Studies on β -Glucuronidase

2. THE PREPARATION AND PROPERTIES OF THREE OX-SPLEEN β -GLUCURONIDASE FRACTIONS

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Analysis of aqueous extracts of acetone-dried ox spleen by the variable solvent solubility test, using ammonium sulphate as the precipitant and laevo-menthyl- β -D-glucuronide as assay substrate, revealed the presence of two β -glucuronidase fractions (Mills, 1948). It was possible to separate these two fractions by repeated ammonium sulphate fractionation at pH 5. The fractions so prepared had optimal activity for the hydrolysis of various glucuronides at pH 4.5 and around pH 5.2 respectively. These findings were confirmed by Kerr, Campbell & Levvy (1949) on mouse organs. On the other hand, Bernfeld & Fishman (1950a) and Sarkar & Sumner (1950) have adduced evidence which they interpret as indicating that β -glucuronidase in calf spleen and liver respectively, is a single component with a single pH optimum.

Since the publication of Mills (1948) it has become increasingly obvious that the presence of only two β -glucuronidase activities in ox spleen, with the pH optima already quoted, could not completely explain the overall β -glucuronidase activity of ox-spleen extracts.

In 1936, Oshima showed that citric and a number of other organic acids inhibited β -glucuronidase. More recently, Karunairatnam & Levvy (1949) showed that mucic and saccharic acids inhibited β -glucuronidase and they stated that D-saccharic, the stronger inhibitor of the two, acted competitively at pH 5.2 in citrate buffer. Spencer & Williams (1951) have shown glucuronate to inhibit β -glucuronidase at pH 5.2, but that its inhibitory power, while greater than that of mucate, is much less than that of saccharate. Becker & Friedenwald (1949) showed ascorbic acid and heparin to be inhibitors of β -glucuronidase at pH 4.5.

In the present paper, data will be presented which show the existence of at least three β -glucuronidase fractions in aqueous extracts of ox spleen, and some of the properties of these fractions will be described along with a study of the action of various inhibitory substances upon them. Preliminary reports of this work have already appeared (Mills & Paul, 1949; Mills, Paul & Smith, 1949).

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METHODS

Assay of β -glucuronidase preparations

(a) The method using (-)-menthyl- β -D-glucuronide has already been described (Mills, 1948).

(b) The method using phenyl- β -D-glucuronide is that described by Mills (1948) with the following slight modification. The controls for the assay are prepared by incubating enzyme preparation and buffer for the requisite time and the substrate is added immediately after the addition of the Folin-Ciocalteu reagent.

(c) The method using phenolphthalein β -D-glucuronide is that described by Mills (1948) with the following slight modification. The method originally employed was developed for highly purified preparations containing little protein. Where crude preparations and tissue homogenate were used, the relatively large amounts of protein present caused the adsorption of some phenolphthalein on the protein precipitated by trichloroacetic acid. This was overcome by stopping the reaction by the addition of 2 ml. glycine-NaOH buffer of pH 10.4 and the removal of any precipitated protein by centrifugation. Samples (2 ml.) of the supernatant were added to a further 6 ml. of the glycine-NaOH buffer of pH 10.4 and the colour determined as previously described. By this method, loss of phenolphthalein by adsorption on the precipitate is completely avoided.

When studying various inhibitory substances the enzyme digests were made up to contain 0.5 ml. buffer solution, 0.2 ml. enzyme solution, 0.1 ml. water or inhibitor solution and 0.2 ml. substrate solution in the case of the phenolphthalein glucuronide method; for the phenyl- and (-)-menthyl-glucuronide methods the digests contained 1.0 ml. buffer solution, 0.3 ml. enzyme solution, 0.2 ml. water or inhibitor solution and 0.5 ml. substrate solution.

The inhibitor solutions were freshly made immediately before use, and the pH of the inhibitor solution was adjusted to that of the assay before addition.

Unless otherwise stated, the following buffers were employed: for pH values of 3.5 and less, the veronal-acetate-HCl buffer of Michaelis (1931), for pH values 3.5-6.0, 0.2 M-acetate buffers, and for pH values of 6.0 and over, 0.1 M-phosphate-citrate buffers. pH Measurements were carried out with the glass electrode and the Muirhead, Tinsley or Marconi pH meters.

Electrophoretic analyses. These were carried out using the Tiselius (1937) apparatus employing the Philpot-Svensson (Svensson, 1939) optical system (Adam Hilger and Co. London). Illumination was provided by a mercury vapour lamp and a green (546 m μ) filter, photographic records being made on Ilford Halftone panchromatic plates. Where

solutions under illumination were red-brown or brown in colour, a yellow (577 m μ . filter and Ilford Rapid process panchromatic or H.P. 3, plates were used.

The cells used were either the double section of 11 ml. capacity or the double section of 3 ml. capacity.

Electrophoresis on filter paper. This was carried out by the method described by Mills & Smith (1951) which is based on that of Durrum (1950).

RESULTS

pH-Activity curves of unfractionated ox-spleen extracts

pH-Activity curves were determined for partially purified unfractionated ox-spleen extracts at stage C of preparation described by Mills (1948) using various concentrations of phenolphthalein β -D-glucuronide as substrate. The range of substrate

Fig. 1. pH-Activity curves of an unfractionated ox-spleen extract at various concentrations of phenolphthalein glucuronide as substrate. A, 0.00025M; B, 0.0005M; C, 0.001M; D, 0.002M; E, 0.003M; F, 0.004M.

concentrations used was 0.00025M to 0.004M and the results are recorded in Fig. 1. The most obvious feature is the change in shape of the pH-activity curve with the change in substrate concentration, particularly around pH 3.5 and pH 6-7. One might conclude from these results that there is present in ox-spleen extracts a β -glucuronidase which is active near 3.5 at low substrate concentrations and that increasing substrate concentrations cause an inhibition of this enzyme activity.

The inhibition of unfractionated ox-spleen extracts by citrate

It was first shown by Oshima (1936) that citric acid, among other organic acids, inhibited β -glucuronidase. In addition to our confirmation of this finding we have found that this inhibition only applies to the enzymes active at low pH values, the enzyme activity at 5.2 not being inhibited. This is

well illustrated by the shapes of the pH-activity curves of a crude ox-spleen extract (stage C, Mills, 1948) examined in acetate and citrate buffers, shown in Fig. 2.

Fractionation of aqueous ox-spleen extracts

Mills (1948) obtained by repeated fractionation with ammonium sulphate at pH 5 a separation of two β -glucuronidase fractions, I and II, with optimal activities for the hydrolysis of *laevo*-menthylglucuronide at pH 4.5 and pH 5.0-5.1 respectively. With fraction I, it has since been found that, using phenolphthalein glucuronide (0.001M) or phenylglucuronide (0.005M) for assay, the ratio of activities at pH 3.5 to that at pH 4.5 was significantly greater than that obtained when *laevo*-menthylglucuronide (0.005M) was used for assay. This would indicate the heterogeneity of fraction I, again suggesting an enzyme with activity around pH 3.5. In view of these findings

Fig. 2. pH-Activity curves of an unfractionated ox-spleen extract using phenolphthalein glucuronide in acetate buffer (A) and citrate buffer (B).

an attempt was made to elaborate this fractionation procedure. In place of the fractional precipitation technique, resort was made to a fractional elution technique starting with the enzyme in a completely precipitated form. It was found, as in the previous work, that pH was a critical factor in the fractionation procedure, much better separation being obtained at pH 8.5 than at lower pH values. The method employed was as follows:

The starting material was an aqueous extract of acetone-dried spleen at stage D of purification of Mills (1948). To 200 ml. of this solution was added solid $(\text{NH}_4)_2\text{SO}_4$ to a final concentration of 2.5M, the pH was adjusted to 8.5 with NH_3 and 2.0 g. Standard Super-Cel (Celite 519A, Johns-Manville Co.) were added/100 ml. solution. After standing at 0° for 2 hr., the material was filtered and the filter cake pressed to remove as much of the supernatant solution as possible. The filter cake was eluted with 4 x 40 ml. 1.8M- $(\text{NH}_4)_2\text{SO}_4$ adjusted to pH 8.5, filtering at each elution. The process was then repeated with 1.6, 1.4, 1.2 and 1.0M- $(\text{NH}_4)_2\text{SO}_4$ solutions. The enzyme in the resulting eluates

was precipitated with $(\text{NH}_4)_2\text{SO}_4$ at a final concentration of 2.5 M, centrifuged at 0° and dissolved in water. The material extracted by the 1.8 M- $(\text{NH}_4)_2\text{SO}_4$ is referred to subsequently as the 1.8 M eluate and similarly with the other extracts. The process was repeated on each eluate a further three or four times. All the original glucuronidase activity was found in those fractions between the limits 1.2 and 1.8 M- $(\text{NH}_4)_2\text{SO}_4$, the most active being the 1.4 and 1.6 M- $(\text{NH}_4)_2\text{SO}_4$ fractions.

The 1.6 M fraction obtained after three such fractionation procedures gave the pH-activity curve shown in Fig. 3, curve A. Activity of this undialysed solution was zero at pH 3.4 (Fig. 3, A);

Fig. 3. pH-Activity curves of ox-spleen fractions produced by elution fractionation. Curve A, 1.6 M- $(\text{NH}_4)_2\text{SO}_4$ fraction after three fractionations before dialysis; curve B is the same after dialysis; curve C, 1.4 M- $(\text{NH}_4)_2\text{SO}_4$ fraction after three fractionations; curve D, original unfractionated material. Assays using phenolphthalein glucuronide.

after dialysis, however, there was considerable activity at pH 3.4 (Fig. 3, curve B) with no alteration in activity at and above pH 4.4. From these experiments we conclude that there is present in ox-spleen extracts a third β -glucuronidase with a pH optimum at about 3.4 and that ammonium sulphate treatment causes a reversible inactivation of this enzyme, activity being regained on dialysis. The additional point revealed by these experiments is that the activity of the β -glucuronidase with a pH optimum at 4.5 (I) is zero at pH 3.4, so that any activity measured at pH 3.4 or below in unfractionated or partially fractionated ox-spleen preparations is confined to this new third β -glucuronidase. This phenomenon is being further investigated in view of the possibility that it is a general salt effect.

The fraction precipitated by 1.4 M-ammonium sulphate gave the pH-activity curve shown in

Fig. 3, curve C, and shows much more activity around pH 5.2 than at pH 4.5. The pH-activity curve of the original stage D preparation is shown for comparison as curve D in Fig. 3. A further two fractionations of each of the two fractions shown in Fig. 3, gave fractions having the pH-activity curves which are shown in Fig. 4, curves A and B. By this fractionation procedure it is thus possible to obtain fractions showing significantly different pH-activity curves with maxima at pH 4.5 and 5.2 respectively.

It was not possible by this fractionation procedure to obtain preparations showing a marked optimum around pH 3.4, but, using organic solvent fractionation, enzymic activity with optimum activity around this pH could be concentrated. Many

Fig. 4. pH-Activity curves of ox-spleen fractions. Curve A, 1.6 M- $(\text{NH}_4)_2\text{SO}_4$ fraction after five fractionations; curve B, 1.4 M- $(\text{NH}_4)_2\text{SO}_4$ fraction after five fractionations; curve C, fraction produced by ethanol precipitation. Assays using phenolphthalein glucuronide.

different conditions for ethanol fractionation were tried, but the method which showed most promise is outlined below.

To 100 ml. ox-spleen preparation (stage D, Mills, 1948) were added 70% ethanol, 1 M-sodium acetate and 1 N-acetic acid to a final concentration of 14% (v/v) ethanol, a pH of 5.0 and a final ionic concentration of 0.05, the temperature being maintained throughout at -5° . After standing for 30 min., the precipitate was removed in the centrifuge at -5° , dissolved in water and dialysed against distilled water to remove any ethanol. This preparation gave the pH-activity curve shown in Fig. 4, C.

The presence of a glucuronidase showing optimal activity at pH 3.4 is clearly seen but it is not claimed that this preparation represents a pure enzymic fraction. Fig. 4 shows that at pH 3.4 the enzyme with optimum activity at 4.5 can be neglected. Kinetic studies carried out on this fraction at pH 3.4 will therefore represent data for the pH 3.4 enzyme alone.

Electrophoretic examinations

An electrophoretic examination of a highly purified but unfractionated glucuronidase preparation, a mixed 1.4M and 1.6M fraction after three of the foregoing $(\text{NH}_4)_2\text{SO}_4$ fractionation procedures, was carried out at pH 8.0 in a phosphate buffer ($I=0.2$). The schlieren diagram is presented in Fig. 5. The main leading boundary (mobility $=3.1 \times 10^{-6}$ cm.²/V. sec.), represents 80% of the total protein and is obviously not homogeneous. This boundary was fractionated into three parts from front to back and all showed β -glucuronidase activity. The very low protein contents of many of the purified fractions rendered

Fig. 5. Electrophoretic analysis of highly purified unfractionated ox-spleen β -glucuronidase. A, ascending (anodic) pattern; B, descending pattern. Buffer, phosphate, pH 8, $I=0.2$.

Fig. 6. Analysis of filter paper electrophoretic run of ox-spleen β -glucuronidase preparations. Assay, using phenolphthalein glucuronide, of 5 mm. lengths of paper. Activity expressed as μ g. phenolphthalein liberated/2 hr. Buffer, phosphate, pH 8, $I=0.05$. Potential $=3$ V./cm. for 22 hr. Curve A, purified, unfractionated β -glucuronidase preparation; curve B, fraction with pH optimum at 5.2.

examination by the conventional electrophoretic method very difficult, only very small refractive index boundaries being obtainable. Electrophoresis was therefore carried out on filter-paper strips, using the method described by Mills & Smith (1951), which allows quantities of the order of 50–100 μ l. of the solution to be examined. Using this technique with a preparation giving a schlieren diagram similar to that in Fig. 5, the result shown in Fig. 6 (curve A) was obtained, where 5 mm. lengths of the paper were assayed for enzymic activity, and the activity plotted against the distance migrated towards the anode. Electrophoresis was carried out at pH 8 in phosphate buffer ($I=0.05$). In this case, where the distance migrated is much greater than in the Tiselius U-tube, the heterogeneity of the enzyme is more obvious, but it is not certain that these results may be

related to those of the classical method. The problems of adsorption on paper and protein-protein interactions in this new medium have yet to be studied. The average mobility of the enzyme activity (3.5×10^{-6} cm.²/V. sec.) is, however, close to the value obtained by the classical method. An examination of a purified fraction having maximum activity at pH 5.2 as shown in Fig. 4, curve B, gave the picture shown in Fig. 6, curve B. Here the homogeneity of the enzyme is much more pronounced than in curve A and corresponds with one of the peaks of activity of the unfractionated preparation. This is confirmatory evidence of the fractionation procedure and of the usefulness of electrophoresis on filter paper as a method of controlling such fractionation procedures.

REACTION CHARACTERISTICS OF THE SEPARATED FRACTIONS

In order to demonstrate that the three fractions obtained are not artifacts simply produced by a modification of the enzyme protein due to fractionation procedures, it is necessary to show that they have significantly different reaction characteristics apart from pH optima. We have therefore studied the effects of variation of substrate concentration upon reaction velocity, the energies of activation and the effects of various inhibitors upon the three ox-spleen β -glucuronidase fractions. From the results of these studies various reaction constants are presented which further emphasize the distinct nature of the three fractions prepared. Table 1 summarizes the pH-optimum characteristics of the three ox-spleen β -glucuronidase fractions.

Table 1. pH optima of ox-spleen β -glucuronidase fractions in 0.2M-acetate buffers

Substrate	Ox-spleen β -glucuronidase fraction		
	I	II	III
(-)-Menthylglucuronide	4.5	5.0 (5.1)*	3.4
Phenylglucuronide	4.5	5.2 (5.3)*	3.4
Phenolphthalein glucuronide	4.5	5.2 (5.3)*	3.4

* Values in 0.1M-citrate buffers.

Effect of substrate concentration on the activity of ox-spleen β -glucuronidase fractions

The effect of variation in the substrate concentration upon reaction velocity was studied for the three separated ox-spleen β -glucuronidase fractions at the optimum pH of the preparation and at 38°. The substrates examined were *laevo*-menthyl-, phenyl- and phenolphthalein glucuronides and the results are recorded in Figs. 7–9.

It will be noted from Fig. 7 that substrate inhibition is shown with phenolphthalein glucuronide and that the intensity of this substrate inhibition differs with the three fractions. Substrate inhibition is also marked in the case of *laevo*-menthylglucuro-

nide, but it is not so evident with phenylglucuronide. Kerr, Graham & Levvy (1948) showed that phenylglucuronide caused marked substrate inhibition of mouse liver and spleen glucuronidase when assayed at pH 5.2. One peculiar feature of the data of these workers is that the enzyme preparations used were completely inactive at substrate concentrations below 2.5×10^{-3} M. In the present work all enzyme preparations showed activity at any substrate concentration above zero.

Fig. 7. Relation between phenolphthalein glucuronide concentration and β -glucuronidase activity (μ g. phenolphthalein liberated/hr.) of the three ox-spleen β -glucuronidase fractions. A, pH 4.5 fraction; B, pH 5.2 fraction; C, pH 3.4 fraction.

Fig. 8. Relation between phenylglucuronide concentration and β -glucuronidase activity (μ g. phenol liberated/hr.) of the three ox-spleen β -glucuronidase fractions. A, pH 4.5 fraction; B, pH 5.2 fraction; C, pH 3.4 fraction.

Substrate inhibition in the case of phenolphthalein glucuronide has already been demonstrated by Talalay, Fishman & Huggins (1946), and in the case of (-)-menthylglucuronide by Fishman (1939). Recently, Spencer & Williams (1951) have demonstrated that *p*-chlorophenylglucuronide causes substrate inhibition of β -glucuronidase.

The variation in substrate inhibition of the three ox-spleen fractions caused by phenolphthalein glucuronide as demonstrated in Fig. 7 explains the variation in the shape of pH-activity curves of crude ox-spleen extracts seen with varying substrate concentrations (see Fig. 1).

The existence of substrate inhibition means that the evaluation of enzyme-substrate dissociation constants by the Michaelis & Menten (1913) method is not reliable since the maximum velocity of reaction observed is not the true maximum velocity and values of K_m calculated from the substrate concentration for 50% maximum velocity will be too low.

Fig. 9. Relation between (-)-menthylglucuronide concentration and β -glucuronidase activity (μ g. glucuronic acid liberated/hr.) of two of the ox-spleen β -glucuronidase fractions. A, pH 4.5 fraction; B, pH 5.2 fraction.

The present data have been analysed by the method of Lineweaver & Burk (1934) for the case of substrate inhibition where two simultaneous equilibria are proposed as existing in the enzymic reaction, namely:

where E =enzyme, S =substrate, ES =active enzyme-substrate complex and ES_n =inactive enzyme-substrate complex. Subsequently $ES \rightarrow E + \text{products}$, while ES_n is inactive.

The reaction velocity equation for this case was shown by Lineweaver & Burk (1934) to be

$$\frac{[S]}{v} = \frac{1}{V_{\max}} \left([S] + K_s + \frac{[S]^n}{K_2} \right), \quad (3)$$

where $[S]$ =substrate concentration, v =observed velocity, $V_{\max}$ =ideal maximum velocity assuming no substrate inhibition, K_s =dissociation constant of ES complex in equation 1, K_2 =dissociation constant of ES_n complex in equation 2, and n =number of substrate molecules combined to one enzyme molecule in the inactive enzyme-substrate complex ES_n (equation 2). At low substrate concentrations the value of $[S]^n/K_2$ in equation 3 will become negligibly small and in this case a plot of $1/v$ against $1/[S]$ will give a straight line with slope of $K_s/V_{\max}$ and ordinate intercept of $1/V_{\max}$. From these two figures it is possible to calculate the value of K_s , the true enzyme-substrate dissociation constant. In Figs. 10-12 the results of the experiments shown in Figs. 7-9 are plotted accordingly.

From the data obtained from Figs. 10-12 we have calculated the values of K_s for the three ox-spleen fractions and the three substrates, phenolphthalein, phenyl- and (-)-menthyl-glucuronides and the results are presented in Table 2.

In the case of substrate inhibition it is assumed that the inhibition is caused by the combination of the active enzyme-substrate complex with further molecules of substrate to form an inactive complex which does not decompose to

Fig. 10. Plot of reciprocal of reaction velocity ($1/v$) against reciprocal of phenolphthalein glucuronide concentration ($1/[S]$) for the three ox-spleen β -glucuronidase fractions. Details as Fig. 7.

Fig. 11. Plot of reciprocal of reaction velocity ($1/v$) against reciprocal of phenylglucuronide concentration ($1/[S]$) for three ox-spleen β -glucuronidase fractions. Details as Fig. 8.

give the products of reaction. It is possible to obtain values for n , the number of molecules of substrate combined per enzyme molecule in the inactive enzyme-substrate complex and for K_2 the dissociation constant of equation 2.

If equation 3 is rearranged into the form

$$\log \left(\frac{[S]}{v} - \frac{K_2}{V_{\max}} - \frac{[S]}{V_{\max}} \right) = n \log [S] - \log K_2 V_{\max}, \quad (4)$$

it will be seen that a plot of the left-hand term against $\log [S]$ yields a straight line of slope $=n$ and intercept at $[S]=1$ of $-\log K_2 V_{\max}$ which gives a value for K_2 .

If the theory is applicable, a plot of $[S]/v$ against $[S] + ([S]^n/K_2)$ will give a straight line.

Fig. 12. Plot of reciprocal of reaction velocity ($1/v$) against reciprocal of $(-)$ -menthylglucuronide concentration ($1/[S]$) for two of the ox-spleen β -glucuronidase fractions. Details as Fig. 9.

Table 2. Values of the enzyme-substrate dissociation constants (K_s) for the three ox-spleen β -glucuronidase fractions, determined at 38° and optimum pH

Substrate	Ox-spleen β -glucuronidase fraction		
	I	II	III
$(-)$ -Menthylglucuronide	4.7×10^{-3}	19×10^{-3}	—
Phenylglucuronide	2.0×10^{-3}	5.1×10^{-3}	1.2×10^{-3}
Phenolphthalein glucuronide	0.8×10^{-3}	2.3×10^{-3}	1.0×10^{-3}

Using the data presented in Fig. 7 for phenolphthalein glucuronide and the three ox-spleen enzymes, an examination has been carried out to determine the values of n and K_2 for the three enzyme fractions and the above substrate. In

Fig. 13 are presented the plots $\log \left(\frac{[S]}{v} - \frac{K_2}{V_{\max}} - \frac{[S]}{V_{\max}} \right)$ against $\log [S]$, and the values of n and K_2 calculated from these graphs are shown in Table 3. The differences in the various constants given in Tables 2 and 3 for the three fractions constitute good evidence for the separate identity of these fractions.

Energy of activation of the ox-spleen β -glucuronidase fractions

The effect of temperature on the rate of enzyme reaction in the region where heat inactivation is negligible has been found in many cases to fit the equation originally proposed by Arrhenius (1889) (cf. Sizer, 1943) namely:

$$\frac{d(\ln k)}{dt} = \frac{A}{RT^2}, \quad (5)$$

or upon integration

$$\ln \frac{k_2}{k_1} = \frac{A}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right), \quad (6)$$

where k = reaction rate at absolute temperature T , R = gas constant, A = energy of activation. Values of energy of activation (A) may be calculated from the slope of the graph in which the logarithm of reaction rate ($\log k$) is plotted against $1/\text{absolute temperature}$. The slope multiplied by the constant 4.58 gives the value of A .

Fig. 13. Plot of $\log ([S]/v - K_s/V_{\max} - [S]/V_{\max})$ against $\log [S]$ for the three ox-spleen β -glucuronidase fractions. A, pH 4.5 fraction; B, pH 5.2 fraction; C, pH 3.4 fraction. Value of n calculated from slope (see text, p. 237).

Table 3. Values of n (number of molecules of substrate in inactive enzyme-substrate complex) and K_s (dissociation constant of inactive enzyme-substrate complex) in the case of phenolphthalein glucuronide

β -Glucuronidase fraction	Optimum pH	n	K_s
I	4.5	2	2.9×10^{-3}
II	5.2	2	7.4×10^{-3}
III	3.4	3	3.1×10^{-6}

The energies of activation were determined for each of the three ox-spleen fractions at their pH optima, using both phenolphthalein and phenylglucuronide as substrate. Enzyme activity was determined at five temperatures between 0 and 40° using substrate concentrations sufficient to ensure maximum activity of the enzyme (0.001M-phenolphthalein glucuronide and 0.03M-phenylglucuronide). The results for the plots of $\log k$ against $1/\text{absolute temperature}$ are recorded in Fig. 14 and the data for values of A obtained by multiplying the slopes of these graphs by 4.58 are shown in Table 4.

It will be seen that the values of the activation energies for the two substrates are in good agree-

ment, and that those for the three fractions are distinctly different. In view of the statement of Sizer (1943) that for certain enzymes changes in pH of reaction or purity of the preparation had no effect on the value of A , the data presented in Table 4 constitute further confirmation of the distinct nature of the three ox-spleen β -glucuronidase fractions.

Fig. 14. Plot of $\log$ of reaction rate ($\log k$) against reciprocal of absolute temperature ($1/T$) for the three ox-spleen β -glucuronidase fractions. I, pH 4.5 fraction; II, pH 5.2 fraction; III, pH 3.4 fraction. A, Phenylglucuronide; B, phenolphthalein glucuronide. Value of activation energy calculated from slopes (see text, p. 238).

Table 4. Activation energies of ox-spleen β -glucuronidase fractions (Cal./mole.)

Substrate	Ox-spleen β -glucuronidase fraction		
	I	II	III
Phenylglucuronide	14700	16000	19300
Phenolphthalein glucuronide	14400	16200	18900

It has been found in the present work that if the substrate concentration is less than that required for maximum activity, lowered values for the energy of activation are found, while at substrate concentrations above that required for maximum activity, constant values for the energy of activation are found.

*Inhibition of the three ox-spleen
 β -glucuronidase fractions*

Inhibition by carboxylic acids

Citrate inhibits the two ox-spleen β -glucuronidase fractions with pH optima at 3.4 and 4.5, but not that with optimum at pH 5.2. This inhibition (Table 5) explains the inhibition of citrate on unfractionated preparations (Fig. 2).

Table 5. *Inhibition of ox-spleen β -glucuronidase fractions by citrate*

(Substrate is phenylglucuronide in acetate buffer. Citrate concentration = 10^{-2} M.)

Substrate concn. (M)	β -Glucuronidase fraction		
	pH 3.4	pH 4.5	pH 5.2
	Inhibition (%)		
10^{-2}	75	65	0
5×10^{-3}	65	50	0

The effects of some other organic acids as inhibitor are recorded in Table 6.

Table 6. *Percentage inhibition of the pH 3.4 ox-spleen β -glucuronidase fraction by various organic acids*

(Substrate is phenylglucuronide (5×10^{-3} M) in acetate buffer at pH 3.4.)

Inhibitor	Concentration (M)						
	10^{-3}	5×10^{-3}	10^{-2}	2×10^{-2}	10^{-1}	5×10^{-1}	10^{-1}
Citrate	75	65	—	—	—	—	—
L-Tartrate	85	—	—	—	—	—	—
Tricarballoylate	5	—	—	—	—	—	—
L-Malate	90	—	—	—	—	—	—
Oxalate	100	90	0	—	—	—	—
Malonate	40	—	—	—	—	—	—
Succinate	25	—	—	—	—	—	—
Glutarate	5	—	—	—	—	—	—
Lactate	5	—	—	—	—	—	—
L-Glutamate	8	—	—	—	—	—	—
D-Gluconate	25	—	—	—	—	—	—
D-Galacturonate	50	30	0	—	—	—	—
D-Mucate	100	100	100	95	75	20	0
D-Saccharate	100	100	100	100	100	90	25

The powerful inhibitory effect of mucic and saccharic acids on β -glucuronidase confirms the findings of Karunairatnam & Levvy (1949) that these two compounds are the most powerful inhibitors of β -glucuronidase so far found. A comparison of the effects of D-saccharate on the three ox-spleen β -glucuronidase fractions is shown in Table 7.

Table 7. *Percentage inhibition of the three ox-spleen β -glucuronidase fractions by D-saccharate*

(Substrate is phenylglucuronide (5×10^{-3} M) in acetate buffer at pH optimum of enzyme studied.)

Concn. of D-saccharate (M)	β -Glucuronidase fraction		
	pH 3.4	pH 4.5	pH 5.2
10^{-3}	100	100	100
2×10^{-4}	100	100	70
10^{-4}	100	60	20
5×10^{-5}	90	20	0
10^{-5}	25	0	0

Four of the inhibitory substances examined above, namely citrate, oxalate, mucate and saccharate, were examined in greater detail with the intention of determining the type of inhibition exerted. The structural similarity of mucate and saccharate with glucuronic acid suggested the possibility of competitive inhibition. The three ox-spleen β -glucuronidase fractions all show substrate inhibition to a greater or less degree depending upon the substrate, and this might complicate the analysis of the results by the graphical method of Lineweaver & Burk (1934).

In the present case we have to consider three possible equilibria existing in the reaction medium, namely:

where E = enzyme, S = substrate, I = inhibitor, ES = active enzyme-substrate complex, ES_n = inactive enzyme-substrate complex and EI = inactive enzyme-inhibitor complex. K_s , K_s and K_I are the dissociation constants for reactions 7, 8 and 9 respectively. In this case the velocity, v , is given by

$$v = V_{\max} [S] / \left([S] + K_s + \frac{[S]^n}{K_s} + \frac{K_s [I]}{K_I} \right). \quad (10)$$

This equation is a combination of the equations describing simple substrate inhibition and simple competitive inhibition (Lineweaver & Burk, 1934). At low substrate concentrations, the term $\frac{[S]^n}{K_s}$ in equation 10 becomes negligibly small, and equation 10 becomes

$$v = V_{\max} [S] / \left([S] + K_s + \frac{K_s [I]}{K_I} \right), \quad (11)$$

which is the equation for simple competitive inhibition as demonstrated by Lineweaver & Burk (1934). This equation may be rearranged thus:

$$\frac{1}{v} = \frac{1}{V_{\max}} + \frac{1}{V_{\max} [S]} \left(K_s + \frac{K_s [I]}{K_I} \right). \quad (12)$$

Plotting the reciprocal of reaction velocity against the reciprocal of substrate concentration in the presence and absence of inhibitor will give, at low substrate concentrations, straight lines which will indicate whether or not the equation for competitive inhibition is obeyed. If $V_{\max}$.

remains unaltered in presence of inhibitor and only the slope of the line is increased, the inhibition is competitive and the value of K_i , the enzyme-inhibitor dissociation constant, may be calculated. If, however, V_{max} decreases in addition to the slope of the line being altered, the inhibition is non-competitive in type.

Fig. 15. Plot of reciprocal of reaction velocity ($1/v$) against ($1/[S]$) for the pH 3.4 ox-spleen β -glucuronidase fraction in the presence of various inhibitors. Substrate, phenolphthalein glucuronide. A, control; B, 3×10^{-3} M-oxalate; C, 10^{-4} M-mucate; D, 10^{-2} M-citrate; E, 2×10^{-5} M-saccharate.

Fig. 16. Plot of reciprocal of reaction velocity ($1/v$) against ($1/[S]$) for the pH 4.5 ox-spleen β -glucuronidase fraction in the presence of citrate. A, control; B, 2×10^{-2} M-citrate. Substrate, phenylglucuronide.

Experiments were carried out with each of the three separated ox-spleen β -glucuronidase fractions in which the velocity of reaction was determined at various substrate concentrations in the presence and absence of the inhibitors. The substrate concentrations chosen were such that a plot of $[S]/v$ against $[S]$ gave a straight line, i.e. where the term $[S]^n/K_s$ is negligible.

The results of these experiments are presented in Figs. 15–18, using the graphical analysis outlined above, where $1/v$ is plotted against $1/[S]$.

Fig. 17. Plot of reciprocal of reaction velocity ($1/v$) against ($1/[S]$) for the pH 4.5 ox-spleen β -glucuronidase fraction in the presence of mucate and saccharate. A, control; B, 2×10^{-4} M-mucate; C, 2×10^{-4} M-saccharate. Substrate, phenolphthalein glucuronide.

Fig. 18. Plot of reciprocal of reaction velocity ($1/v$) against ($1/[S]$) for the pH 5.2 ox-spleen β -glucuronidase fraction in the presence of mucate and D-saccharate. A, control; B, 5×10^{-4} M-mucate; C, 2×10^{-4} M-saccharate. Substrate, phenylglucuronide.

It will be clearly seen from Fig. 15 that for the pH 3.4 fraction, all the four inhibitors examined are competitive in nature, and from Figs. 16 and 17 the same applies for the pH 4.5 fraction. In each case the value for $1/V_{max}$ is unchanged in the presence

of inhibitor and only the slope of the graph is increased. With the pH 4.5 fraction, the inhibition exerted by oxalate was so weak (less than 10% at 2×10^{-2} M-oxalate) that its type could not be established.

With the fraction having an optimum at pH 5.2, citrate and oxalate exert no inhibition at all, but both mucate and saccharate exert non-competitive inhibition (Fig. 18). The findings from all these experiments are summarized in Table 8.

Table 8. *Type of inhibition exerted by various inhibitors on the three ox-spleen β -glucuronidase fractions*

Inhibitor	β -Glucuronidase fraction		
	pH 3.4	pH 4.5	pH 5.2
Citrate	Competitive	Competitive	No inhibition
Oxalate	Competitive	*	No inhibition
Mucate	Competitive	Competitive	Non-competitive
Saccharate	Competitive	Competitive	Non-competitive

* Inhibition of such a low order as to prevent an accurate assessment of type.

From the data presented in Figs. 15–17 the values of K_I , the enzyme inhibitor dissociation constants for the cases of competitive inhibition, can be calculated using equation 12 above, where the increase in the slope of the $1/v$ against $1/[S]$ plot in the presence of inhibitor equals $K_s[I]/K_I$, the values of K_s used being those calculated from the slope and the inverse velocity intercept of the $1/v$ against $1/[S]$ plots in the absence of inhibitor as quoted above. The values of K_I so calculated are presented in Table 9.

Table 9. *Values of the enzyme-inhibitor dissociation constants (K_I) for two ox-spleen β -glucuronidase fractions*

(Expressed as molarity of inhibitor.)

Inhibitor	β -Glucuronidase fraction	
	pH 3.4	pH 4.5
Citrate	7.2×10^{-2}	1.22×10^{-1}
Oxalate	7.4×10^{-2}	—
Mucate	4.1×10^{-4}	1.7×10^{-2}
Saccharate	5.7×10^{-5}	4.8×10^{-3}

By using the method adopted by Eadie (1942) for cholinesterase and by Wills & Wormall (1950) for the suramin inhibition of urease, the number of molecules of inhibitor which combine with each active centre of the enzyme molecule can be calculated. From the Michaelis-Menten equation it is possible to derive the equation

$$\frac{1}{v} = a + b[I]^n, \quad (13)$$

where v = initial velocity, $[I]$ = concentration of inhibitor, n = number of molecules inhibitor per

active centre of enzyme, and a and b are constants. When $1/v$ is plotted against a power of the inhibitor concentration a straight line will be obtained.

Using the available data for the inhibition by saccharate of the enzymes having pH optima at 3.4 and 4.5 (cf. Table 7), the values of n giving straight lines are 2 and 3 for the two enzymes respectively (Fig. 19).

It follows, therefore, that if one molecule of substrate reacts per active enzyme centre and if non-inhibitive substrate concentrations are used, the molecular ratio of inhibitor to substrate for competitive inhibition is 2 in the case of the pH 3.4 β -glucuronidase fraction, and 3 in the case of the pH 4.5 fraction.

Fig. 19. Plot of $(1/v)$ against a power of inhibitor concentration (saccharate) for the pH 3.4 fraction (A) and the pH 4.5 fraction (B).

In the case of the β -glucuronidase fraction with pH optimum at 5.2, where inhibition by saccharate and mucate is non-competitive, the concentration of saccharate to cause 50% inhibition is 1.5×10^{-4} M, inhibition being 100% at 10^{-3} M and falling to zero at 5×10^{-6} M. Mucate is again not such a powerful inhibitor as saccharate, a concentration of 5×10^{-4} M, as used in the experiments recorded in Fig. 18, causing only 36% inhibition.

Inhibition by ascorbic acid

Contrary to the statement of Karunairatnam & Levvy (1949), Becker & Friedenwald (1949) showed ascorbic acid to be an inhibitor of β -glucuronidase preparations. In the present work we have confirmed the findings of the latter workers and have shown ascorbic acid to be a non-competitive inhibitor for all three ox-spleen β -glucuronidase fractions. A typical experiment for the fraction with optimum at pH 4.5 is recorded in Fig. 20. There is clearly a change in both the slope of the graph and the value of $1/V_{\max}$, indicating non-competitive inhibition.

Ascorbic acid inhibits the three ox-spleen fractions to a similar extent, a concentration of 10^{-3} M (17.6 mg./100 ml.) causing approximately 50%

inhibition in all three cases. Becker & Friedenwald (1949) found 2 mg./100 ml. to cause 22 % inhibition and 20 mg./100 ml. to cause 83 % inhibition, results which correspond well with the present work.

Fig. 20. Plot of $(1/v)$ against $(1/[S])$ for the pH 4.5 ox-spleen β -glucuronidase fraction in the presence of ascorbic acid. Substrate, phenolphthalein glucuronide. A, control; B, 10^{-3} M-ascorbic acid.

Fig. 21. Inhibition of pH 4.5 ox-spleen β -glucuronidase fraction by 10^{-4} M-suramin as a function of pH.

Inhibition by suramin

Wills & Wormall (1950) have demonstrated that suramin, a polysulphonic acid trypanocidal drug, inhibits a number of enzymes, the inhibition being pH sensitive and changing from 100 % to zero inhibition over a fairly narrow pH range. The pH at which 50 % inhibition was observed was very close to the isoelectric points of the enzymes studied. Suramin has been examined for its inhibitory effects on the ox-spleen β -glucuronidase fractions and the pH-sensitive type of inhibition has been found in all cases. A plot of the percentage inhibition against pH of the pH 4.5 ox-spleen β -glucuronidase fraction by 10^{-4} M-suramin is given in Fig. 21.

The change from 100 % to zero inhibition occurs over a range of 0.7 pH units with 50 % inhibition at pH 4.9. One interesting feature of the effect of suramin is its activating effect at pH values over 5.2, which has been consistently seen. This activation is seen with some other acidic compounds, and further work is in progress to elucidate its nature.

If the suggestion of Wills & Wormall (1950) is correct, the point of 50 % inhibition (pH 4.9) may be taken as the isoelectric point of this fraction. No matter what preparation of β -glucuronidase is used, the pH for 50 % inhibition always falls near pH 4.9 (pH 4.8–5.1). The inhibition by suramin, measured at the 50 % point, has been shown by the method of Lineweaver & Burk (1934) to be non-competitive.

Fig. 22. Inhibition of pH 4.5 ox-spleen β -glucuronidase fraction by heparin as a function of pH.

Inhibition by heparin

Becker & Friedenwald (1949) showed heparin to have an inhibitory effect upon β -glucuronidase preparations of the order of 50 % at a concentration of 0.4 mg./100 ml. It had previously been shown by Jaques (1943) that heparin combines with proteins and that this combination is influenced by pH. We considered it likely, therefore, that the inhibitory effect of heparin on β -glucuronidase might vary with pH in the same manner as the suramin inhibition.

At a concentration of 0.5 mg./100 ml., heparin showed an inhibition which varied with pH as illustrated in Fig. 22.

An additional factor seen in this case is that the inhibition never reaches zero as in the case of suramin. It is suggested that here there may be two components in the inhibition, one which shows pH sensitivity and which may be related to the sulphate groups of the heparin molecule, and another, not showing variation with pH, which may be related to the carboxyl groups of the heparin molecule.

Effect of miscellaneous compounds on β -glucuronidase

The following substances caused no inhibition of β -glucuronidase preparations at a concentration of 10^{-2} M: zinc, copper, calcium, magnesium and lead acetates; sodium dithionite, pyrophosphate, arsenate, arsenite, fluoride and thioglycollate. Formaldehyde caused only 15% inhibition of the fractions with optima at pH 4.5 and 5.2 at a concentration of 15% (w/v).

DISCUSSION

Evidence has been presented here for the existence in ox spleen of at least three β -glucuronidase fractions, having pH optima at 3.4, 4.5 and 5.2. It has been shown that these activities are capable of fractionation and that the properties of the individual fractions, particularly the energies of activation, effect of substrate concentration and the effect of inhibitors, are sufficiently different for it to be concluded that they are, in fact, three separate enzymes.

In all work in which a particular enzymic activity of a tissue is separated into a number of fractions having different properties a question which must inevitably arise is, are we actually dealing with a mixture of enzymes which pre-exist in the tissue, or are we starting with a parent molecule and breaking it up into sub-units or artifacts which actually have different properties and properties different from the whole? A related question which must also arise is, does not the fractionation procedure simply remove some inhibitory or similar factor originally present, and, in doing so to varying extents in different fractions, thus modify the original activity to produce fractions which appear different, but which actually only differ in amount of inhibitory or other factor present?

Experimental data which give an answer to these questions are those from the results of the pH-activity curves of crude unfractionated ox-spleen extracts at various concentrations of substrate shown in Fig. 1. In view of the results, which show a changing shape of the pH-activity curve as the substrate concentration changes, the natural conclusion is that more than one enzyme exists in ox-spleen extracts and this is very obvious at the extremes of the curves. The variation in shape of pH-activity curves shown in Fig. 1 are those which may be predicted from the behaviour of the separated glucuronidase fractions at varying concentration of substrate shown in Fig. 7. This is good evidence for the belief that the three separated glucuronidases are not artifacts of the separation procedure but are in fact present in the original extracts.

In all experiments so far carried out, evidence has been obtained in favour of the existence of the enzyme active at low pH values and in a few experiments, in particular the one recorded in Fig. 1,

there is evidence for an enzyme active at higher pH values, namely around pH 6.5–7.0. While no attempts to isolate this latter enzyme have so far been made, evidence at present available suggests that such an enzyme probably exists and could possibly be isolated. It has been found that many ox-spleen extracts are very poor in this particular enzyme, but that it is present in much greater amounts in liver. From the data shown in Fig. 1, it appears that this enzyme which shows its activity around pH 6.5–7.0 acts optimally at much higher substrate concentrations than the other three spleen enzymes, namely at around 0.003–0.004M-phenolphthalein glucuronide.

In the recent work of Sarkar & Sumner (1950) on ox-liver glucuronidase, the pH-activity curve of their preparation is claimed to show only one optimum, but it might, from their published data, have two optima.

The evidence from the ammonium sulphate precipitation and elution experiments recorded above give the impression that, while the enzymes acting optimally at pH 3.4 and 4.5 are distinct in their activities, there is some physical affinity between them which makes their separation difficult. In the presence of high concentrations of salts and an adsorptive agent, the pH 3.4 enzymic activity is lost but is regained upon dialysis.

The electrophoretic data recorded in Figs. 5 and 6 indicate their great similarity in nature but also the separability of the ox-spleen enzyme, and these data, along with other results to be presented in a subsequent paper, indicate that the isoelectric points of the ox-spleen glucuronidases all lie in the region of pH 5. Bernfeld & Fishman (1950a) have recently claimed to have obtained an electrophoretically homogeneous preparation of calf-spleen glucuronidase and state (Bernfeld & Fishman, 1950b) that the isoelectric point of this preparation lies between pH 7.5 and 8.0.

The evidence which we have available leads us to believe that glucuronidase may exist in the original tissue as a complex of closely related protein molecules, or possibly a series of protein molecules on a carrier protein molecule, and that while each activity can be separated it is probably only active in the presence of the carrier molecule.

The demonstration, in confirmation of the work of Oshima (1936), of the inhibitory action of citrate is extremely interesting in that it is further evidence in favour of the pre-existence of the separate enzymes in the original tissue, since only the enzymes with pH optima at 3.4 and 4.5 are inhibited and not that with optimum at 5.2.

This raises serious doubts concerning work on glucuronidase carried out in citrate buffer. As has been shown here, citrate acts as a competitive inhibitor and the degree of inhibition of the enzymes

with optima at pH 3.4 and 4.5 will depend not only upon the concentration of citrate, but also on the concentration of substrate. These facts may explain the peculiar shape of the substrate concentration-velocity curve for phenylglucuronide obtained by Kerr *et al.* (1948) in which activity was zero below 0.0025M-phenylglucuronide, a concentration at which all three enzymes show significant activity (cf. Fig. 8). The results of Kerr *et al.* (1948) were obtained at pH 5.2 in citrate buffer using unfractionated mouse-spleen extracts. The figure for the Michaelis constant given by these workers (0.0035M), is considerably too high in view of the citrate inhibition and the inapplicability of the Michaelis & Menten (1913) method of calculation, consequent upon the substrate inhibition shown by Kerr *et al.* (1948).

Figures for enzyme-substrate dissociation constants quoted by other workers (Fishman, 1939; Talalay *et al.* 1946), particularly that for phenolphthalein glucuronide (Talalay *et al.* 1946) where a mixed spleen, liver and kidney preparation was used, must also be revised since they were determined on unfractionated enzymes. The value given by these latter workers in the case of phenolphthalein glucuronide (0.00005M) is much lower than any of those obtained in the present work and, as will be shown in a later paper, much closer to that for the liver glucuronidases.

Turning to the inhibition by mucate and saccharate, it has been shown that these compounds cause competitive inhibition of the enzymes with optima at pH 3.4 and 4.5, while inhibiting the enzyme with optimum at pH 5.2 in a non-competitive manner. Karunairatnam & Levvy (1949) claimed that saccharate at pH 5.2 in citrate buffer caused competitive inhibition of a separated mouse-liver glucuronidase fraction in the same manner as at pH 4.5. This is at variance with the present findings and may be explained in part by the fact that these workers used citrate buffers in their enzyme assays. However, an analysis of the published results of Karunairatnam & Levvy (1949) by the present authors, using the method of Lineweaver & Burk (1934), shows that non-competitive inhibition is actually obtained. The value of K_i quoted by Karunairatnam & Levvy (1949) therefore has no meaning.

More recently Spencer & Williams (1951), when working at pH 5.2, stated that saccharate was a competitive inhibitor of β -glucuronidase. These workers used an inapplicable method in the analysis of their results since they rearranged the Lineweaver & Burk equation for competitive inhibition (equation 11 above) into the form

$$\frac{V_{\max}}{v} - 1 = \frac{K_s}{K_i[S]} \cdot [I] + \frac{K_s}{[S]},$$

and then by plotting $V_{\max}/v - 1$ against $[I]$ obtained a straight line. All that this method of analysis proves is that the intensity of inhibition varies with the inhibitor concentration. They publish no data which would allow a determination of the type of inhibition exerted by saccharate at pH 5.2. In this case also, the value of K_i published has no meaning.

In the work of Karunairatnam & Levvy (1949), oxalate, tartrate and ascorbate are stated to have no inhibitory action on β -glucuronidase, but in the present work all three are shown to inhibit β -glucuronidase, and the last has also been shown, by Becker & Friedenwald (1949), to be an inhibitor of β -glucuronidase.

Examination of the factors necessary for the inhibition of β -glucuronidase reveals that all compounds examined which show inhibitory action are acids. Monocarboxylic acids, with the exception of glucuronic acid, inhibit β -glucuronidase only very weakly, whereas dicarboxylic acids show a much greater inhibitory power. The dicarboxylic acids having a structure resembling glucuronic acid show the greatest effect. It would appear that >CHOH groups in the molecule are necessary for great inhibition by the carboxylic acids, and the more closely the structure resembles that of glucuronic acid, the greater is the inhibition. These findings are in agreement with the findings of Spencer & Williams (1951).

Of the other acidic compounds which are known to inhibit β -glucuronidase, for example ascorbic acid, suramin and heparin, all contain two or more acidic groupings. In view of these findings it is suggested that the active centre in the β -glucuronidases may be basic in character, but may differ in some way in the case of the enzyme with optima at pH 5.2 from that of the enzymes with optima at pH 3.4 and 4.5.

Many discrepancies in the literature of this subject result from the multiple nature of β -glucuronidase.

SUMMARY

1. Evidence has been presented for the occurrence in ox-spleen of three β -glucuronidase fractions having pH optima at 3.4, 4.5 and 5.2.

2. An examination of the effects of the variation in substrate concentration upon reaction velocity, the energies of activation and the effects of various inhibitors, leads to the conclusion that the three fractions may be considered as separate enzymes.

3. An analysis of the inhibition caused by excess substrate indicates that this inhibition is caused by the active enzyme-substrate complex combining with further molecules of substrate to form an inactive complex.

4. It has been shown, in confirmation of Oshima (1936), that various carboxylic acids inhibit β -glucuronidase preparations. Of these, citric acid inhibits the enzyme fractions with pH optima at 3.4 and 4.5 but has no action on the fraction with optimum at pH 5.2.

5. Mucate and saccharate have been shown to exert competitive inhibition on the enzyme fractions with pH optima at 3.4 and 4.5, but to exert non-competitive inhibition on the fraction with optimum at pH 5.2.

6. Suramin and heparin have been shown to be inhibitors of β -glucuronidase, the inhibition being pH-sensitive.

7. The possible nature of the state of the separated enzymes in the original tissue is discussed, as also are the factors necessary for the inhibition of β -glucuronidase.

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Studies on β -Glucuronidase

3. THE INFLUENCE OF AGE, PARTIAL HEPATECTOMY AND OTHER FACTORS ON THE β -GLUCURONIDASE ACTIVITY OF RAT LIVER

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Fishman (1940) showed that administration of borneol to dogs and menthol to mice caused a significant increase in the β -glucuronidase activity of liver, kidney and spleen but not of the testes, ovary, uterus or vagina. Fishman concluded, on the basis of these results, that the observed rise in β -glucuronidase activity was an adaptation to the excess of substrate for the assumed synthesis of glucuronides by β -glucuronidase. Fishman & Fishman (1944) demonstrated that administration of oestrogens to mice caused an increase in uterine β -glucuronidase but had no effect on liver β -

glucuronidase activity. Fishman (1947) extended this work by showing that administration of doses of oestrogens within physiological limits causes a rise in the uterine β -glucuronidase activity of ovariectomized mice, the rise not being antagonized by testosterone. Fishman supposed that β -glucuronidase has a fundamental role in the physiological action of oestrogens. The assumption was made that the enzyme was specific in its action, differing for the uterus on one hand and the liver, kidney and spleen on the other. No such absolute specificity has been shown to exist in the hydrolytic action of β -glucuronidase, the enzyme from uterus, liver, kidney or spleen being capable of hydrolysing

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