LYSOSOMES IN LYMPHOID TISSUE

I. The Measurement of Hydrolytic Activities in Whole Homogenates

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ABSTRACT

Methods have been developed for the quantitative assay of cytochrome oxidase, esterase, and 11 acid hydrolases in rat-spleen homogenates. These methods seem to be applicable also to other lymphoid tissues. Preliminary studies, extended to nine of the acid hydrolases, indicate that these enzymes occur in partly latent and sedimentable form and that they can be unmasked and rendered soluble by some of the treatments that liberate the enzymes from rat-liver lysosomes. The spleen particles appear to be very sensitive to mechanical injury, a property which necessitates special precautions in homogenizing the tissue. Agglutination of spleen particles takes place to a larger extent in 0.25 M sucrose than in 0.15 M KCl.

INTRODUCTION

Lymphoid tissues, especially the spleen, represent one of the richest sources of many of the acid hydrolases known to be associated with lysosomes in other tissues. A lysosomal localization of these enzymes in lymphoid tissues is suggested by the finding that several of them occur partly in latent, particle-bound form, in freshly prepared homogenates of spleen or thymus (6, 10, 22, 26–28, 33). However, various investigators, using different techniques with these tissues, have obtained rather variable patterns for the intracellular distribution of individual acid hydrolases (2, 6, 15, 22, 29, 30) and this fact has even led some workers to question the existence of lysosomes as a distinct group of cytoplasmic particles (22).

Our investigations were started with the aim of obtaining more complete and accurate information concerning the existence, properties, and functions of lysosomes in lymphoid tissues. They were performed principally on spleen, but some experiments were also carried out on thymus and on lymph nodes. The approach has been essentially biochemical and has been extended to about a dozen different enzymes in order to provide a basis as broad as possible for further morphological studies. The present paper describes the results of experiments dealing with the measurement and main kinetic properties of enzymes in whole homogenates. The association of these enzymes with lysosomes and various data concerning the cellular location and functional properties of these particles form the subject of subsequent papers in this series.

MATERIALS AND METHODS

The experiments were performed on Sprague-Dawley rats of either sex weighing between 150 and 300 g. The animals were fed Purina Chow and were permitted access to food and water until the time of the experiment; they were killed by decapitation. The spleen and other lymphoid tissues were quickly removed, immersed in a tared beaker containing ice cold 0.15 M KCl, and weighed.

Some determinations for acid hydrolase activity were made on cell suspensions obtained from thymus. Cells were teased into Tyrode's solution according to

the method described by Fastier (16). The preparation was filtered through several layers of gauze and the cells were collected by centrifuging at 100 *g* for 10 min. They were then washed two or three times and resuspended to the desired volume.

The manner in which the tissue and cell preparations were dispersed and analyzed for various enzymes will be described under Results.

The following substrates were purchased from the Sigma Company (St. Louis, Missouri): β-glycerophosphate (Grade I), cytochrome *c* (Type III), DNA (Type I), hemoglobin (Bovine, Type I, 2x crystallized), nitrocatechol sulfate (Dipotassium salt), *o*-nitrophenyl acetate (Practical grade), *o*-nitrophenyl-β-galactoside, phenolphthalein β-glucuronide (Free acid), and RNA (Commercial grade, yeast). Benzoyl-L-arginine amide hydrochloride and glycyl-L-tyrosine amide acetate were obtained from Mann Research Laboratories (New York) and freed from contaminating ammonia by distillation in the presence of K_2CO_3, followed by neutralization of the carbonate. *p*-Nitrophenyl-α-mannoside was synthesized according to the method of Jermyn (21), with some additional instructions from Dr. James Conchie. The final product had a melting point of 182°–183°C, as compared with 180°–181°C reported by Conchie et al. (7). *p*-Nitrophenyl-*N*-acetyl-β-glucosaminide was synthesized by the method of Findlay et al. (17). The melting point was 201°–202°C, as compared to the value of 204°C given by these authors. Protein was measured according to Lowry et al (23).

RESULTS

Preparation of Homogenates

Phase-contrast microscopy and measurements of latent enzymatic activities were used to assess the quality of spleen homogenates prepared in different ways. A combination of suspension medium and homogenizing device was sought which would provide complete disruption of the tissue and adequate dispersion of subcellular components, while ensuring the best preservation of enzyme latency. Homogenates (10–15 ml per g wet tissue) were prepared either with a Potter and Elvehjem (25) type homogenizer (smooth-walled glass tube with Teflon pestle, manufactured by A. H. Thomas Co., Philadelphia, Pa.), driven at about 1000 rpm, or with the device described by Dounce et al. (14) (all-glass, manufactured by Kontes Glass Co., Vineland, New Jersey). In the latter case, homogenization was started by about 10 up-and-down strokes of a loosely fitting pestle, and completed similarly with no more than three strokes of a tightly fitting pestle. Both types of homogenates had the same appearance under the microscope. Cell breakage appeared to be essentially complete, with the possible exception of small lymphocytes which were difficult to distinguish from free nuclei. However, as shown by the results of Table I, the proportion of acid phosphatase and β-glucuronidase occurring in

TABLE I

Comparison of Homogenizing Devices

In each experiment, halves of the same spleen were homogenized in ice cold 0.25 M sucrose, each with one of the two devices indicated, and the two homogenates were assayed for free and total acid phosphatase and β-glucuronidase activity by the techniques described below. A variance analysis performed on the results of experiments 2 through 4 indicated that the difference between the two homogenizers is highly significant ($P < 0.001$).

	Free activity, % of total activity			
	Potter-Elvehjem		Dounce	
Experiment	Acid phosphatase	β-Glucuronidase	Acid phosphatase	β-Glucuronidase
1	53.5	—	37.7	—
2	73.7	73.9	51.0	50.9
3	66.6	65.5	53.3	51.5
4	63.0	78.0	50.3	53.2

TABLE II

Comparison of Suspension Media

Experiments were carried out as described in Table I; homogenization was performed in all cases with the homogenizer of Dounce et al. (14).

	Free acid phosphatase activity, % of total activity	
Experiment	0.25 M sucrose	0.15 M KCl
1	55.8	58.9
2	52.8	53.4
3	59.2	39.7
4	55.7	54.1
5	55.0	55.0
		Mannitol
6	55.3	60.4*
7	49.8	58.9*
8	47.4	62.2‡
9	49.8	57.7‡

*0.25 M.
‡0.15 M.

free form was 15–20% lower in preparations made with the Dounce homogenizer. There was no evidence that this difference was due to the presence of a higher proportion of intact cells, and it was attributed to better preservation of fragile subcellular particles. For similar reasons, Conchie et al. (8) have recommended Potter-Elvehjem homogenizers with a minimum clearance of 0.23 mm for breaking up spleen tissue.

As shown in Table II, the same degree of latency was observed whether 0.25 M sucrose or 0.15 M KCl was used as suspension medium, whereas mannitol solutions gave less satisfactory results. Microscope examinations and centrifugation experiments indicated that considerably more agglutination of particles took place in sucrose than in KCl solutions. Preference was therefore given to the latter, and all homogenates were prepared in 0.15 or 0.2 M KCl with the Dounce homogenizer. This procedure also gave satisfactory results with thymus and lymph nodes.

The results of Table I suggested that the acid hydrolases of spleen are contained in subcellular particles that are very sensitive to mechanical injury. In confirmation of this interpretation, it has been found in recent experiments that an even higher proportion of latent activity can be obtained by first mincing the spleen before homogenization. These results are shown in Table III.

Kinetics and Assay of Enzymes

In order to establish valid assay conditions for each of the enzymes selected for study, a series of kinetic investigations were undertaken, exploring such variables as enzyme concentration, substrate concentration, ionic strength, incubation time and pH. For brevity's sake, these experiments will not be described in detail. The conditions finally selected for the assays are given in Tables IV and V. Fig. 1 illustrates the relationship between the measured activities and the enzyme concentration and duration of incubation. In Fig. 2 are shown the pH-activity curves of five of the hydrolases.

TABLE III

Influence of Preliminary Mincing of Spleen on Enzyme Latency in Homogenate

In each experiment spleens were minced with a razor blade and then homogenized and analyzed as in experiments of Table II.

Experiment	Medium	Free activity, % of total activity	
		Acid phosphatase	β-Glucuronidase
1	0.25 M sucrose	—	26.2
2	0.2 M KCl	26.4	24.8
3	0.25 M sucrose + 0.2 M KCl	—	29.0
4	0.25 M sucrose + 0.2 M KCl	29.6	—

Two types of assays were developed for the acid hydrolases studied. In measurements of *total activity*, the incubation mixture contained 0.1% of Triton X-100, in addition to the components listed in Table IV. It was verified that this concentration of detergent was sufficient to cause complete loss of latency for all the enzymes. In measurements of *free activity*, osmotic shock was avoided by making up the substrate mixture to the same solute concentration as the suspension medium, and the incubation time was restricted to 10 min. The latter precaution may have been unnecessary, since Rahman (28) has shown that the proportion of latent acid phosphatase activity of spleen preparations remains remarkably unaffected for several hours at pH 5 and 37°C. This stability of the acid phosphatase-containing particles, which contrasts with the lability of liver lysosomes under the same conditions, has been confirmed in our own experiments. *Latent activities* are obtained by subtracting the free from the total activities.

As shown in Fig. 1, all enzymes except acid deoxyribonuclease answered the linearity requirements of a valid assay under the conditions adopted. The kinetics of acid deoxyribonuclease, though not linear, were reproducible and probably reflect the relative insolubility in acid of the first breakdown products of the DNA molecule. In each experiment, a standard reference curve was constructed by running assays at four or five enzyme concentrations, and the results were estimated graphically from the reference curve.

Several of the hydrolases were found to be sensitive to ionic strength. When this was the case, the incubation mixture was supplemented with KCl to give optimal conditions, as shown in Table IV. This was not done for acid ribonuclease, even though this enzyme, like acid deoxyribonuclease, showed a marked increase of activity in the presence of 0.2 M KCl, because this increase was accompanied by a considerable deviation from linearity of the time-course of the reaction.

TABLE IV

Incubation Conditions for Enzyme Assays

All incubations are carried out at 37°C for times ranging between 10 min and 2 hr, except for the esterase and cytochrome oxidase reactions which are run for a few minutes at 25°C in optical cuvettes. E.C. in the second column refers to the enzyme classification by the Commission on Enzymes of the International Union of Biochemistry.

Enzyme	E. C.	Final Volume	Substrate	Buffer	pH	Addition
		ml				
Acid phosphatase	3.1.3.2	1.0	50 mM β-glycerophosphate	0.05 M acetate	5.0	—
Aryl sulfatase	3.1.6.1	1.0	20 mM nitrocatechol sulfate	0.05 M acetate	5.0	0.2 M KCl
Acid deoxyribonuclease	3.1.4.5	1.0	2.5 mg denatured DNA*	0.05 M acetate	5.0	0.2 M KCl
Acid ribonuclease	2.7.7.16	1.0	4.5 mg RNA‡	0.05 M acetate	5.0	—
β-Glucuronidase	3.2.1.31	1.0	1.25 mM phenolphthalein β-glucuronide	0.05 M acetate	5.0	—
β-Galactosidase	3.2.1.23	0.8	5 mM *o*-nitrophenyl-β-galactoside	0.05 M acetate	5.0	0.1 M KCl
N-acetyl-β-glucosaminidase	3.2.1.30	1.0	6 mM *p*-nitrophenyl-*N*-acetyl-β-glucosaminide	0.05 M acetate	5.0	—
α-Mannosidase	3.2.1.24	1.0	6 mM *p*-nitrophenyl-α-mannoside	0.05 M acetate	5.0	—
Cathepsin B	3.4.4.-	1.0	10 mM benzoyl-L-arginine amide	0.1 M citrate	5.0	0.04 M cysteine
Cathepsin C	3.4.4.9	1.0	10 mM glycyl-L-tyrosine amide	0.1 M citrate	5.0	0.04 M cysteine
Cathepsin D	3.4.4.-	1.5	2% denatured hemoglobin§	0.1 M lactate	3.6	—
Esterase‖	3.1.1.6	3.0	2.6 mM *o*-nitrophenyl acetate	0.17 M phosphate	7.4	mM EDTA
Cytochrome oxidase¶	1.9.3.1	3.1	1.35 mg reduced cytochrome *c*	0.03 M phosphate	7.4	—

* Heated for 3–5 min at 95°C, cooled rapidly, dialyzed overnight in the cold against H_2O or 0.1 M acetate buffer, pH 5.0.

‡ Dialyzed overnight in the cold against H_2O or 0.1 M acetate buffer, pH 5.0.

§ Exposed for 1 hr at pH 3.6, 37°C, then dialyzed against 0.1 M lactate buffer, pH 3.6.

‖ Enzyme preparation pretreated with 0.5% of Triton X-100 (final concentration during incubation = 0.067%).

¶ Enzyme preparation pretreated with 0.5% digitonin (final concentration during incubation = 0.065%).

TABLE V

Analytical Procedures for Enzyme Assays

TCA, trichloroacetic acid; PCA, perchloric acid.

Enzyme	Manipulations	Analysis	References
Acid phosphatase	Add 5 ml of 8% TCA, filter	Colorimetric for P_i	3, 18
Aryl sulfatase	Add 3 ml of 2.2% TCA, centrifuge, add enough NaOH to supernatant to make final concentration N	Colorimetric in N NaOH for nitrocatechol (540 mμ)	31
Acid deoxyribonuclease	Add 1 ml of 10% PCA, filter at 0°C after 15 min	UV absorption at 260 mμ	13
Acid ribonuclease	Add 1 ml of 10% PCA + 0.25% uranyl acetate, filter in cold after 1 hr	UV absorption at 260 mμ	13
β-Glucuronidase	Add 3 ml of glycine-NaCl-Na_2CO_3 mixture, pH 10.7, centrifuge	Colorimetric for phenolphthalein (540 mμ)	19
β-Galactosidase *N*-acetyl-β-glucosaminidase α-Mannosidase	Add 2 ml of 2.3% TCA, centrifuge, mix supernatant with buffer pH 10 + enough NaOH to neutralize acid present	Colorimetric for nitrophenol (420 mμ) in glycine-sodium carbonate buffer, pH 10 (0.078 M in glycine)	35 17 7
Cathepsin B Cathepsin C	Add 1 ml of conc. K_2CO_3, cap reaction vessel immediately and recover ammonia in M H_2SO_4 according to Seligson and Hirahara (34)	Colorimetric for ammonia at 625 mμ	32, 34 32, 34
Cathepsin D	Add 2.5 ml of 5% TCA, filter in cold after 30 min	Colorimetric at 660 mμ for reaction in excess NaOH between aromatic degradation products and Folin-Ciocalteu reagent	19
Esterase		Nitrophenol produced by reaction read in optical cuvette (420 mμ) at regular intervals for 6 min	20
Cytochrome oxidase		Reduced cytochrome *c* read in optical cuvette (550 mμ) at regular intervals for 3 min	1, 9

The high rate of spontaneous hydrolysis of *o*-nitrophenyl acetate used as substrate for the measurement of esterase made it impossible to run the reaction under acid conditions and to study the influence of pH on the activity of the enzyme. The contribution of the enzyme to the hydrolysis of the substrate was followed spectrophotometrically at 420 mμ, against a blank containing all components except enzyme. Many solutes, including sucrose, were found to affect the spontaneous rate of hydrolysis of the ester, and blank mixtures were carefully adjusted to the composition of the enzyme preparation.

Total Enzymatic Activities

In Table VI are listed the values obtained for the total enzymatic activities, as measured on normal rats by the methods just described. Values from the literature for lymphoid tissues and liver are given for comparison. In general, the hydrolase activities of spleen compare well with those of liver, but its cytochrome oxidase content is much

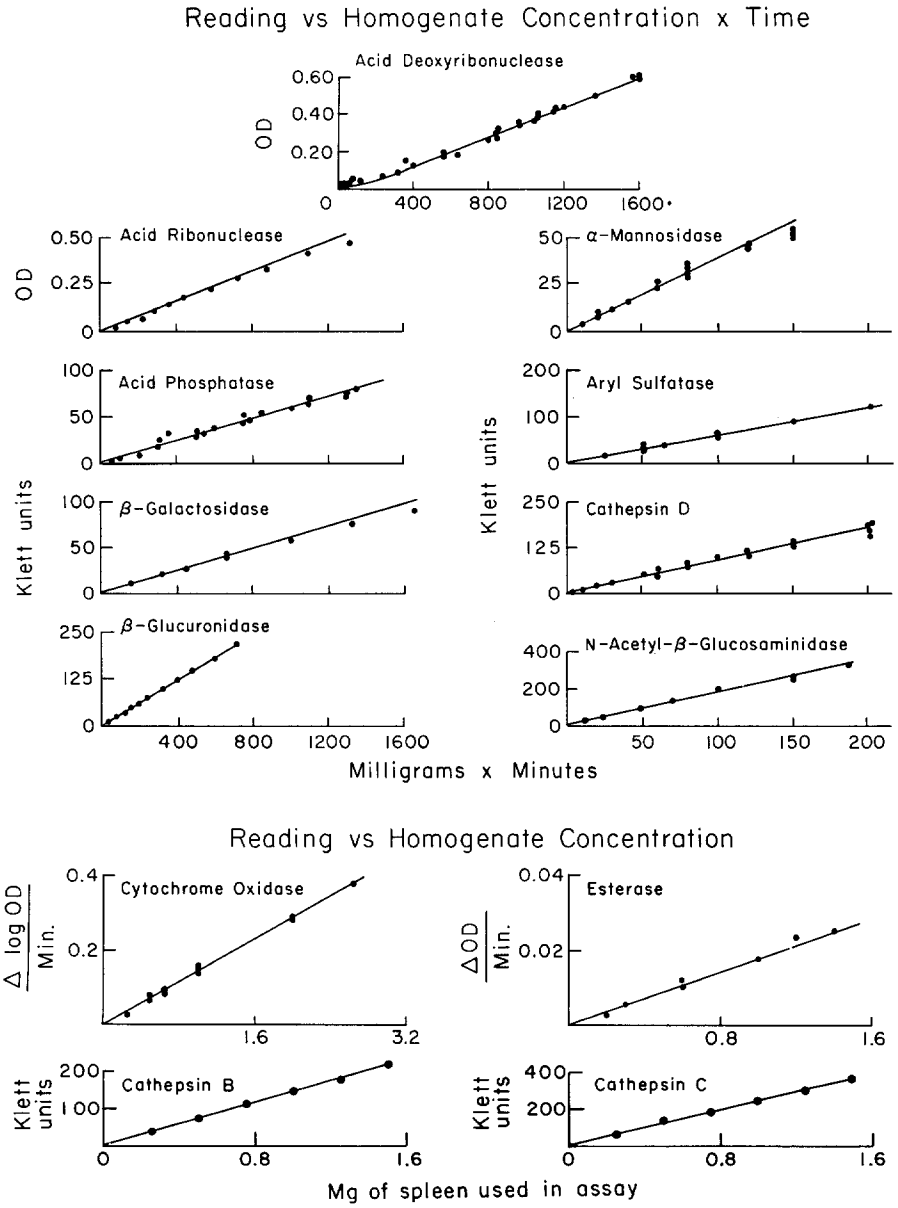

FIGURE 1 Kinetics of spleen enzymes. Upper group of graphs refers to experiments in which incubation time and concentration of homogenate were both varied with the same preparation. Lower group of graphs refers to experiments in which either reaction rates were followed continuously (esterase and cytochrome oxidase) or a single incubation time of 60 min was adopted (cathepsins B and C), with different amounts of the same homogenate. Enzymes were assayed under conditions described in Tables IV and V. OD, optical density; Klett units refer to readings in the Klett-Summerson photocolorimeter.

lower than that of liver. Too few determinations were made on thymus to allow meaningful comparisons, but it would appear that thymus is poorer than spleen in most acid hydrolases.

Enzymatic activities calculated in units per gram of protein are presented in Table VII. Included in this Table are the values obtained for several preparations of teased thymus cells.

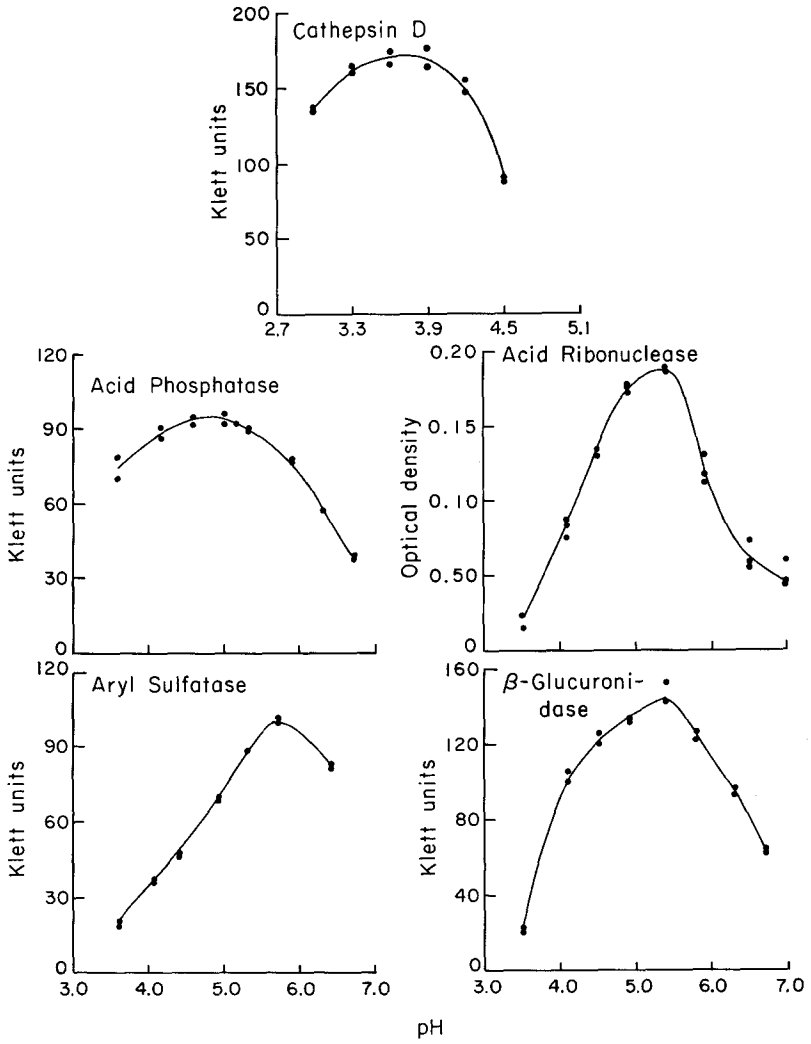

FIGURE 2 Influence of pH on activity of spleen enzymes. The universal ABC (acetate-borate-cacodylate) buffer of de Duve et al. (12) was employed for all enzymes except cathepsin D, for which a 0.1 M lactate-acetate mixture buffered to the required pH with HCl was used. For definition of ordinates, see Fig. 1.

Latency

Table VIII summarizes the values obtained in free activity measurements carried out on fresh spleen homogenates. All the hydrolases tested displayed latency, but in a proportion which varied between 40 and 70% of the total activity, depending on the enzyme. From the larger series of measurements made on acid phosphatase it appears that latency may be retained slightly better in 0.25 M sucrose than in 0.15 M KCl.

The phenomenon of latency has not yet been investigated systematically, but a few experiments suggest that it can be explained, as in liver homogenates, by the presence around the particles containing the acid hydrolases of a membrane essentially impermeable to externally added substrates. The results of Fig. 3 show that Triton X-100, which suppresses latency, also causes the release of bound hydrolases in soluble form, presumably by disrupting the particle membrane. The osmotic sensitivity of the particles is illustrated in Fig. 4. Finally, the results shown in Fig. 5 demonstrate that the proportion of reactive β-glucuronidase activity is not increased by raising the sub-

TABLE VI

Total Activities, Units per Gram Wet Weight

One unit of activity is defined, for the hydrolases, as the amount of enzyme necessary to split one μmole of substrate or to release one μmole of product per minute under the conditions defined in Tables IV and V [products are estimated as mononucleotides for the nucleases, and as tyrosine equivalents for cathepsin D, as explained by de Duve et al. (13)]. The unit of cytochrome oxidase activity is that defined by Cooperstein and Lazarow (9, see also reference 13). Values given are means ± standard deviation.

	Spleen				Thymus				Liver	
			From literature				From literature		From literature	
Enzyme	No. of exps.	U/g	U/g	Ref.	No. of exps.	U/g	U/g	Ref.	U/g	Ref.
Acid phosphatase	61	5.25 ± 1.17	7.3 ± 2.7	4	1	1.46	1.39	4	7.29 ± 0.79	5
									6.05 ± 1.3	13
Aryl sulfatase	20	1.64 ± 0.63			2	0.54 ± 0.02			3.60 ± 0.3	31
Acid deoxyribonuclease	28	4.45 ± 1.59			1	1.18			1.31 ± 0.36	13
Acid ribonuclease	24	5.58 ± 1.22			1	0.78			2.70 ± 0.64	13
β-Glucuronidase	15	1.01 ± 0.19	0.8	24	2	0.25 ± 0.07	0.32	24	0.82	7
									0.78 ± 0.11	13
β-Galactosidase	11	0.78 ± 0.33	0.58	6	1	1.26			0.34	6
									0.34 ± 0.08	35
N-acetyl-β-glucosaminidase	5	1.76 ± 0.60	2.7	6	1	1.80			5.52	6
									6.90 ± 1.1	35
α-Mannosidase	7	0.73 ± 0.32	0.22	6	1	0.36			0.62	6
Cathepsin B	4	2.07 ± 0.17	8.1 ± 1.4	4			1.78	4	1.68 ± 0.30	5
Cathepsin C	3	4.00 ± 0.75	3.9 ± 1.2*	4			1.01	4	6.75 ± 0.42	5
Cathepsin D	18	2.52 ± 1.02	4.1 ± 0.9	4	2	1.92 ± 0.15	2.67	4	1.23 ± 0.07	4
									1.46 ± 0.40	13
Esterase	4	31.2 ± 6.9			1	16.8			~120.0	36
Cytochrome oxidase	9	6.21 ± 1.98			1	4.22			30.6 ± 9.8	13
Protein	34	0.163 ± 0.087‡			2	0.079 ± 0.001‡				

* Glycyl-phenylalanyl amide as substrate.

‡ g protein/g wet weight.

TABLE VII

Total Activities, Units per Gram Protein

For definition of units, see Table VI. Values given are means ± standard deviation.

Enzyme	Spleen		Thymus		Teased thymus cells	
	No. of exps.	U/g protein	No. of exps.	U/g protein	No. of exps.	U/g protein
Acid phosphatase	6	33.0 ± 10.2	1	18.2	3	14.2 ± 4.23
Aryl sulfatase	8	9.83 ± 3.22	2	6.80 ± 0.42	3	3.35 ± 0.78
Acid deoxyribonuclease	10	23.9 ± 5.37	1	14.8	3	7.58 ± 2.78
Acid ribonuclease	6	29.6 ± 14.7	1	9.75	3	6.00 ± 1.16
β-Glucuronidase	6	6.94 ± 1.61	2	3.20 ± 0.96	3	2.44 ± 0.78
β-Galactosidase	3	3.75 ± 0.92	1	15.7	3	9.66 ± 2.94
N-acetyl-β-glucosaminidase	3	13.6 ± 3.13	1	22.5	3	10.6 ± 3.29
α-Mannosidase	4	4.36 ± 2.64	1	4.50	3	3.14 ± 0.83
Cathepsin B	4	12.7 ± 1.00				
Cathepsin C	3	24.6 ± 4.60				
Cathepsin D	8	18.3 ± 0.79	2	24.1 ± 2.07	3	16.9 ± 3.95
Esterase	4	231 ± 23.4	1	210	2	139 ± 51.1
Cytochrome oxidase	9	12.8 ± 3.57	1	52.7		

TABLE VIII

Free Activities

Homogenization was performed in all cases with the homogenizer of Dounce et al. (14). Values given are means ± standard deviation.

Enzyme	Free activity, % of total activity			
	In 0.25 M sucrose		In 0.15 M KCl	
	No. of exps.	Free activity	No. of exps.	Free activity
Acid phosphatase	24	51.8 ± 5.4	15	55.3 ± 6.2
Aryl sulfatase	1	30.5		
Acid deoxyribonuclease	4	44.8 ± 15.0	1	55.9
Acid ribonuclease	5	45.6 ± 6.0		
β-Glucuronidase	7	54.0 ± 4.5	1	55.0
β-Galactosidase	1	55.8		
N-acetyl-β-glucosaminidase	1	45.8		
α-Mannosidase	1	59.0		
Cathepsin D*	2	39.6 ± 16.1		

* Measured at pH 5.0.

strate concentration to several times the Michaelis constant of the enzyme. Such an increase would be expected if the particle membrane were sufficiently permeable to the substrate to allow internal enzyme molecules to contribute significantly to the free activity (11).

DISCUSSION

By establishing valid assay conditions for a dozen spleen hydrolases known or suspected to be associated with lysosomes in other tissues, the results described in the present work have paved the way to a comprehensive biochemical investigation of these particles in lymphoid tissues. Preliminary data indicating that the enzymes are associated at least partly with particles possessing the typical latent properties of lysosomes have also been collected, and methods have been worked out for the homogenization of the tissues without excessive damage to the particles. Further results obtained

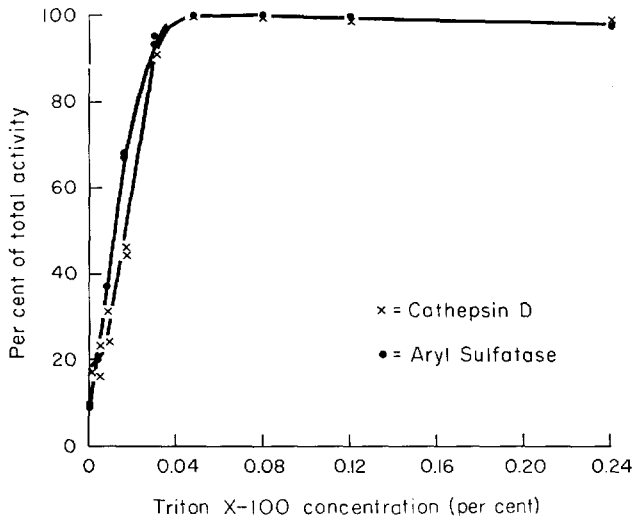

FIGURE 3 Influence of concentration of Triton X-100 on release of cathepsin D and aryl sulfatase in fresh spleen homogenate. Unsedimentable activities were measured on supernatant separated by centrifuging homogenate for 30 min at 40,000 *g* after addition of Triton X-100 to concentration indicated, and are expressed as percentage of total activity.

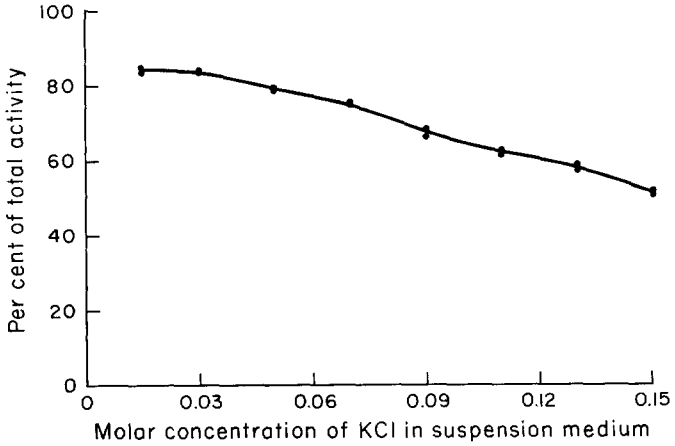

FIGURE 4 Influence of temporary exposure to different KCl concentrations on free acid phosphatase activity of fresh spleen homogenate. Portions of the same homogenate were kept for 15 min at 0°C at KCl concentration indicated, and were then brought back to the initial 0.15 M concentration and analyzed for free and total acid phosphatase activity.

with these techniques are described in the following papers of this series.

If account is taken of the differences in some of the assay techniques employed, the quantitative results recorded in this paper agree fairly well with those of other workers (see Table VI). The greatest discrepancy occurs with the results of Bouma and Gruber (4) whose values for cathepsin B in spleen are four times as high as ours, even though the techniques used by both groups are almost identical and lead to the same values for cathepsin C (in our experience, glycyl-phenylalanine amide, the substrate used by Bouma and Gruber, gives slightly lower values for the latter enzyme than glycyl-tyrosine amide). Most probably, the cause of this difference lies in the animals themselves, but it must be pointed out that we found commercial lots of benzoyl-L-arginine amide, the substrate for

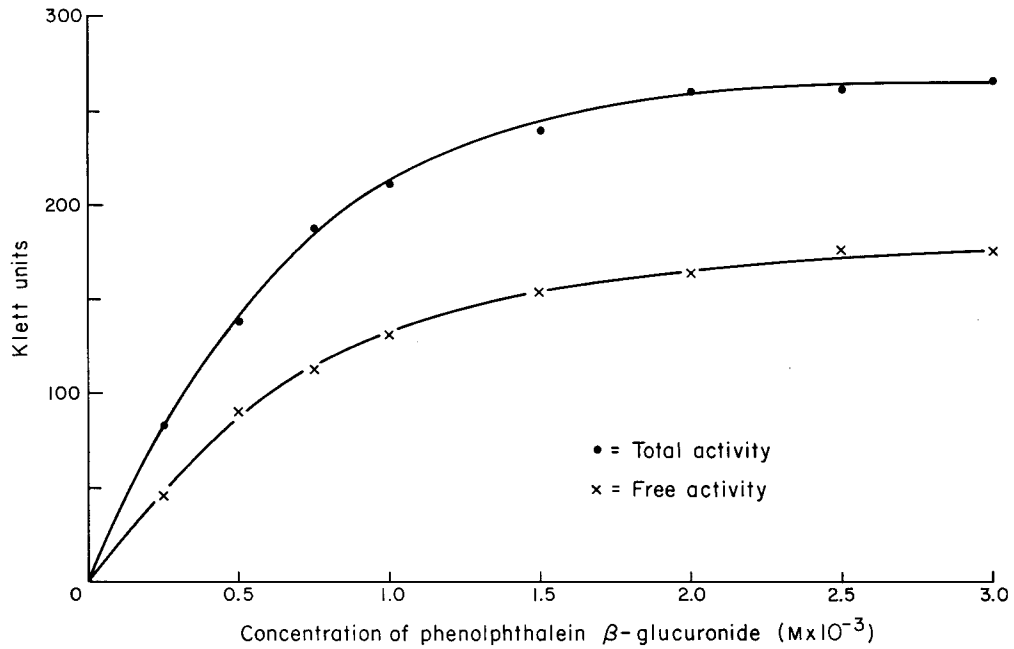

FIGURE 5 Influence of substrate concentration on free and total β-glucuronidase activity in fresh spleen homogenate.

cathepsin B, heavily and variously contaminated with free ammonia. All our substrates were purified to give very low ammonia blanks.

Our results on enzyme latency agree with the findings of Conchie and Hay (6) and Levvy and Conchie (22) who have noted the partial latency and sedimentability of five glycosidases in rat-spleen homogenates. However, these authors report higher free-activity values than those recorded in this paper and offer some evidence that the particle membrane may have a significant degree of permeability to the substrates. In our opinion, these discrepant results are probably due to the use by the Scottish workers of Potter-Elvehjem homogenizers and of longer incubation times. Our own results on β-glucuronidase (Fig. 6) suggest that the membrane of *intact* particles is essentially impermeable to phenolphthalein β-glucuronide. In this and several other respects, the spleen particles resemble liver lysosomes. They could differ from the latter by a greater mechanical sensitivity, but this point will bear further investigation, in view of the results indicating that a preliminary mincing of the tissue may allow the preparation of homogenates almost comparable to hepatic homogenates in terms of latency of acid hydrolases. This suggests that the mechanical resistance of the tissue as a whole, rather than the fragility of the particles themselves, may, by imposing the use of greater disruptive forces, be the main factor responsible for the high degree of particle breakage in spleen homogenates.

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