- Bray, H. G., Hybs, Z., James, S. P. & Thorpe, W. V. (1953). *Biochem. J.* **53**, 266.
- Dippy, J. F. J. (1939). *Chem. Rev.* **25**, 151.
- Dyson, G. H., George, H. J. & Hunter, R. F. (1926). *J. chem. Soc.* p. 3043.
- Parke, D. V. & Williams, R. T. (1951). *Biochem. J.* **48**, 624.
- Parke, D. V. & Williams, R. T. (1955). *Biochem. J.* **59**, 415.
- Pauly, H. (1921). *Z. anorg. Chem.* **119**, 271.
- Roberts, E. & Turner, E. E. (1927). *J. chem. Soc.* p. 1843.
- Robinson, D., Smith, J. N. & Williams, R. T. (1953). *Biochem. J.* **55**, 151.
- Seikel, M. K. (1944). *Org. Synth.* **24**, 47.
- Spencer, B. & Williams, R. T. (1950*a*). *Biochem. J.* **47**, 279.
- Spencer, B. & Williams, R. T. (1950*b*). *Biochem. J.* **47**, 284.
- Stekol, J. A. (1936). *J. biol. Chem.* **113**, 279.
- West, H. D. & Mathura, G. R. (1954). *J. biol. Chem.* **208**, 315.
- Zbarsky, S. H. & Young, L. (1943). *J. biol. Chem.* **151**, 211.

Tissue Fractionation Studies

3. FURTHER OBSERVATIONS ON THE BINDING OF ACID PHOSPHATASE* BY RAT-LIVER PARTICLES

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A considerable part of the unspecific acid phosphatase of rat liver is bound to cytoplasmic granules which are recovered mainly with the mitochondria, but also to some extent with the microsomes (Berthet & Duve, 1951). Recent experiments have shown that these granules form a separate group, entirely distinct from the cytochrome oxidase-containing mitochondria and from the glucose 6-phosphatase-bearing microsomes (Duve, Gianetto, Appelmans & Wattiaux, 1953; Appelmans, Wattiaux & Duve, 1955).

Fresh preparations show very little enzymic activity when assayed under conditions which preserve their morphological integrity; they are easily activated by a number of treatments, which all cause the simultaneous release of the enzyme in soluble form. Investigations bearing on this phenomenon led to the conclusion that acid phosphatase is retained within the granules by a membrane-like barrier, impermeable to sucrose and glycerophosphate, but not to a number of other substances, such as glycerol, glucose and various inorganic salts (Berthet, Berthet, Appelmans & Duve, 1951). The granules appeared to behave as typical osmotic systems.

The present paper deals with a more detailed study of the facts observed previously. In general, the new results afford additional support to the earlier conclusions. However, some of the concepts on which these were based have had to be re-examined.

METHODS

Washed mitochondria were isolated from 0.25M sucrose homogenates of rat liver by a slight modification of the method described previously (Berthet & Duve, 1951). The pressing of the liver through a wire mesh was omitted

and higher field-time values were used to ensure a better yield of phosphatase-containing granules. Using a Spinco Model L preparative ultracentrifuge, with rotor no. 40 ($r_{\max} = 8.1$ cm., $r_{\min} = 4.8$ cm.), the 1:10 cytoplasmic extract, free of nuclei, was usually centrifuged at 160000 g-min.; ($S_{\min} = 3500S$); the resuspended particles were washed twice at 130000 g-min. ($S_{\min} = 4400S$). (The use of the composite unit g-min. has been proposed by Duve & Berthet (1953). It is necessitated in the present case by the fact that the centrifugings are performed essentially by means of up-and-down runs, full advantage being taken of the force developed during acceleration and deceleration. The term $S_{\min}$ refers to the sedimentation constant, expressed in Svedberg units (10^{-13} sec.), of the lightest spherical particles which are completely sedimented under the conditions used.)

Acid phosphatase was measured as before, with β -glycerophosphate as substrate. Various commercial preparations were used, after it had been ascertained by means of periodate titration that they contained no more than 5% of the α isomer (which, unlike the β isomer, is significantly hydrolysed by the microsomal glucose 6-phosphatase).

Most of the assays were carried out in 10 min. at pH 5 and 37°, or for longer periods at pH 6.1 and 0°. As already shown (Berthet & Duve, 1951), little thermal activation occurs under these conditions, allowing for a selective estimation of the amount of free enzyme present in the incubation mixture. The total enzyme content of the preparations was measured in a similar manner on suspensions previously treated for 3 min. in a Waring Blendor (usually after tenfold dilution with distilled water), care being taken to prevent excessive heating of the preparation during this treatment. The reaction was stopped by the addition of 8% (w/v) trichloroacetic acid and inorganic P estimated on the filtrates by the method of Fiske & Subbarow (1925).

RESULTS

Osmotic activation

As reported previously (Berthet *et al.* 1951), treatment of granules with distilled water causes a rapid release of their acid phosphatase activity. The same

* Part 2 of this series: Berthet, Berthet, Appelmans & Duve (1951).

phenomenon occurs, but in a more progressive manner, when the granules are kept at 0° in 0.25M solutions of various ionized or non-ionized substances. The rate of release at 0° is negligible in 0.25M sucrose or β -glycerophosphate, or in media containing 0.25M sucrose in addition to a substance such as NaCl or KCl, which alone is unable to prevent the activation. The osmotic properties of the granules were deduced from the results of these experiments.

In most of the investigations mentioned above, the release of the phosphatase was followed by determining the amount of unsedimentable enzyme present after centrifuging off the particulate material at high speed. Owing to adsorption phenomena, this method does not measure the total activity set free. This drawback has been overcome in the present studies by assaying the free activity of the preparations in the presence of the particles. In addition, advantage has been taken of a technique involving the graded osmotic lysis of the granules, to investigate some of the finer aspects of this phenomenon.

Graded osmotic lysis. Fig. 1 shows the influence of sucrose concentration on the rate of liberation of inorganic P by granules incubated at pH 6.1 and 0° in the presence of 0.0125M sodium β -glycerophosphate. The reaction proceeds linearly with time in all cases, but at a rate which increases with decreasing sucrose concentration. Thus each decrease in sucrose concentration causes the rapid release of

a finite fraction of bound enzyme, and no additional activation occurs during the subsequent incubation.

The general shape of the activation curve is shown in Fig. 2, in which the rates, expressed in percentage of the maximal rate achieved by the blender-treated preparation, are plotted against sucrose concentration. This graph includes the results of a parallel experiment made in the presence of 10^{-4} M 2,4-dinitrophenol. It is seen that this compound, which has been found to activate the latent ATPase of mitochondria (Hunter, 1951; Potter & Recknagel, 1951; Lardy & Wellman, 1953), is without effect on the osmotic activation of acid phosphatase.

Osmotic protection by glycerophosphate. In a similar experiment, run at two different concentrations of glycerophosphate, the curve obtained with the higher substrate concentration was parallel to the other, but shifted to the left by an abscissa value corresponding to a difference in sucrose concentration of approximately 0.03M (Fig. 3). Since this shift is due to the presence of 0.0125M glycerophosphate in excess, it follows that 12.5 μ moles of glycerophosphate show the same protective effect on the granules as 30 μ moles of sucrose. At pH 6.1, 12.5 μ moles of glycerophosphate ($pK_a = 6.37$, according to Kiessling, 1934) are equilibrated by 17.1 μ equiv. of sodium, and will create an osmotic pressure equal to that of 29.6 μ moles of sucrose dissolved in the same volume. The data of Fig. 3, therefore, demonstrate in a quantitative manner that the protection afforded by glycerophosphate is of osmotic nature, a conclusion which had already

Fig. 1. Influence of sucrose concentration on the acid phosphatase activity of a mitochondrial preparation. Incubation at 0° in 0.0125M sodium β -glycerophosphate, pH 6.1, and sucrose at concentration indicated.

Fig. 2. Osmotic activation curve. ●, data of Fig. 1; ○, duplicate experiment, with 10^{-4} M 2,4-dinitrophenol.

been drawn from earlier experiments in which large amounts of glycerophosphate were used.

Transient protection by monovalent salts. The effect of inorganic salts has also been studied by the new technique. Fig. 4, which should be compared with Fig. 1, shows the rate of liberation of inorganic P when 0.15M-NaCl is present in addition to the other components. The rates are initially equal, but

Fig. 3. Influence of glycerophosphate concentration on the osmotic release of acid phosphatase. Experimental conditions as in Figs. 1 and 2, with substrate concentrations indicated.

Fig. 4. Transient osmotic protection by NaCl. Experimental conditions as in Fig. 1, but with 0.15M-NaCl in all samples.

increase progressively in the samples which are hypotonic with respect to sucrose, to reach finally the value which would have obtained immediately if no NaCl had been present. Obviously, the salt affords only a transient osmotic protection, which

Fig. 5. Osmotic activation in solutions of KCl. Preincubation at 0° and pH 6.1 (0.002M cacodylate buffer) in KCl at concentration indicated on graph. Free acid phosphatase measured in 10 min. at pH 5 and 37° in 0.05M glycerophosphate, 0.05M acetate buffer, 0.25M sucrose and KCl at the concentration used for the preincubation.

Fig. 6. Irreversibility of osmotic activation. Preincubation 30 min. at 0° in sucrose concentration indicated on the abscissa. Free acid phosphatase measured in 10 min. at 37° in 0.006M glycerophosphate adjusted to pH 5 and sucrose at same concentration as during preincubation (●) or 0.25M (○).

disappears as the concentration within the granules becomes equilibrated by diffusion with that in the medium.

In the experiment of Fig. 5, the granules were kept at 0° and pH 6.1 in two different concentrations of KCl, sucrose being 0.025M in the mixture. Free acid phosphatase was measured at regular intervals at pH 5 and 37°. The substrate added contained KCl at the same concentration to prevent the bursting of salt-enriched granules (Berthet *et al.* 1951). The results are consistent with the hypothesis that diffusion is the factor limiting the rate of activation when the granules are suspended in salt solutions. However, attempts to measure quantitatively the permeability constant of the granules to various salts in experiments of this type failed, owing to the difficulty of establishing the exact relationship between the penetration of salt and the release of enzyme.

Irreversibility of osmotic activation. In order to study the possible degree of reversibility of the activation which occurs in hypotonic media,

duplicate samples of granules were preincubated for 30 min. at 0° in the presence of different sucrose concentrations, following which the free enzyme was estimated at pH 5 and 37°. Dilute glycerophosphate was used, in order to minimize the contribution of the substrate to the osmotic pressure. In one series, the sucrose concentration was kept constant during the preincubation period and the actual assay. In the other, sufficient sucrose was added with the substrate to make its concentration 0.25M in all cases during the enzyme test. As is shown in Fig. 6, this procedure did not decrease the amount of enzyme made available by hypotonic conditions and it may be concluded that osmotic activation is entirely irreversible.

Reproducibility of osmotic activation. In Fig. 7, the results of six different experiments have been plotted against the minimum effective osmotic pressure of the medium to which the granules had been exposed. Some differences appear to exist between individual preparations with respect to their resistance to osmotic disruption. However,

Fig. 7. Reproducibility of osmotic activation. ●, experiment of Fig. 2; ⊙, same with 10^{-4} M dinitrophenol; ○, experiment of Fig. 3, 0.0125M glycerophosphate; ×, experiment of Fig. 6; ▲, △, data of three experiments, in which the free activity was measured in 10 min. at 37° and pH 5 in 0.25M sucrose, after 15 min. exposure at 0° to varying osmotic pressures. For the experiments of Figs. 2 and 3, the glycerophosphate present has been considered as iso-osmolar sucrose in the abscissa.

differences in technique may have to be considered as well, for the two upper curves in Fig. 7 were obtained at 0° and pH 6.1, whereas the four lower ones were established at 37° and pH 5. As will be shown below, there is some evidence of a greater contribution of the internal enzyme to the free activity at pH 6.1.

Thermal activation

Berthet *et al.* (1951) have shown that granules incubated at 38° in isotonic sucrose progressively release acid phosphatase in unsedimentable form. However, the true amount of enzyme set free was not measured and no attention was paid to the pH of the incubation mixture. It was therefore decided to repeat these experiments under more controlled conditions.

Effect of pH. Equal quantities of a mitochondrial suspension were incubated at 37° in individual test

tubes, containing 0.25M sucrose and either 0.1M cacodylate buffer, pH 6.1, or 0.1M acetate buffer, pH 5. The free activity was measured at regular intervals by adding equal volumes of a mixture containing 0.1M glycerophosphate adjusted to the same pH and 0.25M sucrose, and incubating for 10 min. Similar tests were made on blender-treated preparations. Fig. 8 shows the results of a representative experiment of this type.

It is seen that the activation proceeds more rapidly at pH 5 than at pH 6.1. This difference is not, however, apparent during the early phase of the process, and the initial free activity of the preparation is actually higher at pH 6.1 than at pH 5. As will be shown later (Fig. 9) this discrepancy is explained by the difference in the pH/activity curves of the free and total activities and it may be concluded that thermal activation is at all times more rapid at pH 5 than at pH 6.1.

Fig. 8. Influence of pH on thermal activation. For experimental conditions, see text. ○, intact granules, pH 5; ●, intact granules, pH 6.1; □, blender-treated granules, pH 5; ■, blender-treated granules, pH 6.1.

Kinetic properties of the free enzyme

As shown in this and previous papers, homogenates or mitochondrial preparations always contain a certain amount of free phosphatase, representing usually 10–20 % of the total activity or sometimes more if the granules have been damaged in the course of isolation. Only part of this activity is recovered in soluble form when the granules are centrifuged off, but strong evidence was obtained that the part which remains attached to the sediment is fundamentally different from the native bound form and must be considered as free enzyme secondarily adsorbed to the surface of the granules, or perhaps also partly trapped within 'ghosts' of injured granules (Berthet *et al.* 1951). In the present study an attempt has been made to compare the kinetics of the free activity of mitochondrial suspensions with those of completely disrupted preparations.

Influence of substrate concentration. Raising the concentration of glycerophosphate, even within the range where the enzyme is almost saturated, hardly

Table 1. Influence of substrate concentration on free and total activity at pH 5

Free activities measured on intact granules, incubated 10 min. at 37° in 0.25M sucrose and 0.05M acetate buffer, pH 5; total activities measured similarly on blender-treated granules. Results expressed as $\mu\text{g. P/10 min./g. liver}$.

Glycero-phosphate concn. (m-moles/l.)	Expt. 1 ^a			Expt. 2		
	Total activity	Free activity	Free activity (% of total)	Total activity	Free activity	Free activity (% of total)
3	300	44.6	14.9	297	40.3	13.6
6	460	54.5	11.8	435	51.7	11.9
12	600	62	10.3	623	67.5	10.8
24	745	71.3	9.6	802	87.8	10.9
36	855	82.1	9.6	920	102	11.1
60	935	89	9.5	1028	120	11.7
90	955	97.5	10.2	1070	131	12.2
120	958	99	10.3	1082	133	12.3

causes a greater proportion of the total activity to appear as 'free' enzyme. This fact, which is shown clearly in Table 1, furnishes strong corroborative evidence of the essential impermeability of the granules to glycerophosphate. A slight relative increase of free activity is noted at very low substrate concentrations, suggesting that glycerophosphate may, by itself, exert some kind of action on the granules. This action could possibly affect their permeability, but a more likely explanation is

that it consists simply in a protective effect against thermal activation during the assay.

Influence of pH. As is shown in Fig. 9, a distinct difference between free and total enzyme becomes evident when the pH is varied. As a result, the proportion of total enzyme estimated as free activity is not constant, but varies from 9% at pH 5 to 14% at pH 6.1 and 23% at pH 6.9. An increase in the rate of destruction of the granules during the assay can hardly account for these results, since a less acid pH value should have a protective effect (Fig. 8). On the other hand, this explanation could account for the slight relative increase in free activity observed in the very acid range.

Influence of temperature. The temperature coefficient of the reaction was measured at pH 6.1 in order to minimize the danger of activation during the enzyme assay. The liberation of inorganic P proceeded linearly with time, even with the suspension of granules, and made it possible to obtain accurate values for the velocity of the reaction at each temperature. These values furnish very satisfactory Arrhenius plots, as shown in Fig. 10. However, the slope of the two lines is not the same. Using the method of least squares, it was calculated that the energy of activation is $10\,300 \pm 250$ cal./mole for the blender-treated preparation, and $11\,800 \pm 250$ cal./mole for the free activity of the intact suspension. The difference between the two values is statistically significant ($P < 0.01$).

DISCUSSION

The new data presented here are compatible with the sac-like representation proposed in an earlier paper for the acid phosphatase-containing granules (Berthet *et al.* 1951). However, since it has now been found that these granules are entirely distinct from those which contain cytochrome oxidase (Duve *et al.* 1953; Appelmans *et al.* 1955), the conclusions arrived at cannot any more be taken to apply to the mitochondria in general. Conversely, morphological observations may have no bearing on the properties of the acid phosphatase-containing granules, for the latter are probably much less abundant than the oxidizing type in the usual mitochondrial fractions. As was pointed out previously, the two approaches lead to similar conclusions with respect to the permeability properties of the 'mitochondrial' membrane. Moreover, examples of structural hindrance, recalling the facts observed with acid phosphatase, have been described for other enzymes, such as glutamic dehydrogenase (Hogeboom & Schneider, 1953), aconitase (Dickman & Speyer, 1954) and succinoxidase (Tyler, 1954), which probably belong to true mitochondria. These facts suggest that the

Fig. 9. Influence of pH on free and total activity. Assays performed in 10 min. at 37°, in 0.05 M glycerophosphate, 0.05 M acetate and 0.05 M cacodylate, adjusted to desired pH. ○, intact granules, assayed in 0.25 M sucrose; ●, blender-treated granules.

Fig. 10. Influence of temperature on free and total activity at pH 6.1. ○, free activity measured on intact granules incubated in 0.05 M glycerophosphate, pH 6.1, and 0.25 M sucrose; ●, total activity, measured similarly on blender-treated granules.

two types of granules may not be too dissimilar, but the possibility remains that significant differences may show up in other studies. For instance, the reversibility of the water activation of succinoxidase, observed by Tyler (1954), is in contrast with the essential irreversibility of the osmotic release of acid phosphatase, reported in this paper.

Some additional comments are necessary with respect to the interpretation of the data. In the earlier work, a sharp distinction was made between free and bound activity. The former was attributed exclusively to diffusible enzyme, either present in true solution or adsorbed upon the outer surface of particulate material, whilst the enzyme present in intact granules was taken to be entirely inaccessible to its substrate, owing to the impermeability of the membrane to β -glycerophosphate. The fact that no release of enzyme occurred when the granules were kept in 0.25M glycerophosphate was brought forward in support of this contention. In the present work, the osmotic nature of the protection afforded by glycerophosphate has been established in a quantitative manner with small concentrations of substrate, thus strengthening the earlier evidence. However, attention must be called to a possibility which was not taken into account in the previous reasoning.

It is true for any inert substance that it can only afford osmotic protection if it is unable to penetrate through the membrane. Otherwise it will enter together with sufficient water to maintain osmotic equilibrium, and will cause the granules to swell as they do in distilled water, but at a rate limited by the rate of diffusion of the solute. Examples of this phenomenon have been described by Berthet *et al.* (1951) and in the present paper (Figs. 4, 5). However, if the solute which penetrates is altered inside the granules and converted into products for which there exists a negative concentration gradient, causing them to diffuse out of the granules, perfect osmotic protection remains compatible with some degree of permeability, provided the action of the enzyme and the back-diffusion of the products of reaction are able to keep pace with the inward diffusion of the substrate, thus preventing the latter from accumulating within the granules.

Since this could be the case for glycerophosphate, the possible contribution of the enzyme within the particle to the free activity must be re-examined. A consideration of all the data available indicates that this contribution, if it exists at all, must be very small at pH 5. The free activity under these conditions can be less than 10 % of the total activity and is due mainly to dissolved and adsorbed phosphatase (Berthet *et al.* 1951), leaving very little for the possible contribution of the internal enzyme. In addition, if the latter were somewhat accessible

to external substrate, one would expect its contribution to increase with increasing glycerophosphate concentration. That such is not the case is indicated by the results of Table 1.

On the other hand, a few facts suggest that the inaccessibility between internal enzyme and external substrate may become less complete as the pH increases. At least such a hypothesis could account for the anomalous pH/activity curve of the free activity (Fig. 9) and for the difference in the temperature coefficients of the free and total activities, as measured at pH 6.1 (Fig. 10). It is possible that the bivalent glycerophosphate ion enters the granules more easily than the univalent ion. Of interest in this respect is the finding by Cleland (1952) that isolated sarcosomes are more permeable to the bivalent than to the univalent inorganic phosphate ion. Dickman & Speyer (1954) have recently reported a similar difference in the pH/activity curve of mitochondrial and soluble aconitase, which they also ascribe to selective permeability. In their case, the permeability to isocitrate appeared to decrease with increasing pH.

Attention must be called to the peculiar shape of the osmotic activation curves described in this paper. As is shown in Fig. 7, the process is very gradual and the lysis curves, unlike those obtained in studies on erythrocytes (Hunter, 1940), extend practically over the entire range of osmotic pressure below isotonicity. This fact suggests that great differences must exist between individual granules with respect to their resistance to osmotic disruption.

Thermal activation has been ascribed to autolytic changes of the membrane, possibly occurring in an autocatalytic manner (Berthet *et al.* 1951). The effect of pH on this process (Fig. 8) is consistent with the above hypothesis, since the preparation contains a cathepsin, with a strongly acid pH optimum, which is released in the same manner as acid phosphatase (Duve *et al.* 1953). However, if the observation of Cleland (1952) that the permeability of heart sarcosomes to sucrose increases with decreasing pH is applicable to liver granules, the phenomenon could also be osmotic in nature and occur as a consequence of the penetration of sucrose and water within the particles.

SUMMARY

1. The osmotic activation of the bound acid phosphatase of rat liver has been studied as a function of the sucrose concentration of the medium to which the granules are exposed. The lysis curves are S-shaped and extend over the entire range of osmotic pressure below isotonicity, indicating great variability in the resistance of individual granules to osmotic disruption.

2. The partial activation which occurs in hypotonic media takes place very rapidly, does not show a further increase if the time of exposure at 0° is prolonged and is not reversed by a return to isotonicity.

3. Inorganic univalent salts afford only transient osmotic protection. In contrast, part of the sucrose can be replaced in the medium by an iso-osmolar quantity of β -glycerophosphate, with no adverse effect on the stability of the granules. 2:4-Dinitrophenol is without significant influence on the activation of acid phosphatase.

4. The activation which occurs when the granules are incubated at 37° in 0.25M sucrose is much more rapid at pH 5 than at pH 6.1.

5. The internal enzyme appears to be entirely inaccessible to external glycerophosphate at pH 5, but seems to contribute significantly to the free activity of intact preparations as the pH is raised, or as the temperature is increased at pH 6.1.

6. The results obtained afford additional support for the sac-like representation of the acid phosphatase-containing granules arrived at in earlier work. It is pointed out that a limited degree of permeability of the membrane to glycerophosphate remains compatible with perfect osmotic protection by this compound, as long as its rate of inward diffusion does not exceed the rate at which it can be

hydrolysed within the granules and eliminated in the form of glycerol and inorganic phosphate.

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REFERENCES

- Appelmans, F., Wattiaux, R. & Duve, C. de (1955). *Biochem. J.* **59**, 438.
 Berthet, J., Berthet, L., Appelmans, F. & Duve, C. de (1951). *Biochem. J.* **50**, 182.
 Berthet, J. & Duve, C. de (1951). *Biochem. J.* **50**, 174.
 Cleland, K. W. (1952). *Nature, Lond.*, **170**, 497.
 Dickman, S. R. & Speyer, J. F. (1954). *J. biol. Chem.* **206**, 67.
 Duve, C. de & Berthet, J. (1953). *Nature, Lond.*, **172**, 1142.
 Duve, C. de, Gianetto, R., Appelmans, F. & Wattiaux, R. (1953). *Nature, Lond.*, **172**, 1143.
 Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
 Hogeboom, G. H. & Schneider, W. C. (1953). *J. biol. Chem.* **204**, 232.
 Hunter, F. E. jun. (1951). *Phosphorus Metabolism*, **1**, 377. Baltimore: The Johns Hopkins Press.
 Hunter, F. T. (1940). *J. clin. Invest.* **19**, 691.
 Kiessling, W. (1934). *Biochem. Z.* **273**, 103.
 Lardy, H. A. & Wellman, H. (1953). *J. biol. Chem.* **201**, 357.
 Potter, V. R. & Recknagel, R. O. (1951). *Phosphorus Metabolism*, **1**, 377. Baltimore: The Johns Hopkins Press.
 Tyler, D. B. (1954). *Fed. Proc.* **13**, 154.

Tissue Fractionation Studies

4. COMPARATIVE STUDY OF THE BINDING OF ACID PHOSPHATASE, β -GLUCURONIDASE AND CATHEPSIN BY RAT-LIVER PARTICLES

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In a study of the β -glucuronidase of mouse liver, Walker (1952) has described a number of observations which resemble in a striking fashion the findings made in this laboratory on the unspecific acid phosphatase of rat liver (Berthet & Duve, 1951; Berthet, Berthet, Appelmans & Duve, 1951; Appelmans & Duve, 1955). Like acid phosphatase, β -glucuronidase occurs largely in particulate form; it is partly unreactive when in the bound state and is rendered simultaneously soluble and fully active by all the treatments which have been found to cause a similar release of acid phosphatase. After it was verified in preliminary experiments that the findings of Walker (1952) also apply to the β -

glucuronidase of rat liver, it was decided to make a parallel study of the release of the two enzymes, using the methods previously worked out for the investigations on acid phosphatase.

In a search for other enzymes of the same type in rat liver, it was found that cathepsin, whose intracellular distribution recalls in some respects that of acid phosphatase (Maver & Greco, 1951), also exhibits the same general pattern of behaviour. Accordingly, the release of this enzyme was also studied in a number of comparative experiments with acid phosphatase. These studies, which have been reported elsewhere in condensed form (Duve, Gianetto, Appelmans & Wattiaux, 1953), have revealed a close parallelism, both qualitative and quantitative, in the manner of release of the three enzymes.

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