water bath, and after filtration through four thicknesses of cheese-cloth were rapidly frozen in a dry ice–acetone bath. Frozen homogenates were either stored at −20° for subsequent use or immediately thawed rapidly by intermittent immersion with vigorous shaking in a water bath at 37°. Care was taken not to allow the temperature of the homogenate to rise above 4–5°. Thawed homogenates were immediately transferred to an ice-water bath. When homogenates were used without storage, incubation with substrate was usually begun within 20–30 min after the death of the animal.

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Studies of small intestine during development

II. The intracellular location of intestinal β-galactosidase

Recent studies have indicated that intestinal β-galactosidase (EC 3.2.1.23) can be sedimented with the "nuclear" and microsomal fractions derived from an homogenate of intestinal mucosa[1]. In liver several acid hydrolases are contained within a lysosome, which when disrupted will release the active enzymes[2]. This study was designed to determine more accurately the distribution of β-galactosidase by comparing its cellular location with that of acid phosphatase (EC 3.1.3.2) and β-glucuronidase (EC 3.2.1.31) (typical lysosomal enzymes) and also with alkaline phosphatase (EC 3.1.3.1), an enzyme associated with the brush border[3].

Rats of the Wistar strain, 2–4 days of age were used. The method of preparation of the intestinal homogenate, the centrifugal fractionation (except for the use of a somewhat higher force, 600 × *g*, for sedimentation of the nuclear fraction) and the β-galactosidase assay, using lactose as substrate have been described[1]. Homogenates

of liver were treated in the same manner. β-Glucuronidase and acid phosphatase were assayed as described by GIANETTO AND DE DUVE[4]. Alkaline phosphatase using β-glycerophosphate as substrate was determined by the method of MOOG[5] with Tris buffer instead of Veronal–carbonate buffer. To test for "activation" of lysosomal enzymes the cellular fractions were assayed in the presence of 0.2% deoxycholate. This concentration of deoxycholate slightly inhibited activity of intestinal β-glucuronidase but the extent was ascertained by comparing activity in the presence of deoxycholate with that obtained after pre-incubation in hypotonic media. The activities of β-glucuronidase and β-galactosidase were slightly lower (approx. 20%) in homogenates made with 0.25 M sucrose as compared to those prepared with 0.65 M mannitol but there was no effect on the cellular distribution of any of the enzymes using either homogenizing medium. Typical specific activities of the three acid hydrolases in fractions of young-rat intestinal homogenate, and their recovery in each fraction are shown in Table I. The observed distributions of acid phosphatase and β-glucuronidase are quite similar except that there is more β-glucuronidase associated with the soluble fraction. Distribution of β-galactosidase in the various

TABLE I

SPECIFIC ACTIVITY AND RECOVERY OF ACID HYDROLASES IN SUBCELLULAR FRACTIONS OF RAT INTESTINAL HOMOGENATE

μmoles of glucose or inorganic phosphate or μg phenolphthalein released per mg protein per 10 min.

Fraction	Enzyme					
	β-Galactosidase		Acid phosphatase		β-Glucuronidase	
	Specific activity	Recovery (%)	Specific activity	Recovery (%)	Specific activity	Recovery (%)
Whole homogenate	1.9	100	0.21	100	20	100
Nuclear	4.8	58	0.24	28	19	23
Mitochondrial	1.1	7	0.29	17	24	15
Microsomal	5.1	25	0.34	15	30	14
Soluble	0.58	10	0.13	22	22	37
Total		100		82		89

fractions differs quite strikingly from that of the other two enzymes. In the presence of deoxycholate there was no increase in activity of the three enzymes in various fractions from intestine. Since these results are markedly different from those found for adult-rat liver[3], livers from young rats (2 days old) were fractionated, and the activities of these same enzymes were determined with and without deoxycholate. The activity of β-galactosidase was very low in this tissue and could not be measured accurately in crude preparations except in the mitochondrial fraction (0.008 μmoles glucose released/10 min/mg protein). There was a similarity of distribution of β-glucuronidase and acid phosphatase in the various fractions and the activity of the two enzymes (β-galactosidase also, in liver mitochondria) was markedly increased after distruptive treatment with deoxycholate. The ratio of activity after treatment of the mitochondrial fraction with deoxycholate to that before treatment was as follows: β-galactosidase, 6.2; acid phosphatase, 5.5; and β-glucuronidase, 4.4. These results are similar to those already described for adult-rat liver[3].

From the distribution studies it was concluded that β-galactosidase is not associated with the same type of particle in intestinal mucosa as are β-glucuronidase and acid phosphatase. Also, there is no indication chemically that these latter two enzymes are associated with a lysosomal-like particle in intestine. The cellular distribution of β-galactosidase was also compared with that of alkaline phosphatase since histochemically this phosphatase has been shown to be associated with the intestinal epithelial brush border[3]. The distribution of the two enzymes is similar (Table II) although a higher percentage of alkaline phosphatase than of β-galactosidase has consistently been found in the soluble fraction of the cell. This might be interpreted as indicating that alkaline phosphatase is less tightly bound to a particle. There is no "activation" of intestinal alkaline phosphatase by deoxycholate.

There has been considerable work in recent years concerning the intracellular location of various enzymes in different tissues (for references see reviews by NOVIKOFF[6] and DE DUVE[7] and the demonstration by DE DUVE *et al.*[2] of a separate cell particle in liver, the lysosome, as the locus of a number of acid hydrolases stimulated further work in this field. In liver, the major acid hydrolases, β-glucuronidase, acid phosphatase, acid ribonuclease (EC 2.7.7.16), acid deoxyribonuclease (EC 3.1.4. 5)

TABLE II

SPECIFIC ACTIVITY AND DISTRIBUTION OF β-GALACTOSIDASE AND ALKALINE PHOSPHATASE IN SUBCELLULAR FRACTIONS OF YOUNG RAT (2 DAYS OLD) INTESTINAL HOMOGENATE

Glucose or inorganic phosphate (μmoles) released per mg protein per 10 min.

Fraction	Enzyme			
	β-Galactosidase		Alkaline phosphatase	
	Specific activity	Recovery (%)	Specific activity	Recovery (%)
Whole homogenate	2.3	100	12	100
Nuclear	5.1	52	24	47
Mitochondrial	0.97	4	12	10
Microsomal	3.9	14	25	17
Soluble	0.54	7	8	19
Total		77		93

and cathepsin are contained in a particle which effectively separates them from their substrates and from which they can be released in fully active form by rupture of the membrane of the particle. In kidney as well, these enzymes have been shown to be contained within lysosomes or "droplets" as they were originally termed[8] and GREENBAUM *et al.*[9] have described lysosomes in rat mammary tissue. It has been hypothesized[7] that lysosomes have a digestive function related particularly to phagocytosis and pinocytosis. In an extensive fractionation procedure of mouse pancreas VAN LANCKER AND HOLZER[10] found a distribution of acid phosphatase similar to that found in liver with a 2-fold activation when assayed in the presence of Triton X-100. In other tissues, however, these enzymes have not always been found associated with such particles. In heart muscle for instance[11] fully active acid phosphatase has been found largely in the particulate fractions. In rat ventral prostate this enzyme is distributed throughout all fractions and its activity can be increased

by only 40% by homogenizing in hypotonic solution or by repeated freezing and thawing[12]. The significance of the differences in activation of β-glucuronidase and acid phosphatase in intestine as shown here as compared with liver and kidney is a matter for conjecture but may be related to the different functions performed by these tissues.

In the intestine, β-glucuronidase and acid phosphatase have a similar distribution pattern which differs from that of β-galactosidase and alkaline phosphatase indicating that they are probably not contained in or upon the same particles. Of considerable interest in this regard is the histochemical demonstration by BARKA[13] of different intracellular loci for the acid and alkaline phosphatases of mouse intestinal epithelium. The acid phosphatase was found in small granules of the "terminal web" whereas the enzyme active at alkaline pH was concentrated in the brush border and Golgi zone. Alkaline phosphatase has also been shown by electron microscopy to be associated with the brush border and intracellular granules of the intestinal epithelial cells[14]. Invertase (EC 3.2.1.26) may also have both a brush border membrane[15] and intracellular granule location[16]. The histochemical technique used by DAHLQVIST AND BRUN[16] demonstrated that invertase activity is not particularly concentrated within the brush border but is concentrated in granules of different sizes in the apical part of the cytoplasm.

From the data presented it appears that β-galactosidase and alkaline phosphatase occupy similar sites within the intestinal epithelial cell. The observed bimodal cellular distribution of activity of β-galactosidase (lactase) may represent association of the enzyme with larger granules after synthesis within or upon microsomal particles or it may result from mechanical disruption of large particles during homogenization.

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