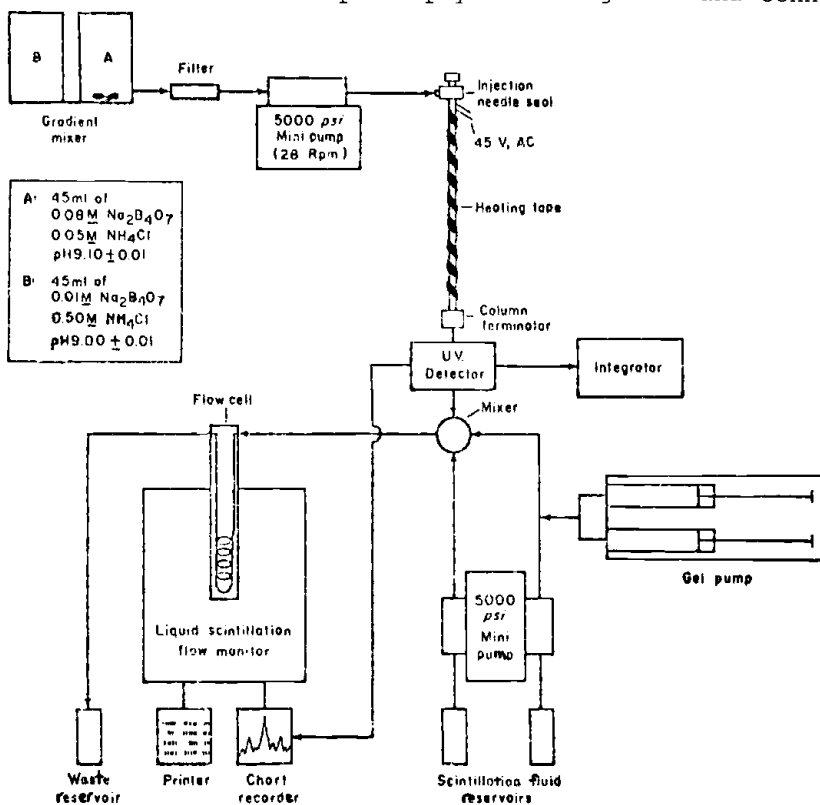


Fig. 1. Schematic illustrations of instrument connections.

to one channel of a chart recorder. After measurement of UV absorption the effluents were transported to a nonmechanical mixer where it was mixed with 2 vol. of scintillation fluid pumped by a duplex Mini Pump. The scintillation fluid pumped by one side of the Mini Pump was mixed with 30 segments (60 μ l each) per minute of 6% polyacrylamide gel delivered by the gel pump. Segments of gel separated the liquid stream and thus prevented the spreading of separated compounds. In principle, the gel segments functioned as a series of pistons which wiped the traces of passing samples off the walls of the tubing, and at the same time prevented spreading of the samples and maintained a discrete sample transport. The mixture of the column effluent, scintillation fluid and the gel was passed through a hollow tube flow cell mounted in a liquid scintillation flow monitor. The accumulated counts were documented at pre-set intervals by a printer. At the same time the counts per minute were recorded on the chart recorder with the UV profile.

III. RESULTS

Examples of the resolution of a standard mixture of radioactive and nonradioactive purine bases, nucleosides and nucleotides is illustrated (Fig. 2). The profile of nonradioactive components documented by UV absorbance is presented by a continuous curve. The profile of radioactive components is documented by steplike curves.

The identity of the eluted compounds is indicated at the top of the peaks. With the exception of the GR HX and XMP ATP pairs, other components in this profile were well separated. The peaks illustrated by the UV profile represented approximately 75 nmoles of each of the 16 standards. The peaks documented by the isotope counter represented 80-100 picomoles of radioactive standards. In this sample, radioactive X, XR and GMP were left out. Small amounts of radioactive impurities which were present in some of the standards, especially guanine, and eluted in an early peak.

In order to establish the molecular causes of abnormal purine metabolism in various hyperuricemic patients we incubated the skin fibroblasts of the patient with different ^{14}C precursors and used our chromatographic system to establish how these precursors were utilized by the cell. The cells grown in tissue culture were incubated with various radio-

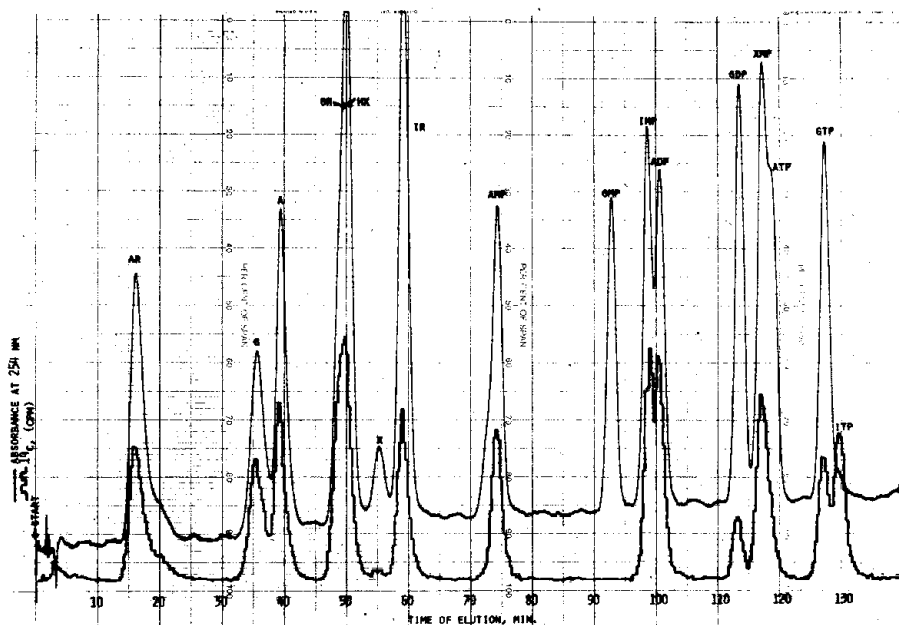


Fig. 2. Separation of artificial mixtures of nonradioactive and radioactive standards. Profiles of radioactive and nonradioactive standards recorded simultaneously by a two-pen chart recorder connected to the UV detector and liquid scintillation flow monitor. The analyzed sample contained 75 nmoles of each nonradioactive and 80-100 picomoles of radioactive standards.

active precursors and extracted with a small volume of perchloric acid. Fifty microliter aliquots were used for chromatographic analysis. As shown in Figure 3, extracts of normal skin fibroblasts incubated with ^{14}C -hypoxanthine, contained many components. However, there were only 13 identifiable radioactive purine metabolites. This analysis proved that under our incubation condition all known metabolic pathways of purine metabolism were operable.

As shown in Table I the analysis of acid soluble extracts from the cells of individuals who were overproducing uric acid revealed dramatic differences. The total amount of ^{14}C -hypoxanthine utilized by the cells of some patients was considerably different than in normal cells. As shown

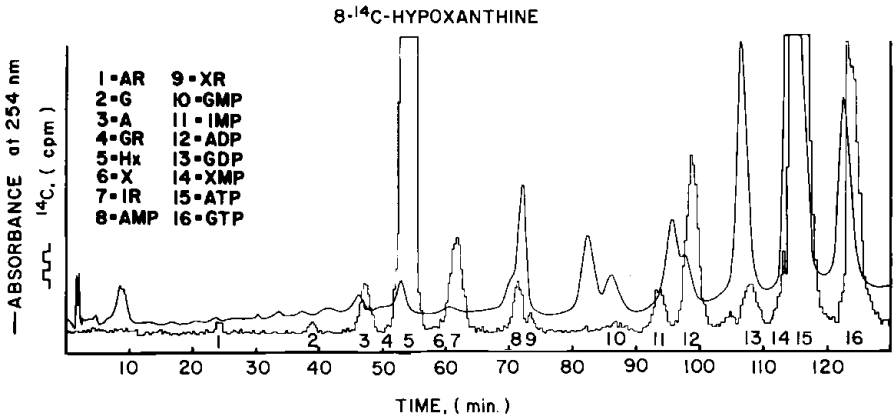


Fig. 3. Chromatographic profiles of purine metabolites extracted with acid from skin fibroblasts pulsed with ¹⁴C-hypoxanthine. 50 μ l aliquot of extract from 7.4×10^6 cells of a normal control.

in Table 1, cells of the patient Bu utilized 88% less of hypoxanthine than those of Ba, a normal control. Cells of patient S utilized about 3 times more ¹⁴C-hypoxanthine than normal cells. Furthermore, cells of each patient utilized the precursor in a different way. The normal Ba, converted the bulk of hypoxanthine to ATP and GTP. Cells of S produced large amounts of ATP, ADP, AMP and IR but in contrast they produced a very small amount of GTP. Cells of patient GG produced large amounts of AMP, ADP, GMP, GDP and IR and less than normal amounts of ATP and GTP. It is clear that molecular abnormalities in purine metabolism in these patients are distinctly different. This type of analysis helped to establish the exact molecular cause of abnormal purine metabolism in some of these and other patients. Most of these findings will appear soon in the literature.

Table 1. Incorporation of $8^{14}C$ hypoxanthine by skin fibroblasts of hyperuricemic patients with different clinical manifestations

Purine Meta bolite	NORMAL N=8 n=8				L.N. N=4 n=10				H. Chr. B. N=1 n=2				S.M. N=1 n=3			
	nM	UV	pM	^{14}C	nM	UV	pM	^{14}C	nM	UV	pM	^{14}C	nM	UV	pM	^{14}C
A	4.6		15.7		1.7		4.1		1.8		0		1.0		37.8	
AR	0.1		3.9		0.1		3.4		0		7.1		<0.1		1.7	
AMP	7.2		127.6		5.0		3.4		1.3		8.0		4.8		181.7	
ADP	14.1		238.9		12.4		6.2		7.3		22.3		11.3		764.4	
ATP	43.1		911.1		43.4		10.5		60.4		41.1		51.7		3382.2	
(HX)*	(3.0)				(2.7)				(6.3)				(2.9)			
IR	2.3		132.1		2.2		49.7		0.6		52.5		1.3		203.0	
IMP	0.1		17.2		3.5		2.9		0		0		4.8		34.9	
X	0		0		0		0		0		0		0		0	
XR	0		2.2		0		0		0		0		0		0	
XMP	0		7.8		0		0		0		0		0		0	
G	1.6		3.2		1.0		0		1.1		0		0.2		0	
GR	1.1		3.9		0.8		1.7		0.4		0.3		0.3		6.5	
GMP	5.7		21.7		6.1		1.0		3.7		3.2		3.5		21.2	
GDP	11.2		44.7		12.2		6.3		11.8		5.8		14.7		68.8	
GTP	8.9		122.9		10.7		3.4		11.7		15.0		6.7		139.6	
Total	100.0		1652.9		100.0		92.6		100.0		154.7		100.0		4841.9	

N = number of patients

n = number of determinations

nM = determined by analyzer, averages

pM ^{14}C = calculated from dpm using specific activity of precursor

* = mainly extracellular precursor carried over on the cell surface

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