substrates, ADP–glucose and ADP–ribose have been estimated as $2.9 \cdot 10^{-4}$ M and $1.3 \cdot 10^{-3}$ M, respectively. With ADP–glucose as substrate the pH optimum is between 7.8 and 8.4.

Rat skin, liver and kidney contain particulate or nuclear pyrophosphatases of broad specificity which are activated by magnesium[8]. Substrates which are hydrolyzed by the particulate pyrophosphatase from rat liver microsomes or nuclei are UDP–glucuronic acid, UDP–glucose, UDP–*N*-acetylglucosamine, GDP–mannose, NAD^+, NADH, $NADP^+$ and FAD[9]. Pyrophosphatases with broad specificity and activated by Mg^{2+} have also been extracted from various microbial sources[1,2]. Heating extracts for 5 min at about 58° is often required for full activity of the microbial pyrophosphatases.

The distinguishing property of the pyrophosphatase described herein is a unique specificity for adenosine substituted on one of the phosphate groups and either a glycosidic (glucose, mannose or ribose) or ester (NAD) linkage on the other phosphate group and stimulation by a divalent cation.

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Neuraminidase in human intestinal mucosa

Neuraminidase (*N*-acetyl neuraminate glycohydrolase, EC 3.2.1.18) is present[1,2] in animals, bacteria, myxo viruses and some tumor tissues. Although this enzyme has been detected in human plasma[1] and brain[1] no study has been undertaken with other human tissues. The present investigation was prompted by the reports[3] that while hepatic, renal and placental alkaline phosphatases (orthophosphoric monoester phos-

phohydrolase, EC 3.1.3.1) undergo a reduction in the anodal migration by a pretreatment with bacterial or viral neuraminidase, alkaline phosphatase from human intestinal mucosa is resistant to neuraminidase[3]. The intestinal alkaline phosphatase, which has been the subject of thorough investigation in this laboratory during the last several years[3], does not exhibit a reduction in its electrophoretic mobility after incubation with neuraminidase, an enzyme specific for cleaving sialic acid residues. This communication examines the presence of endogenous neuraminidase in the human intestinal mucosa, describes some of the properties of intestinal neuraminidase including the hydrolysis of *N*-acetyl neuramin-lactose and human placental alkaline phosphatase (proven to be a sialoprotein[3,4]) and also suggests the probable role it may play in making intestinal alkaline phosphatase neuraminidase resistant.

Homogenates (10%) of human intestinal mucosa and of other *post-mortem* tissues were made in ice-chilled 0.05 M Tris–HCl buffer (pH 8.6) with a mechanically driven all-glass homogenizer. The digest for measuring neuraminidase activity contained 0.1 ml *N*-acetyl neuramin-lactose solution (8 μmoles/ml) as substrate; 0.1 ml 2 M acetate buffer (pH 5.5); 0.02 ml 0.1 M $CaCl_2$; 0.02 ml 0.1 M $MgCl_2$ and 0.1 ml of the tissue homogenates. Samples (0.1 ml) were withdrawn from the digests after incubation for 60 min (or other desired interval of time) at 37° and immediately mixed with 0.1 ml of sodium metaperiodate reagent and assayed for free sialic acid as described before[4] using the thiobarbiturate color reaction of Warren. The control digests containing buffered homogenates without neuramin-lactose and those with substrate alone in acetate buffer, respectively, were run concurrently and the readings, if any, were

TABLE I

NEURAMINIDASE ACTIVITIES IN HUMAN INTESTINAL MUCOSA

The activity of neuraminidase was expressed as μmoles sialic acid liberated per g of tissue in 1 h from *N*-acetyl neuramin-lactose in acetate buffer (pH 5.5) at 37°.

Sample No.:	*1*	*2**	*3*	*4*	*5*	*6***	*7***	*8*
Neuraminidase activity***	7.7	48.7	2.5	2.5	3.8	5.1	6.9	27.1

* The liver, bone, kidney and brain tissues of this subject had neuraminidase in them in relatively smaller amounts than intestine.

** The activities given here are those of mucosa obtained from large intestine (colon).

*** The neuraminidase activities in the nonintestinal tissues of liver, bone, kidney, spleen, lung and brain of Subjects 1, 3, 4, 5 and 8 were measured and found to be zero under the present experimental conditions.

subtracted from those of the test digests. A correction was also made for the reduction of the intensity of sialic acid color by the presence of neuramin-lactose.

The activities of neuraminidase in about 60 specimens of liver, bone, kidney, small and large intestine, spleen, lung and placenta were measured. In 5 out of 6 subjects studied (Sample No. 1, 2, 3, 4, 5 and 8 of Table I) the activity was not detectable at all under the present assay conditions in all those nonintestinal tissues (liver, bone, kidney, spleen and lung), the alkaline phosphatases of which were found to be neuraminidase sensitive. The Tris homogenates of human placental tissues also did not exhibit any neuraminidase activity.

The neuraminidase activities of several human intestinal mucosa are presented in Table I.

The intestinal neuraminidase preparation was made as follows: A 10% homogenate in Tris buffer of pooled large intestinal mucosa was digested with 1% trypsin for 48 h at pH 7.0. The trypsin-digested material was spun at 40 000 rev./min for 1 h in Beckman Spinco Model L centrifuge. The pH of the supernatant maintained at 0° (ice-bath) was adjusted to 5, and precooled (−30°) methanol was very slowly added to make 60% methanol. The mixture was allowed to settle for 2 days in the refrigerator and spun at 105 000 × *g* for 1 h. The residue obtained was dissolved in water and used as human intestinal neuraminidase in experiments, the results of which are presented in Fig. 1.

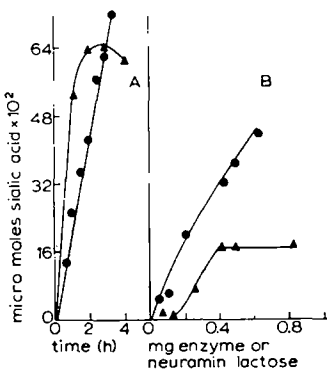

Fig. 1. Hydrolysis of *N*-acetyl neuramin-lactose (●) and human placental alkaline phosphatase (▲) by human intestinal neuraminidase. A. The velocity of sialic acid release as a function of time of incubation. The digest contained either 1 mg of neuramin-lactose or 1.6 mg of placental alkaline phosphatase (mixture of A and B variants[3], specific activity, 100 units/mg protein). B. The release of sialic acid as a function of substrate (*N*-acetyl neuramin-lactose or human placental alkaline phosphatase) concentration. The time of incubation was 150 min.

The residues obtained by the differential centrifugation of a 20% homogenate of the intestinal mucosa (No. 8 of Table 1) at 1100 × *g* for 10 min, 12 000 × *g* for 45 min, 40 000 × *g* for 90 min, 105 000 × *g* for 120 min, were called nuclear, mitochondrial, lysosomal and microsomal fractions, respectively. The fractions were washed with the media used, centrifuged and reconstituted in 0.05 M Tris–HCl buffer (pH 8.6). A typical set of results on the pattern of subcellular distribution of neuraminidase compared with that of alkaline phosphatase is presented in Table II.

Table I shows that human intestinal mucosa contain variable but appreciable amounts of neuraminidase. We also found that the gastric juice, intestinal and duodenal secretions as well as ileostomy drainage, which contained neuraminidase resistant alkaline phosphatase, had measurable amounts of neuraminidase. Unlike liver, bone, placenta and other tissues the pH of the intestinal and gastric secretions are acidic around 5.6, the optimum pH for neuraminidase[3] for the removal of sialic acid from intestinal alkaline phosphatase. The subcellular distribution pattern of intestinal neuraminidase (Table II) is similar to that of intestinal alkaline phosphatase with the largest amount of both enzymes in the supernatant fractions. Fig. 1 illustrates that

TABLE II

SUBCELLULAR DISTRIBUTION OF NEURAMINIDASE AND ALKALINE PHOSPHATASE IN HUMAN INTESTINAL MUCOSA

Intestinal fractions	*Percentage distribution of enzyme activity*			
	Neuraminidase Medium of homogenization		*Alkaline phosphatase* Medium of homogenization*	
	0.25 M sucrose	*2% citric acid (pH 6)*	*0.2 M $NaHCO_3$*	*2% citric acid (pH 6)*
Nuclear	7.1	13.7	1.6	3.4
Mitochondrial	4.3	18.7	10.5	18.8
Lysosomal	1.4	11.2	9.4	11.5
Microsomal	13.0	7.3	13.3	1.0
Supernatant	74.0	49.0	65.5	65.6

* The alkaline phosphatase activity was measured by assaying phenol released from 18 mM phenyl phosphate in 0.05 M carbonate–bicarbonate buffer (pH 9.8) at 37°.

the preparation of human intestinal neuraminidase can split off sialic acid from *N*-acetyl neuramin-lactose and also from purified preparation of human placental alkaline phosphatase, which has been characterized as a neuramino-protein[3,4].

Under the present circumstances it may be reasonable to suggest that the mucosa of human small and large intestines are good sources of neuraminidase. Human alkaline phosphatases in duodenum, small intestine, colon, ileostomy drainage and other gastro-intestinal tissues are neuraminidase resistant probably because endogenous neuraminidase has already induced *in vivo* cleavage of the sialic acid residues of the enzyme. Any further treatment *in vitro* with purified *Vibrio cholerae* or influenza virus neuraminidase does not lead to any more reduction in the electrophoretic migration as the neuraminidase sensitivity of these alkaline phosphatases has already been impaired *in vivo*.

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