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THE CHANGE IN SHAPE OF THE pH-ACTIVITY CURVE OF ACID PHOSPHATASE IN THE LIVER AND SPLEEN OF MICE AFTER INTRAPERITONEAL ADMINISTRATION OF MACROMOLECULAR SUBSTANCES

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Abstract—The increase in the activity of acid phosphatase occurring as a result of storage of macromolecular substances in the liver and spleen of mice was studied. Dextran and polyvinylpyrrolidone were injected intraperitoneally in mice of the 0_{20} strain. The activity of acid phosphatase was investigated in liver and spleen homogenates between pH 2·9 and pH 7·1. It was shown that in the mice used, there were present three different non-specific acid phosphatases, characterized by different pH optima. As a result of the incorporation of the macromolecular substances, the activity of the entire enzyme complex rises. The increase in activity, however, not being equally large in all the acid phosphatase enzymes. In the liver, after administration of dextran, the activity of the enzyme with an optimum at pH 5·8 showed relatively the largest increase.

INTRODUCTION

DURING histochemical investigation of the localization of acid phosphatase activity in normal and pathological human tissue, it was found that macrophages present in chronically inflamed lungs and in the neighbourhood of a degenerating corpus luteum were characterized by a high acid phosphatase activity.[1] These macrophages have, in addition, stored a great deal of material, probably originating from degenerating and necrotic cells.

On the basis of these observations the assumption was made that the incorporation of macromolecular material is accompanied by an increase in the acid phosphatase activity in the cell. 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[2]. In order to study further this possible correlation between storage of macromolecular substances and an increase in acid phosphatase activity, mice were intraperitoneally injected with various macromolecular metabolically inert substances. It was found that part of the injected materials was stored in the liver and spleen.[3] The acid phosphatase activity in the liver and spleen of animals which had been injected with the various macromolecular substances was significantly higher than that of the control animals.[3, 4] The results of the investigations were thus in agreement with the assumed correlation between the storage of macromolecular substances and increase in acid phosphatase activity.

This acid phosphatase activity was determined at pH 4·8. Since the activity is strongly dependent on pH, it was of importance to study at other pH values of the incubation milieu the variation in acid phosphatase activity resulting from intraperitoneal administration of macromolecular substances. In the investigation reported below, the pH activity curves of acid phosphatase were studied in the liver and spleen of control and injected mice. The investigation demonstrated that as a result of the storage of macromolecular substances the shape of the pH-activity curves of acid phosphatase shows important changes.

METHOD

Mice from 6 to 8 months old of the O_{20} strain (Amsterdam) were used. After intraperitoneal administration of macromolecular substances, the acid phosphatase activity was studied in liver and spleen homogenates.

Substrate

The substrate used was sodium-β-glycerophosphate $Na_2C_3H_7O_6P.\ 5H_2O$.

The product contained 2·1% of the α-isomer (determined according to Toal and Phillips[5]). The substrate solution contained 50 mg glycerophosphate per ml and was brought to pH 7·0 with dilute hydrochloric acid.

Macromolecular substances

(1) *Dextran*, with an average molecular weight of 200,000. The dextran was obtained from Poviet & Co., Amsterdam. From the dextran a 6% sterile solution in 0·9% NaCl was prepared.

(2) *Polyvinylpyrrolidone*, a polymer of vinylpyrrolidone, with an average molecular weight of 640,000 (Bayer, Leverkusen). From this substance a 6% sterile solution in 0·9% NaCl was prepared.

(3) *Polyvinylpyrrolidone*, with an average molecular weight of 50,000 (Bayer, Leverkusen). A 6% sterile solution in 0·9% NaCl was prepared.

Injection scheme

Five groups of animals were examined, each group consisting of four or five animals. The animals were killed on the 10th day after the first injection. The activity of acid phosphatase was determined in homogenates of the livers and spleens. One control group (I) received no treatment. A second control group (II) was given daily intraperitoneal injection of 1 ml of 0·9% NaCl solution for nine days and fasted for 18 hr before being killed on the 10th day. Group III was given daily intraperitoneal injections of 1 ml of dextran solution for nine days and fasted for 18 hr before being killed on the 10th day. Group IV was given daily intraperitoneal injections of 1 ml of polyvinylpyrrolidone solution (640,000) for nine days, and fasted for 18 hr before being killed on the 10th day. Group V was given daily intraperitoneal injections of 1 ml of polyvinylpyrrolidone solution (50,000) for nine days and fasted for 18 hr before being killed on the 10th day.

Preparation of the homogenates

Small quantities of tissue (200 mg) taken from various parts of the liver and from the whole spleen were weighed rapidly on a torsion balance. The tissue was homogenized for $1\frac{1}{2}$ min in a ground-glass Potter-Elvejhem homogenizer in about 20 ml

of twice-distilled water. After homogenizing, more distilled water was added to bring the tissue concentration to 100 mg tissue per 20 ml water. (Because of the limited weight of the spleens of the control mice (Groups 1 and II) it was necessary, in order to have sufficient homogenate solution, to dilute the spleen homogenates of these animals to 50 mg tissue/20 ml water). To ten parts of this solution was added one part of a 1% solution of Triton-X-100 (a gift from Rohm and Haas & Co., Philadelphia, U.S.A.) to achieve maximal solubilisation and availability of the enzymes for interaction with its substrate. The homogenates to which Triton-X-100 had been added were allowed to stand for 30 min at 4°.

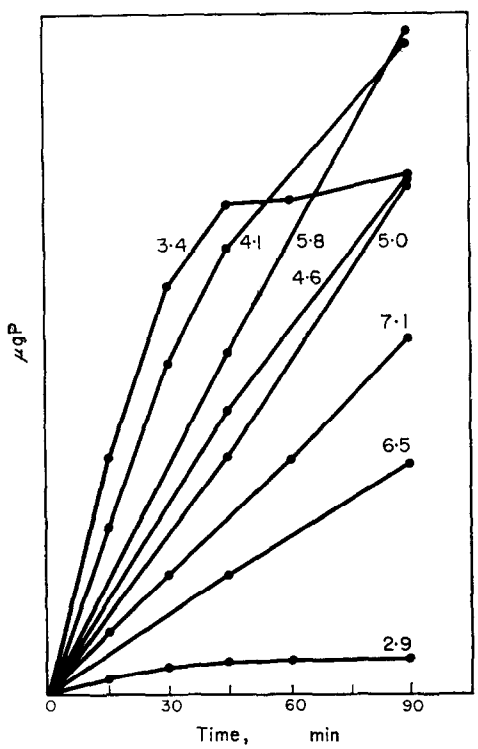

FIG. 1. Liver

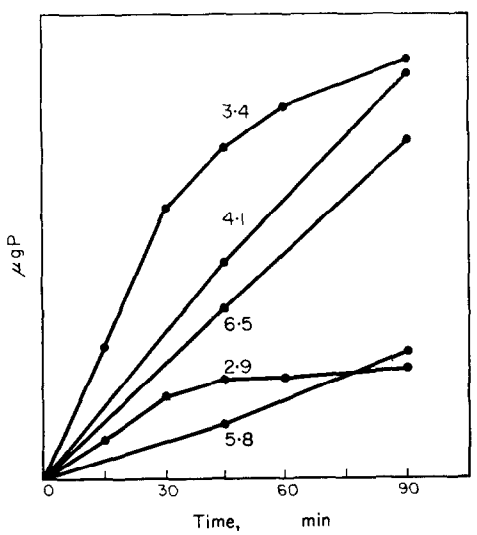

FIG. 2. Spleen

FIGS. 1. and 2. Correlation between quantity of phosphate liberated by enzymic splitting of the glycerophosphate and the incubation time, for various pH values of the incubation medium. The solid lines in the figures are obtained from control mice (Group I). (Because more than one homogenate was used in these experiments, the activities at the various pH values are not mutually comparable).

Acid phosphatase activity determinations

The activity of acid phosphatase at a fixed pH was determined by incubating 1·5 ml of the homogenate with 7·5 ml of a 0·2 M acetate buffer and 1·5 ml substrate solution. Incubation was done in glass-stoppered test tubes. Inorganic phosphate was determined in aliquots of the incubation mixture before and after the incubation period, according to Lindberg and Ernster.[6]

Buffer solutions

The activity of acid phosphatase is strongly dependent on the type of buffer solution brought into the incubation flasks.[7] For this reason, in the present study use was made of only one type of buffer solution, namely, 0·2 Molar buffer solutions of acetic acid and sodium-acetate. The pH values of the buffer solutions were: 1·8, 2·2, 3·6, 4·4, 4·8, 5·0, 5·3, 6·0 and 7·1 (0·1 N HCl was used to bring the first two of these buffer solutions to the desired pH). The pH values of the incubation mixtures obtained with these buffer solutions were 2·9, 3·4, 4·1, 4·6, 5·0, 5·3, 5·8, 6·5 and 7·1 respectively. The fact that several of these buffer solutions possessed only limited buffering capacity explains the slight deviation of the pH values of the incubation mixtures from those of the buffer solutions. Tests showed that the differences in pH values of the incubation solutions before and after incubation were never greater than 0·05 pH units.

RESULTS

Incubation time

Experiments in which the quantity of phosphate liberated by the enzyme was determined after various incubation intervals showed that the stability of the enzymes is dependent on the pH of the incubation solutions. In these experiments an investigation was made of the activity of acid phosphatase in liver and spleen homogenates of control animals, and of animals injected with macromolecular substances. It was shown that the stability of the acid phosphatase enzyme complex does not change under the influence of intraperitoneal injections of the various macromolecular substances used. From the figures below it can be seen that the stability of the enzyme is lowest in the incubation solutions with pH values below 4·4. The data collected in these figures are derived from control mice (Group I). Mice of Group II and the mice injected with macromolecular substances give, in relation to the inactivation of the enzyme, identical pictures.

An incubation time of 30 min was used. With this incubation period any inactivation of the enzyme in the incubation flasks with the lowest pH values would in any case be very slight.

pH activity curves

Figs. 3–10 give the pH activity curves. The activity of the acid phosphatase enzyme complex is plotted against the pH. By the activity is understood the calculated quantity of phosphorus, expressed in μg, liberated by the entire organ during an incubation time of one hour and the resultant value divided by the weight of the mouse. This method provides the most easily comparable results.[8]

Figs. 3 and 4 show the activity of acid phosphatase in the liver and spleen of mice injected with 0·9 per cent NaCl in water (Group II). Since the acid phosphatase activity in the liver and spleen of the untreated animals (Group I) gave the same results as those found for Group II, no figures are given for Group I. Figs. 5 and 6 show the acid phosphatase activity in the liver and spleen of the mice injected with dextran (Group III). Figs. 7–10 show the acid phosphatase activity in the liver and spleen of mice injected with polyvinylpyrrolidone 640,000 or 50,000 (Group IV and Group V).

DISCUSSION

The pH activity curves of the acid phosphatase enzyme complex in five mice injected with physiological saline (Group II) are shown in Figs. 3 and 4. It can be clearly seen that for homogenates of both liver and spleen the pH activity curves show three peaks at about the same pH values: pH 3·4, 5·0 and 5·8. In the liver

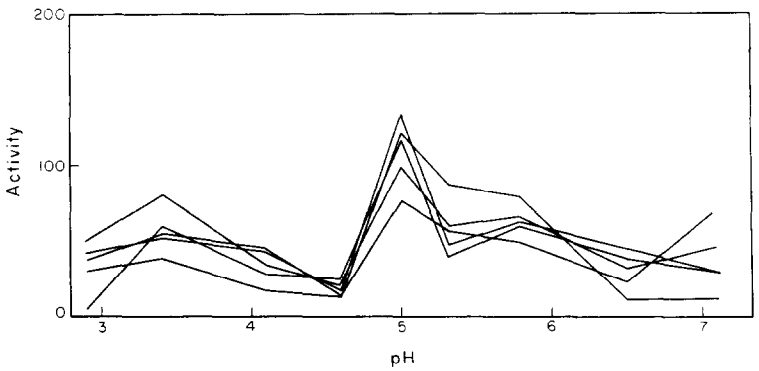

FIG. 3. Liver

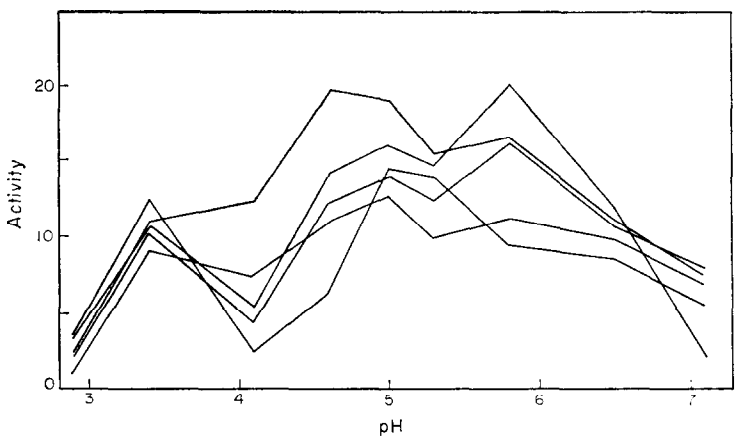

FIG. 4. Spleen

FIGS. 3 and 4. The activity of acid phosphatase in the liver and spleen of 5 mice injected with 0·9% NaCl in water (Group II). Activity is μg P (liver)/g (mouse)/hr, and μg P (spleen)/g(mouse)/hr.

homogenates the peak at pH 5·0 is by far the highest. In the spleen homogenates the difference in the height of the peaks is less extreme. The pH activity curves of the acid phosphatase enzyme complex for the control mice (Group I) which received no treatment are identical with the curves given in Figs. 3 and 4. It follows from this that the injections with physiological saline produced no effect on the activity of the enzyme complex acid phosphatase. This is in agreement with previous observations.[3]

These results show the presence of at least three types of non-specific acid phosphatase in liver and spleen homogenates of mice of the 0_{20} strain. The optimal activity of these three enzymes lies in the neighbourhood of the pH values 3·4, 5·0 and 5·8.

The presence of three different types of non-specific acid phosphatase has already been demonstrated by Folley and Kay.[8] For their investigations they made use of

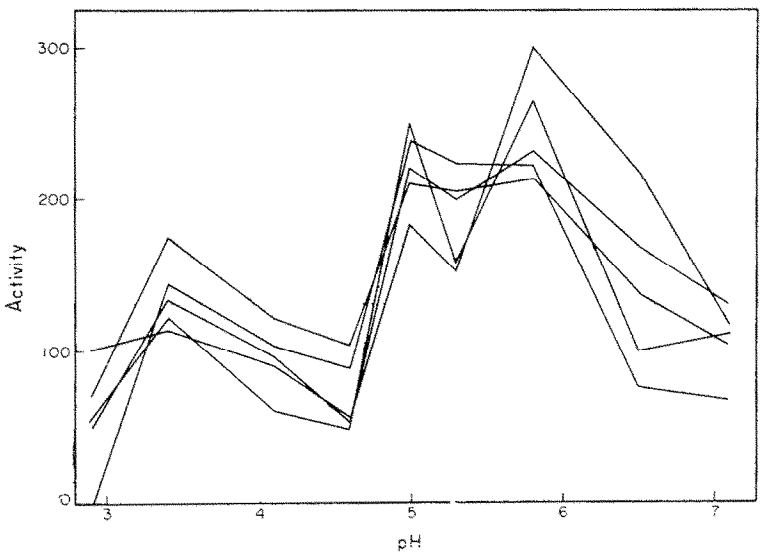

FIG. 5. Liver

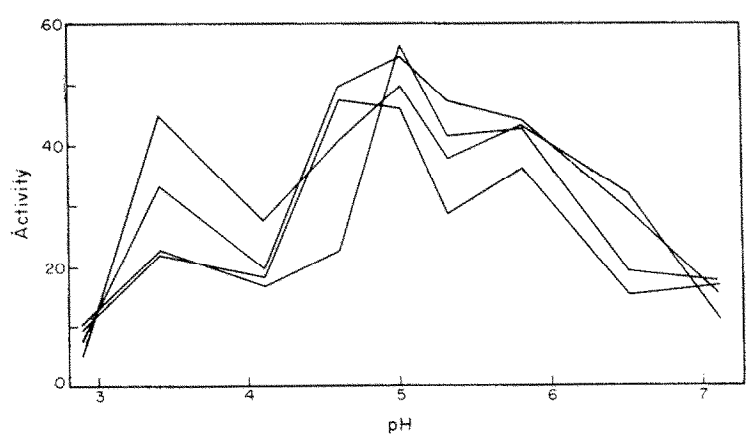

FIG. 6. Spleen

FIGS. 5 and 6. The activity of acid phosphatase in the liver (5 mice) and spleen (4 mice) injected with a dextran solution (Group III).

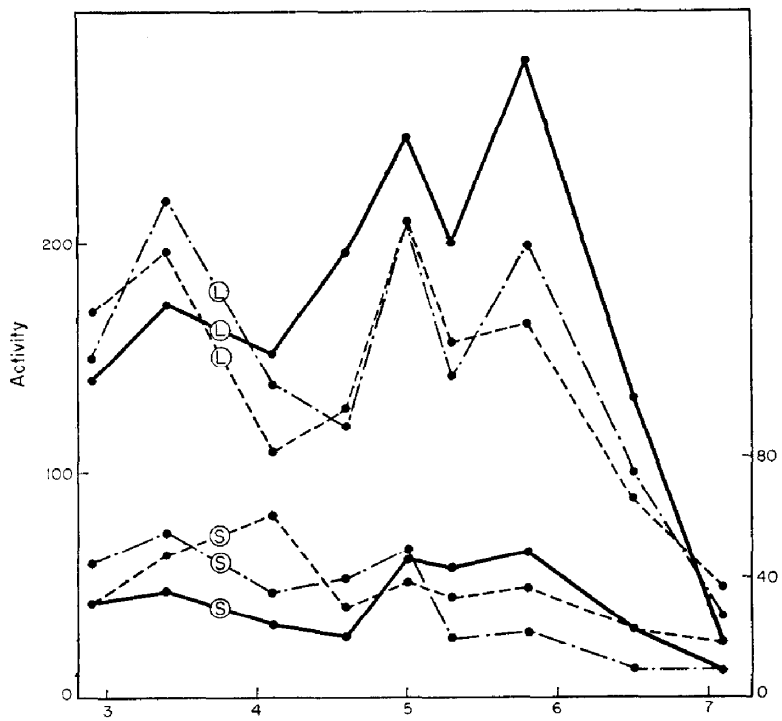

FIG. 7

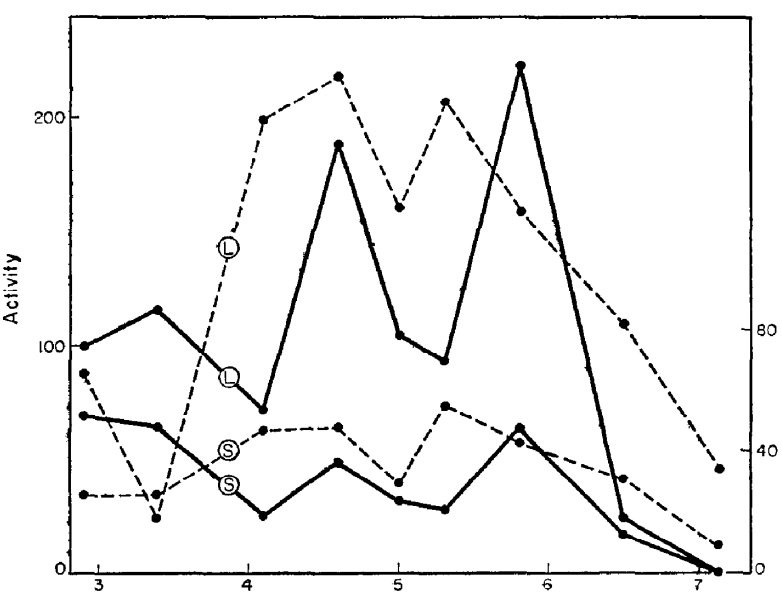

FIG. 8

FIGS. 7 and 8. The acid phosphatase activity in liver and spleen of mice injected with a solution of PVP with an average molecular weight of 640,000 (Group IV). The 40–80 scale applies to the spleens and the 100–200 scale to the livers. In Fig. 7 the acid phosphatase activity in the liver and spleen of three mice are shown by ·————·, ·— — —·, and ·—·—·—· and in Fig. 8 the activity in 2 mice is shown by ·————· and ·— — — —·. L = liver, S = spleen. Horizontal scale represents pH values.

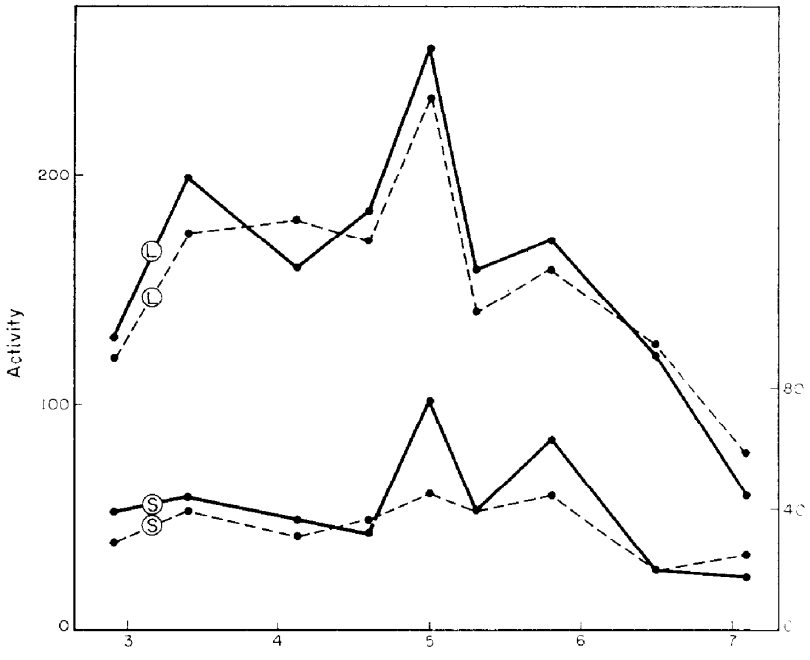

FIG. 9

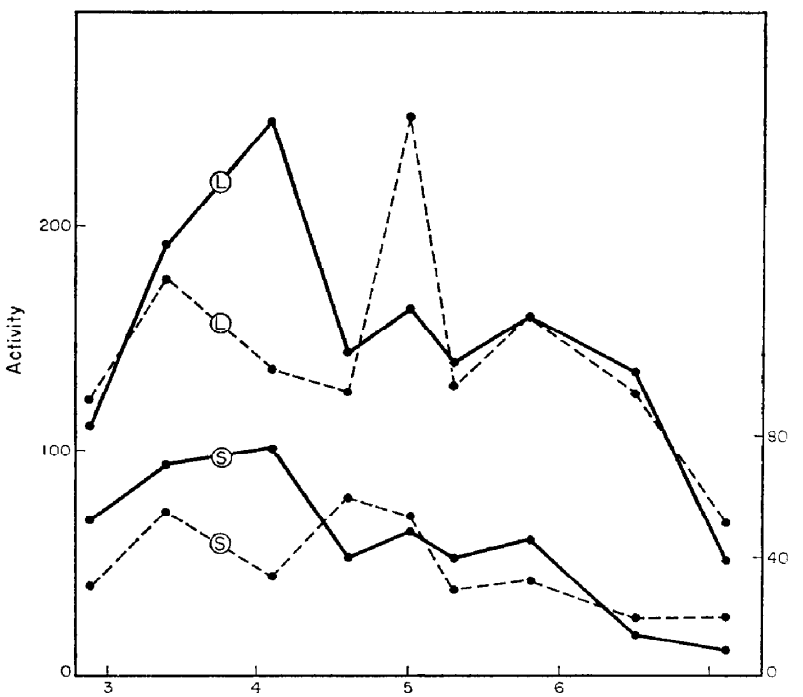

FIG. 10

FIGS. 9 and 10. The acid phosphatase activity in the liver and spleen of mice injected with a solution of PVP with an average molecular weight of 50,000 (Group V). The 40–80 scale applies to activity in the spleens, the 100–200 scale to the livers. In both Fig. 9 and Fig. 10 the acid phosphatase activity in the liver and spleen of 2 mice are shown by ·———· and ·— — — —·. L = liver, S = spleen. Horizontal scale represents pH values.

liver homogenates from rats. The three types of acid phosphatase are characterized by their dissimilar pH optima and their dissimilar behaviour in relation to Mg^{2+} ions. Investigations carried out by Roche[9] and by Goodlad, Mills and Smith[7, 10] demonstrated in rat liver homogenates at least two different non-specific acid phosphatases, one with a pH optimum between 3·5 and 4·0 and another with a pH optimum between 5·0 and 5·5. In addition, Goodlad and Mills considered it probable that in rat livers still a third type of non-specific acid phosphatase with a pH optimum between 6·0 and 7·0 is present. All these investigations, however, were done with rat livers. In the present investigation the work was done with homogenates of the liver and spleen of mice.

Figs. 5 and 6 show the pH activity curves of mice injected with a dextran solution (Group III). Here again three peaks can be distinguished which lie at the same pH values as those for the control groups (I and II). In comparison with the activity in both organs of the controls mice, it is clear that the activity of the enzyme complex acid phosphatase has risen over the entire pH range from pH 3·4 to pH 7·1 under the influence of the incorporation in both organs. The increase in activity is not the same for all pH values, however. In the liver homogenates the increase is found especially in the activity of the acid phosphatase with a pH optimum of about 5·8. This means that the three types of acid phosphatase are activated to different degrees. The elevation in activity of the enzymes might be visualized as a reflection of an increased quantity of enzyme or of an alteration in the properties of the enzyme in the sense of increased catalytic activity, or a combination of both.

In contrast with the liver, no characteristic change in the shape of the pH activity curves of the spleen homogenates as an effect of the incorporation of dextran is established, although a rise in the activity can be observed.

The distinct change in the shape of the pH activity curves in the liver after incorporation of dextran resembles to some extent the results which Norberg[11] obtained with rat livers. He showed that after partial hepatectomy the shape of the pH activity curve of rat liver acid phosphatase varies during the process of regeneration. Goodlad and Smith[10] reported that rat liver acid phosphatase activity assayed at pH 5·5 showed a 50 per cent increase in animals maintained on a low protein, low choline diet, although the activity assayed at pH 3·7 showed no change. He does not, however, give a complete activity curve for the acid phosphatase.

Three peaks are also by the pH activity curves of the liver and spleen of the mice which received PVP (Groups IV and V). The activity of the enzyme complex rose over the pH range from pH 2·9 to pH 6·5 under the influence of the incorporation of PVP. At pH 7·1, however, no increase in activity is found. In contrast with the mice which were injected with dextran, it cannot be said that a particular type of acid phosphatase is most highly activated. This is different for each mouse injected with PVP. The shapes of the pH activity curves for liver and spleen for each mouse belonging to Groups IV and V do agree somewhat. This can be seen most clearly in Fig. 8, in which for one mouse the three peaks for both liver and spleen are shifted to the left. Taken as a whole, the rise in activity at pH 2·9 for the mice injected with PVP is greater than for the mice injected with dextran.

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REFERENCES

1. R. G. J. WILLIGHAGEN, Thesis, Leiden (1960).
2. B. N. HALPERN (Editor), *Symposium on the Physiopathology of the Reticulo-endothelial System*. Blackwell, Oxford (1958).
3. A. E. F. H. MEIJER and R. G. J. WILLIGHAGEN, *Biochem. Pharm.* **8**, 389
4. P. VAN DUIJN, R. G. J. WILLIGHAGEN and A. E. F. H. MEIJER *Biochem. Pharm.* **2**, 177 (1959).
5. J. S. TOAL and I. J. PHILLIPS *J. Pharm., Lond.* **1**, 869 (1949).
6. O. LINDBERG and L. ERNSTER, *Methods of Biochemical Analysis*. (edited by D. GLICK). Vol. III Interscience Publishers, New York (1956).
7. G. A. J. GOODLAD and G. T. MILLS, *Biochem. J.* **66**, 346 (1957).
8. S. J. FOLLEY and H. D. KAY, *Ergebn. Enzymforschung*. **5**, 159 (1936).
9. J. ROCHE, *The Enzymes*, Vol. 1, p. 275 (Edited by J. B. SUMMER and K. MYRBÄCK), New York. Academic Press (1950).
10. G. A. J. GOODLAD, G. T. MILLS and E. E. B. SMITH, *Biochem. J.* **49**, VI (1951).
11. B. NORBERG, *Acta Physiol. Scand.* **19**, 246 (1949).