Isolation of Acid Soluble Nucleotides from Rabbit Intestinal Mucosa.* (24984)

L. S. Dietrich and Ira M. Friedland

Dept. of Biochemistry, University of Miami School of Medicine, Coral Gables, Fla.

In studies on the action of drugs it was essential to isolate the acid soluble nucleotides in a manner that ratios of 5'-monophosphates to 5'-polyphosphate nucleotide derivatives approximate those found *in vivo*. Previously published procedures for isolation of acid soluble nucleotides from intestinal mucosa(1,2) necessitate several minutes between death of animal and isolation and acid denaturation of mucosa tissue. Thus, although these procedures gave adequate qualitative results, the quantitative aspects of analyses were suspected. Subsequently a procedure for isolation of acid soluble nucleotides of intestinal mucosa which completely eliminates any time lag between death of animal and acid fixing of mucosa tissue was developed.

Materials and methods. Young adult male albino rabbits were used. The animals were anesthetized with ether and the abdomen opened with long midline incision. Mucosa tissue was isolated by the following 2 procedures: a) The small intestine was ligated at a point approximating beginning of jejenum. The intestine was then severed at about 3 feet caudal to ligation. A 19 gauge hypodermic needle was inserted just inferior to point of ligation and 150 ml of ice-cold 3% perchloric acid slowly passed through lumen using 100 ml syringe. The acid-bathed segment of intestine was then excised and dropped into ice-cold 3% perchloric acid. After few minutes exposure to acid the intestine was removed and portions taken for histological examination. The remaining sections were blotted dry, cut open lengthwise, flattened out on blotting paper and the denatured mucosa removed by delicate scraping with forceps, Peyer's patches being excluded(3). The mucosa was then weighed and homogenized in 9 volumes of 3% perchloric acid. b) The identical intestinal region was excised without preliminary bathing with perchloric acid, cut lengthwise and flattened out on blotting paper. Intestinal contents were wiped away with absorbent cotton and water. The mucosa was removed in identical manner as in acid-fixed tissue, weighed and homogenized in 9 volumes of ice-cold 3% perchloric acid. The acid soluble nucleotides were isolated from perchloric extracts as previously reported(4). The solution containing concentrated acid soluble nucleotides was applied as spots to sheets of Whatman 3 MM paper and developed in first dimension with acid ammonium isobutyrate(5). Areas occupied by AMP, ADP and ATP wre cut out and quantitatively analysed as previously reported(4). Qualitative estimations of all components of acid extracts were made after chromatography in the second dimension with modified Markham and Smith solvent #3(6) [Aqueous $(NH_4)_2SO_4$ (564.8 g/liter) : water : isopropanol : 0.1 M versenate (78:19:2:1)].

Results. Qualitatively the same nucleotides were present regardless of whether or not the mucosa was bathed *in situ* with perchloric acid. These are the same as previously reported for rat(1) and rabbit(2) intestinal mucosa, *i.e.*, the acid extracts in both cases contained mono-, di, and triphosphates of adenosine, guanosine, cytidine and uridine together with inosine monophosphate, di-, triphosphopyridine nucleotide, several uridine diphosphate derivatives and a number of unidentified compounds containing adenine. Quantitatively the differences are very significant. Intestinal mucosa fixed *in situ* with perchloric acid consistently exhibited lower AMP and higher ADP and ATP level than mucosa tissue removed without preliminary perfusion (Table I). These results may be explained by the fact that perchloric acid denatures viable, well nurtured mucosa cells that have up to moment of fixation received ample supplies of oxygen and metabolites from the

* Supported in part by grants from Nat. Vit. Fn. and U.S.P.H.S.

TABLE I. Effect of Perchloric Acid Fixation of Rabbit Intestine *In Situ* on Major Acid Soluble Adenosine Derivatives of the Mucosa.*

Treatment	μmoles/g mucosa wet wt AMP	ADP	ATP
None	1.16 (.98, 1.34)	.32 (.39, .26)	.49 (.47, .51)
Perchlorate-treated	.10 (.16, .04)	.31 (.35, .27)	1.28 (.95, 1.60)

* All calculations are based on reported molar absorbance index for adenosine derivations, at 260 mμ(12). Values are avg of 2 experiments. Individual values are in parentheses. See *Materials and methods* for experimental details.

mesentery circulation. Thus concentration of 5′-mono and 5′-polyphosphate nucleotide derivates should approach that which occurs *in vivo*. These data are consistent with studies reported with tumor(7), lung(7), liver(8), and yeast(9), tissues in which most of the adenosine derivatives were present as 5′-polyphosphates. The same picture exists in mucosa when other nucleotide 5′-mono and 5′-polyphosphate nucleotides are compared. However in these cases quantitative values are unavailable since in *in situ* fixed tissues the amounts of guanosine-, cytidine-, and uridine-monophosphates present were so low that they could not always be detected. However, with mucosa tissue isolated without preliminary fixation, quantities of these 5′-mono-phosphate compounds were consistently observed. This is in keeping with observations of Siekevitz and Potter(10) who showed that non-adenosine 5′-mono- and 5′-polyphosphate nucleotide derivatives are in equilibrium with 5′-mono- and 5′-polyphosphate adenosine compounds. Impairment of high energy bond synthesis by production of cellular anoxia such as would occur in removal of intestine and subsequent isolation of respiring mucosa should produce a state of energy deficiency. This would be evident in a manner similar to that observed in respiring mitochondria treated with dinitrophenol or cyanide. In these cases(11) concentration of 5′-polyphosphate adenosine nucleotides decreased and AMP increased. Such was the case with mucosa tissue isolated without preliminary acid fixation (Table I). It is of interest that the morphology of mucosa tissue obtained by both procedures is normal (unpublished data). This may indicate that in non-perfused intestine ATP synthesis, although greatly diminished, continues at a rate fast enough to maintain cellular integrity.

Summary. A method is described which permits isolation of acid soluble nucleotides of the small intestinal mucosa. This procedure entails bathing intestine mucosa, *in situ*, with ice cold perchloric acid. In this manner, mucosa tissue is fixed while still in optimal nutritional state. The tissue so obtained gives a nucleotide pattern which both quantitatively and qualitatively resembles that of other normal well nurtured tissues.

Abbreviations used are: Adenosine mono-, di-, triphosphate, AMP, ADP, ATP.

1. Daoust, R., Cantero, A., *Cancer Res.*, 1955, v15, 734.

2. Keir, H. M., Davidson, J. N., *Arch. Biochem. Biophysic*, 1958, v77, 68.

3. Stevens, C. E., Daoust, R., Leblond, C. P., *J. Biol. Chem.*, 1953, v202, 177.

4. Dietrich, L. S., Friedland, I. M., Kaplan, L. A., *ibid.*, 1958, v233, 964.

5. Magasanik, B., Vischer, E., Doniger, R., Elson, D., Chargaff, E., *ibid.*, 1950, v186, 37.

6. Markham, R., Smith, J. D., *Biochem. J.*, 1951, v49, 401.

7. Dietrich, L. S., Kaplan, L. A., Friedland, I. M., Martin, D. S., *Cancer Res.*, 1958, v18, 1272.

8. Hurlbert, R. B., Schmitz, H., Brumm, A. F., Potter, V. R., *J. Biol. Chem.*, 1954, v209, 23.

9. Schmitz, H., *Biochem. Z.*, 1954, v325, 555.

10. Brumm, A. F., Potter, V. R., Siekevitz, P., *J. Biol. Chem.*, 1956, 220, 713.

11. Siekevitz, P., Potter, V. R., *ibid.*, 1955, v215, 237.

12. Bock, R. M., Ling, N. S., Morell, S. A., Lipton, S. H., *Arch. Biochem. Biophysic*, 1956, v62, 253.

Received April 27, 1959. P.S.E.B.M., 1959, v101.