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INCREASED ACTIVITY OF ACID PHOSPHATASE AND β-GLUCURONIDASE IN THE LIVER AND SPLEEN OF MICE AFTER INTRAPERITONEAL ADMINISTRATION OF VARIOUS MACROMOLECULAR SUBSTANCES

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Abstract—In the present investigation the macromolecular substances dextran, polyvinylpyrrolidone, Periston and carbon were injected into the peritoneal cavity of mice. The acid phosphatase and β-glucuronidase activities of the liver and spleen were investigated biochemically and histochemically. The localization of the macromolecular materials stored in these organs was investigated histochemically.

Histochemical results. In the liver the macromolecular substances were stored in the cytoplasm of the Kupffer cells, and dextran also in the cytoplasm of the parenchymal cells. In the spleen the macromolecular substances were exclusively stored in the cytoplasm of the reticulum cells.

In the livers of the control animals, activity of the two enzymes was found almost exclusively in the Kupffer cells. In the livers of injected animals, activity of the two enzymes was seen in both the Kupffer and the parenchymal cells. In the spleen, only the reticulum cells surrounding the follicles show acid phosphatase and β-glucuronidase activity.

Biochemical results. The total activity of acid phosphatase and β-glucuronidase in the liver and spleen of mice injected with the specified macromolecular substances was significantly increased compared with control animals which were not injected or which received injections of physiological saline solution. The total activity of both enzymes in both organs showed no variation as a result of fasting or injection with physiological saline solution. A possible correlation is also indicated between the activity of acid phosphatase and of β-glucuronidase per unit weight for both liver and spleen in those mice which were not injected with macromolecular substances. This correlation could, however, not be demonstrated after the injection of the macromolecular substances. As a result of the storage of the macromolecular substances, the weights of the liver and of the spleen rose significantly.

INTRODUCTION

DURING a previous histochemical investigation, Willighagen[1] observed that the activity of acid phosphatase is sharply increased in macrophages present in chronically inflamed lungs and in the neighbourhood of a degenerating corpus luteum. These macrophages also show a great deal of material deriving from degenerating and necrotic tissue. These observations suggested that the incorporation of macromolecular substances may be correlated with a rise in the activity of acid phosphatase.[2] The possible existence of a relation between storage in cells and a high acid phosphatase activity seemed further strengthened by the high acid phosphatase activity found in reticuloendothelial cells such as those in the liver and spleen, which are known for their capacity to store many materials of microscopic and

macromolecular dimensions in their cytoplasm.[3] In order further to investigate this hypothetical correlation between storage of macromolecular substances and increased acid phosphatase activity, an investigation was made of the activity of acid phosphatase in mouse livers after the mice had been injected with dextran.[4]

Using histochemical methods it was found that after intraperitoneal administration the dextran is incorporated both in the parenchymal cells and in the Kupffer cells of the liver, and that this storage was indeed accompanied by a rise in acid phosphatase activity in the parenchymal cells. An increase in enzyme activity was also observed in liver homogenates.[4] Although it is known that dextran is broken down very slowly in the liver of the animal organism[5–7] and although there are still no indications that the acid phosphatase enzyme complex plays any part in the metabolism of the monomer of dextran (glucose), the possibility that the increase in the acid phosphatase activity is connected with a metabolic digestion of the ingested substance cannot be entirely excluded. This suggested the use of metabolically inert macromolecular substances such as polyvinylpyrrolidone and carbon particles in a subsequent study.[8, 9]

Investigations by de Duve *et al.*[10] showed that in rat liver cells the acid phosphatase enzyme complex is found primarily in lysosomes. In addition to acid phosphatase, lysosomes contain still other hydrolytic enzymes. It seemed to us of interest to determine not only the acid phosphatase activity but also the activity of another enzyme found in lysosomes. Our choice fell on the enzyme β-glucuronidase. In the investigation described in this paper, the activity of acid phosphatase and of β-glucuronidase was studied histochemically and biochemically in the spleen as well as in the liver. Determinations with the biochemical method yielded quantitative data concerning the changes in activities of the two enzymes in tissue homogenates as a result of the storage of the macromolecular materials. These results, however, represent mean values for a variety of cellular elements and extracellular fluids. To translate these results in terms of the structural units of tissue, histochemical methods are used to determine in which of these units the activity is located.

EXPERIMENTAL

Mice of the O_{20} (Amsterdam) strain from 4 to 6 months old, were used. After intraperitoneal administration of the macromolecular substances, the acid phosphatase and β-glucuronidase activity in the liver and spleen were biochemically and histochemically investigated. In addition, the cellular storage sites of the various macromolecular substances was studied histochemically.

Macromolecular substances

(1) *Dextran*, average molecular weight 160,000. The dextran was obtained from Poviet & Co., Amsterdam. From the dextran a 6 per cent sterile solution in 0·9 per cent NaCl was prepared.

(2) *Polyvinylpyrrolidone*, a polymer of vinylpyrrolidone with an average molecular weight of 640,000 (Bayer, Leverkusen). A 6 per cent solution in 0·9 per cent NaCl was prepared.

(3) *Periston*, a polymer of vinylpyrrolidone; average molecular weight 17,000. The Periston was supplied as a 4 per cent solution in 0·9 per cent NaCl (Bayer, Leverkusen).

(4) *Carbon particles*. From an India-ink suspension (Günther Wagner; Hannover, Tusche no. C11/1431a) a 2 per cent suspension of carbon particles in 0·9 per cent

NaCl solution was prepared. The average diameter of the carbon particles was, according to specification, 25 mμ.[11]

Injection scheme

Seven groups of animals were used, each group consisting of seven animals. The animals were killed on the tenth day after the first injection. Acid phosphatase and β-glucuronidase activity was determined in homogenates of liver and spleen. One control group (I) received no treatment. A second control group (II) was fasted for 18 hr before being killed, an ample supply of drinking water being available. The third group (III) was given a daily intraperitoneal injection of 1 ml of 0·9 per cent NaCl solution for 9 days and fasted for 18 hr before being killed on the tenth day. Group IV was given daily intraperitoneal injections of 1 ml of dextran solution for 9 days and fasted for 18 hr before being killed on the tenth day. Group V was given daily injections of polyvinylpyrrolidone solution for 9 days and fasted for 18 hr before being killed on the tenth day. Group VI was given daily injections of 1 ml of Periston for 9 days and fasted for 18 hr before being killed on the tenth day. Group VII was given daily injections of 1 ml of carbon suspension for 5 days and fasted for 18 hr before being killed on the tenth day. (Because the O_{20} mice did not react well to nine carbon injections, only five injections were given, on the tenth, eighth, sixth, fourth and second day before the animal was killed).

Histochemistry

Pieces of liver and spleen tissue were quickly frozen on dry ice and series of sections with a thickness of 10 μ were made in a cryostat. The sections were fixed in an aqueous solution consisting of 10 ml of a 4 per cent aqueous solution of formaldehyde and 10 ml of a 2 per cent aqueous solution of $CaCl_2 \cdot 2H_2O$.

To this fixative 80 ml of a 6 per cent aqueous solution of dextran was added as an osmotic protection.[12] Acid phosphatase activity was demonstrated in these sections with Gomori's method.[13] Histochemical activity of β-glucuronidase was demonstrated with Fishman and Baker's method[14] on cryostat sections and also on formalin fixed frozen sections according to Holt.[15] For the demonstration of dextran, sections were fixed in absolute ethanol and stained with the alcoholic periodic acid Schiff method.[16] We were unable to stain polyvinylpyrrolidone histochemically, but in the haematoxylin-stained sections an accumulation of basophilic substance could be seen in the Kupffer cells. This may indicate the site of the stored polyvinylpyrrolidone. The sections used for the demonstration of the carbon particles were fixed in a 4 per cent aqueous solution of formaldehyde.

Biochemistry

Small quantities of tissue (200 mg) taken from different parts of the liver and from the whole spleen were weighed rapidly on a torsion balance. The tissue was homogenized for 1½ min in a ground-glass Potter–Elvejhem homogenizer in about 20 ml of twice distilled water. After homogenization, more distilled water was added to bring the tissue concentration to 100 mg tissue per 20 ml water. To 10 parts of this solution were added 1 part of a 1 per cent solution of Triton-X-100 (a gift from Rohm and Haas & Co., Philadelphia, U.S.A.) to achieve maximal solubilization of the enzymes and availability of the substrates.[17] The homogenates to which Triton-X-100 had been added were allowed to stand for 30 min at 4 °C.

Acid phosphatase activity determinations. Acid phosphatase activity at pH 4·8 was determined as reported previously.[4] Disodium β-glycerophosphate was used as substrate in both the histochemical and biochemical experiments. The product contained 2·1 per cent of the α-isomer (determined according to Toal and Phillips[18]).

β-Glucuronidase activity determinations. The activity of the enzyme β-glucuronidase in the homogenate was determined using phenolphthalein-β-D-monoglucuronide as a chromogenic substrate, the liberated phenolphthalein being determined colorimetrically. The substrate phenolphthalein-β-D-monoglucuronide was prepared according to Fishman *et al.*[19] The estimations were carried out as described by Talalay *et al.*[20] at 37 °C and pH 4·5. Each determination was made in triplicate with a single control.

Triton-X-100 in the concentration used was found not to disturb the acid phosphatase and β-glucuronidase determinations. Aliquots of the homogenates were analyzed for total nitrogen by the Kjeldahl method.[21]

RESULTS

Histochemistry

Liver. The dextran was found to be stored in the cytoplasm of the Kupffer and parenchymal cells in the form of small droplets. The parenchymal cells located around the central vein stored more dextran than those lying peripherally. In the sections stained with haematoxylin, a weakly basophilic substance was observed in the cytoplasm of the Kupffer cells of animals which had been injected with polyvinylpyrrolidone. This is probably the location of the polyvinylpyrrolidone storage, since the livers of animals which were not injected with this substance did not show this phenomenon. Carbon particles were found stored in the cytoplasm of the Kupffer cells and not in the cytoplasm of the parenchymal cells. We did, however, observe that compact fields of histiocytic cells were present in the liver which showed signs of intense phagocytosis of carbon.

The livers of the control mice (Groups I, II and III) showed a high degree of acid phosphatase activity in the cytoplasm of the Kupffer cells. The acid phosphatase activity in the cytoplasm of parenchymal cells of the control animals, however, was low. The livers of mice injected with the macromolecular substances show high acid phosphatase activity in the cytoplasm of both the Kupffer cells and the parenchymal cells.

The methods described in the literature for histochemical determination of β-glucuronidase are still far from ideal. Even with the Fisher and Baker method used in this investigation, the localization of the enzyme was not sharp, while the intensity of the histochemical stain was not sufficiently representative of the activity of the enzyme in the tissue slices. In spite of these difficulties, it was clear that in the liver activity of β-glucuronidase is to be found in the same locations as activity of acid phosphatase. For the reasons just mentioned, however, it was impossible to determine accurately whether there was a variation of the activity of β-glucuronidase as a result of the intraperitoneal administration of macromolecular substances. In the mice injected with macromolecular substances the Kupffer cells were swollen and had increased in number.

Spleen. In the spleen the macromolecular materials were present only in the reticulum cells. Most of the macromolecular material was stored in the cytoplasm of the

reticulum cells surrounding the follicles, while only a few of the reticulum cells of the reaction centres of the follicles had stored. Only the reticulum cells show acid phosphatase and β-glucuronidase activity. After injection with the macromolecular materials the reticulum cells showed a swollen appearance and a high activity of acid phosphatase and β-glucuronidase. Because of the already high activity of these enzymes in the reticulum cells of the control animals, it was impossible to determine whether this activity had risen as a result of the incorporation of the injected substances.

Biochemistry

The results of the acid phosphatase and β-glucuronidase determinations are given in Tables 1 and 2.

In order to compensate for fluctuations in hepatic and splenic weight and for the use of animals of varying body weight, the specific activity of the enzymes acid phosphatase and β-glucuronidase was multiplied by the weight of the intact liver or spleen and divided by the body weight of the animal. This calculation normalized values to give the total enzyme activity in the two organs per g body weight.

TABLE 1. THE AVERAGE QUOTIENTS Q AND THE AVERAGE ENZYME ACTIVITIES OF THE LIVERS OF THE VARIOUS GROUPS OF MICE ($\pm$ s.d.)
(s.d. $= \sqrt{[\Sigma (X - \bar{X})^2/(n-1)]}$)

Group	Q*	Activity†	
		Acid phosphatase	β-Glucuronidase
		pH 4·8	pH 4·5
I. Controls (not fasted)	49·2 ± 2·4	109 ± 17	269 ± 51
II. Controls (fasted)	37·9 ± 2·3	111 ± 16	234 ± 47
III. Controls (0·9% NaCl)	40·1 ± 2·9	128 ± 18	259 ± 52
IV. Dextran, m.w. = 160,000	49·4 ± 2·5	287 ± 69	421 ± 60
V. PVP, m.w. = 640,000	44·4 ± 6·2	275 ± 49	388 ± 48
VI. PVP, m.w. = 17,000	47·9 ± 3·4	261 ± 65	409 ± 114
VII. Carbon φ 250 Å	48·5 ± 2·8	224 ± 47	358 ± 59

* $Q = \frac{\text{g(liver)}}{\text{g(mouse)}} \times 1000.$

† Activity $= \frac{\mu\text{g P(liver)}}{\text{g(mouse).hr}}$, or $\frac{\mu\text{g phenolphthalein (liver).}}{\text{g(mouse).hr}}$

Nitrogen determinations in liver and spleen showed that the nitrogen content per 100 mg wet tissue remains practically constant in all seven groups of mice irrespective of treatment.

Table 3 gives the enzyme activity per mg N.

DISCUSSION

It may be concluded from the quotient Q in Tables 1 and 2 that:

(1) As a result of fasting, liver and spleen weights drop.

(2) As a result of the incorporation of macromolecular materials liver and spleen weights rise (compare Group III with Groups IV, V, VI and VII).

TABLE 2. THE AVERAGE QUOTIENTS Q AND THE AVERAGE ENZYME ACTIVITIES OF THE SPLEENS OF VARIOUS GROUPS OF MICE ($\pm$ s.d.)

Group	Q*	Activity†	
		Acid phosphatase	β-glucuronidase
		pH 4·8	pH 4·5
I. Controls (not fasted)	2·4 ± 0·3	14·4 ± 2·6	43·8 ± 2·9
II. Controls (fasted)	1·9 ± 0·4	14·6 ± 1·6	43·1 ± 8·6
III. Controls (0·9% NaCl)	2·0 ± 0·5	13·2 ± 1·7	50·6 ± 9·3
IV. Dextran, m.w. = 160,000	6·8 ± 1·3	62·9 ± 12·5	104·0 ± 29·1
V. PVP, m.w. = 640,000	5·0 ± 0·8	45·7 ± 5·7	93·5 ± 11·7
VI. PVP, m.w. = 17,000	3·2 ± 0·5	28·9 ± 4·8	73·1 ± 8·1
VII. Carbon ϕ 250 Å	6·6 ± 1·2	38·0 ± 7·5	138·0 ± 31·6

* $Q = \frac{\text{g(spleen)}}{\text{g(mouse)}} \times 1000.$

† Activity = $\frac{\mu\text{g P(spleen)}}{\text{g(mouse).hr}}$, or $\frac{\mu\text{g phenolphthalein (spleen)}}{\text{g(mouse).hr}}$

TABLE 3. THE AVERAGE ENZYME ACTIVITIES PER MG N OF LIVER AND SPLEEN, OF THE VARIOUS GROUPS OF MICE ($\pm$ s.d.)

Group	Acid phosphatase activity (μg P/hr per mg N)		β-Glucuronidase activity (μg phenolphthalein/hr per mg N)	
	liver	spleen	liver	spleen
I. Controls (not fasted)	87 ± 13	214 ± 32	216 ± 73	650 ± 90
II. Controls (fasted)	99 ± 14	282 ± 36	208 ± 41	830 ± 128
III. Controls (0·9% NaCl)	107 ± 16	310 ± 34	217 ± 45	925 ± 147
IV. Dextran, m.w. = 160,000	211 ± 47	348 ± 76	309 ± 52	575 ± 121
V. PVP, m.w. = 640,000	231 ± 44	331 ± 47	283 ± 42	677 ± 109
VI. PVP, m.w. = 17,000	199 ± 38	336 ± 60	286 ± 80	851 ± 122
VII. Carbon ϕ 250 Å	155 ± 32	352 ± 39	247 ± 43	832 ± 203

(3) The total acid phosphatase activity and the total β-glucuronidase activity in the liver and spleen do not vary as a result of fasting or injection with 0·9 per cent NaCl solution.

(4) The total acid phosphatase activity and the total β-glucuronidase activity in the liver and spleen rise significantly as a result of the incorporation of the macromolecular substances used (significance for the physiological saline mice of Group III: $P \leqslant 0{\cdot}05$).

The combination of biochemical and histochemical results thus show that a considerable increase in the acid phosphatase activity and β-glucuronidase activity of mouse liver homogenates and spleen homogenates accompanies storage of macromolecular substances in both organs. In the liver the increase in activity of acid phosphatase and β-glucuronidase is localized in the parenchymal and Kupffer cells. In the spleen the increase in activity for both enzymes is localized in the reticulum cells. The elevation in activity of the two enzymes might be visualized as a reflection

of an increased quantity of enzyme or as an alteration in the properties of the enzyme in the direction of increased catalytic activity, or as a combination of both.

Since the calculated total enzyme activity of acid phosphatase and β-glucuronidase of the whole organ does not change as a result of fasting or injection with physiological saline, it can be excluded that the rise in enzyme activity in the animals injected with the macromolecular substances is a result (or partial result) of a change in the glycogen level or of a stress factor. A direct metabolic effect can also be excluded because the rise in enzyme activity takes place not only with the macromolecular substance dextran, which is largely but not completely metabolically inert, but also with the metabolically inert substances Periston, polyvinylpyrrolidone and carbon.

The rise in enzyme activity is probably not caused by the ingestion process in the cell. This conclusion is based on data reported in a previous publication[4] which indicated that some days after the last injection the acid phosphatase activity is still very high, as well as on the fact that a rise in enzyme activity can be demonstrated by the biochemical determination method only after an injection series lasting at least three days.

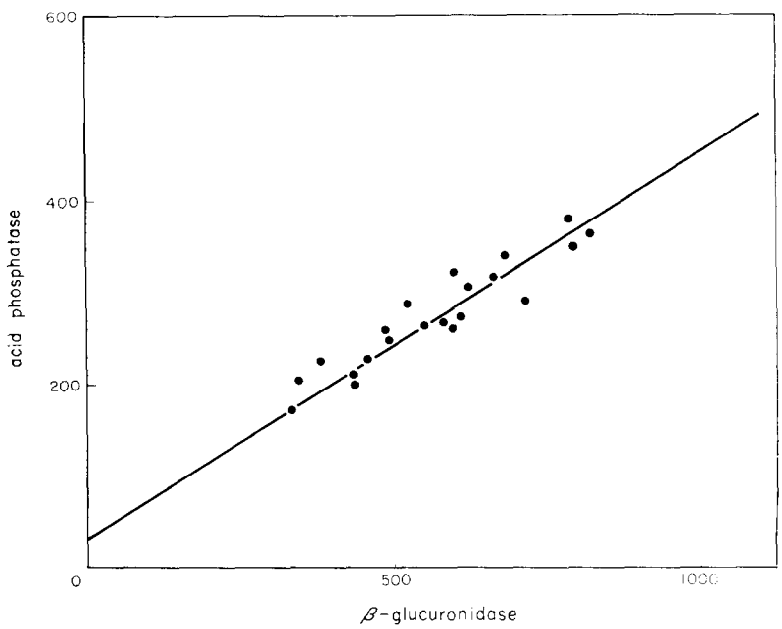

FIG. 1. Correlation between acid phosphatase activity and β-glucuronidase activity per 100 mg wet liver tissue of the first three groups of mice. Acid phosphatase activity: μg P/100 mg wet liver per hr. β-Glucuronidase activity: μg phenolphthalein/100 mg wet liver per hr.

It is therefore probable that our results indicate the existence of a special relationship between acid phosphatase and β-glucuronidase activity and the cellular storage of certain substances. The phenomenon of phagocytosis or pinocytosis followed by storage in different types of cells such as found in rat liver and rat kidney tissue has been related to small cytoplasmic granules.[22, 23] Granules of this type, as far as they have been studied, have been found to be rich in acid phosphatase and β-glucuronidase.[10]

According to various authors, the activity of glucose-6-phosphatase and the activity of fructose-1 : 6-diphosphatase, enzymes which play an important part in carbohydrate metabolism, are highly dependent upon the nature of the animals nutrition. It has also been shown that as a result of fasting the activity of glucose-6-phosphatase rises, while the activity of fructose-1 : 6-diphosphatase drops.[24] Since it appears from the present data that the total acid phosphatase activity and the total β-glucuronidase activity do not vary as a result of fasting, neither of these enzymes would seem to play an important role in glycogeen metabolism.

In Fig. 1 for the first three groups of mice the acid phosphatase activity per 100 mg wet liver tissue is plotted for each mouse individually against the β-glucuronidase activity per 100 mg wet liver tissue. In Fig. 2 the same is done for the spleen. The distribution of the points suggests a possible relationship between the acid phosphatase and the β-glucuronidase activities. This correlation is statistically significant as demonstrated with Fisher's χ^2 test ($P \leqslant 0{\cdot}05$). The lines drawn in the figures are calculated from the position of the points according to Youden.[25] Enzyme determinations in liver from other, unpublished investigations with O_{20} mice also indicate a possible correlation between the activity of acid phosphatase and β-glucuronidase.

FIG. 2. Correlation between acid phosphatase activity and β-glucuronidase activity per 100 mg wet spleen tissue of the first three groups of mice. Acid phosphatase activity: μg P/100 mg wet spleen per hr. β-Glucuronidase activity: μg phenolphthalein/100 mg wet spleen per hr.

This correlation could not be statistically demonstrated for either the liver or the spleen in the four groups of mice which were injected with macromolecular substances.

Finally, it may be remarked that the rise in acid phosphatase activity observed in the present investigation is of the same order of magnitude as the rise in acid phosphatase activity described in a previous investigation in which Swiss mice were used.[4]

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