Clinical and Experimental Studies on Acid Adenosine Triphosphatase, with Special Reference to Lysosomes and Isozyme

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ACID adenosine triphosphatase activity in human serum has been found to be increased in patients with various diseases including essential hypertension^{1,2}. However, the origin and the property of the enzyme were almost unknown and therefore, clinicopathological interpretation of the increased enzyme activity in the sera of patients was hardly possible. For the purpose of obtaining information concerning the problem, biochemical analyses were made by the author and N. SAITO M. D.

METHODS AND MATERIALS

Tissue Fractionation.

The liver and the kidney of female Sprague Dawley rats were subjected to the tissue fractionation as described before³ according to SHIBKO and TAPPEL, and SAWANT et al.

Preparation of Organ Extract.

Human or Wistar rat organs were homogenized in three-fold weight of cold deionized water with a Waring blender or a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenates were freeze-thawed three times, in order to disrupt subcellular particles, and centrifuged at 10^5 G for 60 minutes, using a Hitachi preparative ultracentrifuge Model 55PA. Clear supernatant solutions were separated.

Electrophoresis.

Preparative zone electrophoresis was carried out using Pevikon C-870 (Stockholms Superfosfat Fabriks A.-B., Stockholm, Sweden), according to the method of MÜLLER-EBERHARD, H. J.⁴ A plastic tray ($40 \times 15 \times 1.5$ cm) was filled with a homogenous suspension of washed Pevikon in Veronal buffer (ionic strength 0.05, pH 8.6). The final block thickness was 0.6 cm. An amount of 6 to 7 ml serum was put into a Visking cellophane tube and concentrated by fanning in the air to about 2.5 ml. The concentrated serum was mixed with a small amount of Pevikon

and placed into a 0.9-cm-wide slot which was cut across the block approximately 10 cm from the cathodal end. The potential gradient was approximately 10 V/cm and the duration of the run was about 14 hours at 4°C in a cold room. One-centimeter-wide sections running the width of the block were removed and placed into a sintered glass funnel. Then a volume of 6 ml of Veronal-acetate-HCl buffer pH 3.0 was poured into the funnel and eluted by suction.

Gel-Filtration.

Sephadex G-200 (Pharmacia, Uppsala, Sweden) was swelled in a buffer solution (0.05M tris-maleate, 0.1M NaCl, pH 7.5) for a week and a column was prepared according to FLODIN and KILLANDER⁵ in a cold room. The gel bed had the dimensions of 3.7 by 61.5 cm. Approximately 7 ml of serum was dialyzed against the buffer in the cold room overnight and applied to the column between the gel surface and the buffer. Elution was carried out under a hydrostatic pressure of approximately 30 cm of the buffer. The flow rate was about 19 ml per hour. Fractions of 12.5 ml were collected in a gravimetric fraction collector (Toyo Industrial Co. Model SF-200A).

DEAE-cellulose Column Chromatography.

The anion-exchange cellulose column (diameter 1.75 cm, length 23 cm) was prepared as described by PETERSON and SOBER⁶ by packing a suspension of DEAE-cellulose (Serva, 0.54 mEq/g) in tris-chloride buffer (the starting buffer 0.05M, pH 8.0). Materials (sera and organ extracts) were applied to the column after dialysis against the starting buffer overnight at 4°C. About 10 fractions were eluted with the starting buffer into which nonadsorbent proteins broke through. Then a linear chloride gradient was started by the two-chamber device⁷. The limiting buffer was 0.05M tris-chloride buffer pH 8.0 containing 0.3 or 0.5M NaCl. At the end of the gradient elution, tris-chloride buffer (0.05M, pH 8.0) containing 1.0M NaCl was applied. Portions of 12.5 ml were collected.

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Enzyme Assay.

Acid adenosine triphosphatase (ATPase). Serum acid as well as alkaline adenosine triphosphatase activity was determined by the method of SAITO¹.

For the determination of the enzyme activity in fractions which were obtained by electrophoresis, 0.5 ml of substrate solution (0.8 gm Na₂ATP Sigma Co. per 100 ml, neutralized with tris) was added to 4 ml of the eluate. Final pH was 5.4. Incubation was carried out at 37°C for 30 minutes.

As to the material from gel-filtration, 4 ml of the eluate, a suitable amount of maleic acid solution (0.5 ml of 0.18M or 0.2 ml of 0.4M) and 0.5 ml of the substrate solution were mixed, then incubated at 37°C for 60 to 90 minutes. The final pH was 5.1.

In regard to the enzyme activity of the fraction by DEAE-cellulose column chromatography, the

substrate solution (0.5 ml) and 0.23M acetic acid solution (0.5 ml) were put into 4 ml of the eluate, obtaining a final pH 4.9. The reaction mixture was incubated at 37°C for 30 minutes.

At the end of the incubation, of ice cold 20 per cent (W/V) trichloroacetic acid solution was added to the incubation mixture. After standing for 10 min. in ice cold water, the reaction mixture was filtrated through the doubled filters (Toyo Filter Paper Co. No. 5c). The filtrate was subjected to the phosphorous determination by NAKAMURA⁸. Controls with the substrate, the buffer and trichloroacetic acid solutions alone were employed. The non-enzymatic inorganic phosphorus was subtracted.

Acid phosphatase. Acid phosphatase activity in the fraction from Pevikon was measured by the method of KING and KING⁹.

Fig. 1. Two examples of sera from patients in electrophoresis.

The eluate of Sephadex gel filtration or DEAE-cellulose column chromatography was subjected to acid phosphatase assay according to BESSY, LOWRY and BROCK¹⁰, using tris acetic acid buffer at final pH of 5.0. The concentration of liberated *p*-nitrophenolate was determined at 410 m μ .

Determination of Marker Enzymes.

Methods for the determination of various enzymes in subcellular fractions are described in the previous

paper³.

Protein Determination.

The concentration of protein was determined by the method of LOWRY¹¹ et al., employing bovine albumine (Armour Lab.) as a standard. Relative protein concentration was determined, measuring the ultraviolet absorption at $\lambda=280$ to 300 m μ .

Determination of Chloride.

Determination of the chloride concentration of the

Fig. 2. Combined diagrams of results in nine electrophoresis.

Fig. 3. Gel-filtration, normal serum.

eluted fraction from DEAE-cellulose column was carried out by the method of SCHALES and SCHALES¹², using a standard NaCl solution (Daiichi Pure Chemicals Co.).

Preparation of Sodium free ATP solution.

Adenosine triphosphate solution containing very

little sodium was prepared by treating the sodium salt of adenosine triphosphate as described by WHEELER and WHITTAM¹³.

RESULTS

Electrophoresis

Fig. 4. Gel-filtration, serum from a patient with nephrotic syndrome.

Fig. 5. Gel-filtration, serum from a patient with juvenile hypertension.

Fig. 1 shows the results of electrophoresis of two sera from patients with hepatocerebroathy and renal hypertension as examples. Results of electrophoresis of sera from patients with various diseases are presented together in

Fig. 2 in which the peak of albumin is taken as 100 per cent distance from the origin. Acid ATPase activity is distributed electrophoretically in α to β -globulin regions. Activities of acid ATPase and acid phosphatase are some-

Fig. 6. DEAE-cellulose column chromatography, serum from a patient with essential hypertension and arteriosclerosis.

Fig. 7. DEAE-cellulose column chromatography, serum from a patient with systolic hypertension.

what dissociated. The resolution of serum proteins is not so satisfactory owing to the large load of protein and relatively low ionic strength of the buffer.

Gel-filtration

The author's own serum was subjected to gel-filtration as a normal example (Fig. 3). Fig. 4 and 5 show the protein, acid ATPase and acid

Fig. 8. DEAE-cellulose column chromatography, serum from a patient with chronic hepatitis.

Fig. 9. DEAE-cellulose column chromatography, serum from a patient with pulmonary hypertension.

phosphatase patterns on the gel-filtration of sera from patients with nephrotic syndrome or juvenile hypertension, respectively. A major acid ATPase activity is localized between the

Fig. 10. DEAE-cellulose column chromatography, rat serum.

Fig. 11. DEAE-cellulose column chromatography, combined serum of two dogs.

second and the third peaks of serum proteins near the latter and a minor acid ATPase activity is found in the first peak. Acid ATPase activity is different from acid phosphatase in the distribution pattern. The molecular weight

of the major acid ATPase is estimated to be approximately 9×10^4 according to ANDREWS¹⁴. The molecular weight of the minor activity would be equal to or more than about 2×10^5 .

Fig. 12.

Fig. 13. Kidney from a patient with chronic renal failure, linear gradient of chloride concentration was not obtained because of stopping of amagnetic stirrer during elution.

DEAE-cellulose Column Chromatography

The elution diagrams of sera from several patient are presented in Fig. 6 to 9, revealing at least three peaks of acid ATPase activities which are different from acid phosphatase activities. The diagrams of rat and dog sera are

found in Fig. 10 and 11, respectively. It is remarked with interest that the rat serum contains a distinct acid ATPase activity which breaks through the column in the starting buffer, whereas the dog serum has no activity to correspond with it.

Fig. 14.

Fig. 15.

Acid ATPase, acid *p*-nitrophenyl phosphatase and protein distribution patterns of various human organ extracts are shown in Fig. 12 to 19. In the elution diagrams of human liver,

kidney, spleen and aorta extracts, one or two distinct acid ATPase activities are revealed in fractions close on No. 5 fraction. These enzymes are eluted into the fractions in which

Fig. 16.

Fig. 17. Pancreas cancer.

the chloride concentration remains yet unchanged (Fig. 12 to 15). There has not been found any peak of acid ATPase activity of human organs which corresponds definitely to the

most prominent activity of human serum. Some rat organ extracts have plural acid ATPase activities also. The patterns of the enzymes [of rat organs seem to be rather simpler than that

Fig. 18.

Fig. 19.

of human organs (Fig. 20 to 23).

Tissue Fractionation Studies

The specific activities of four enzymes in the subcellular fraction of Sprague-Dawley rat liver are shown in Fig. 24. In accordance with the

Fig. 20.

Fig. 21.

previous study³ using Wistar rats, lysosomal fraction had the highest specific activity of acid ATPase. Fig. 25 shows pH-activity curves of the four subcellular fractions as to dephosphorylating activity against ATP. Lysosomal fraction demonstrated a major activity at pH 5 and a minor activity at pH 9. The mitochondrial

and microsomal fractions showed the maximum activity at pH 9 and less activity at pH 7 or 5. Soluble fraction scarcely had activity at pH 9 and only a minimal activity at pH 5.

Effect of Na^+ , K^+ and Mg^{++} on ATPases of Rat Liver.

Fig. 22.

Fig. 23.

Lysosomal fraction (F III) was dialysed against 0.25M sucrose solution in order to adjust the sucrose concentration to other fractions. Acid ATPase activity of each fraction was meas-

Fig. 24. Enzyme activities in subcellular fractions of Sprague-Dawley rat liver.
white column: succinoxidase
dotted column: acid phosphatase
black column: acid ATPase
striped column: Glucose-6 phosphatase

Fig. 25. ATP phosphohydrolase pH activity curves in four subcellular fraction of rat liver.

- lysosomal fraction
- .....□ mitochondrial fraction
- ×——× microsomal fraction
- .....○ soluble fraction

ured in 3.1ml of medium with final concentration of 0.13M tris-acetic acid buffer at final pH 5.0, 2.1mM ATP and 8.1mM sucrose. Mg^{++} or in addition Na^+ and K^+ were added to the reaction mixture. The microsomal fraction only was activated by the addition of Mg^{++} (Fig. 26). Therefore, the pH-activity curve of microsomal fraction was reexamined, adding the cations into the reaction mixture. As shown in Fig. 27, microsomal ATP-phosphohydrolase was activated between pH 4 to 10 by the addition of Mg^{++} . The degree of the activation was most prominent at pH 7. However, further addition of sodium and potassium ions into

Fig. 26. Effect of cations on acid ATPase activities in subcellular fractions.
white column: no addition of cation
stippled column: 4mM Mg^{++}
cross-hatched column: 4mM Mg^{++} + 120mM Na^+ + 20mM K^+

Fig. 27. Effect of cations on pH activity curve in microsomal fraction.

- A: No addition of cation
- B: 4mM Mg^{++}
- C: 4mM Mg^{++} + 120mM Na^+ + 20mM K^+

the reaction mixture did not enhance the activity at all the pH tested.

ATPase Activities of Sera from Patients with Various Diseases

Sera of patients with various diseases had increased values of acid and alkaline ATPase activities (Table I). The data of normal subjects for controls were derived from SAITO's report¹. Serum acid and alkaline ATPase levels of patients with essential hypertension, con-

gestive heart failure including (Classes III and IV (NYHA), hyperthyroidism, coronary insufficiency or myocardial infarction, and diabetes mellitus were significantly increased. Groups of patients with congenital heart diseases, and valvular diseases including Classes I and II showed no increase in them. The mean serum acid ATPase level of patients with nephritis (acute, chronic and nephrotic syndrome) was found to be increased, although the value was small.

Fig. 28. Effect of cations on human serum acid ATPase activity.

TABLE I ACID AND ALKALINE ATPase ACTIVITIES IN VARIOUS DISEASES

	acid ATPase		alkaline ATPase	
	No. of case	mean $\pm$ SD*	No. of case	mean $\pm$ SD*
normal	38	6.6 $\pm$ 1.9	36	6.8 $\pm$ 2.4
hyperthyroidism	9	10.0 $\pm$ 1.6 $P < 0.001$	9	11.2 $\pm$ 5.4 $P < 0.001$
diabetes mellitus	11	10.9 $\pm$ 4.2 $P < 0.001$	10	10.7 $\pm$ 6.0 $P < 0.005$
essential hypertension	23	11.7 $\pm$ 2.9 $P < 0.001$	20	9.3 $\pm$ 3.0 $P < 0.005$
hypertension with renal disease	14	8.7 $\pm$ 3.8 $P < 0.02$	14	8.7 $\pm$ 2.6 $P < 0.02$
congestive heart failure (class III & IV)	21	11.1 $\pm$ 3.2 $P < 0.001$	21	11.0 $\pm$ 5.9 $P < 0.001$
valvular disease (class I & II)	27	7.6 $\pm$ 2.2 $P > 0.05$	27	8.3 $\pm$ 4.6 $P > 0.05$
congenital heart diseases without cardiac failure	21	8.0 $\pm$ 2.9 $P > 0.05$	21	8.4 $\pm$ 4.8 $P > 0.05$
myocardial infarction & angina pectoris	17	11.6 $\pm$ 5.0 $P < 0.001$	17	10.1 $\pm$ 4.0 $P < 0.001$
(nephritis)	16	8.9 $\pm$ 2.3 $P < 0.001$	15	8.3 $\pm$ 2.5 $P < 0.05$

SD* = standard deviation

Significance of difference between means of serum acid or alkaline ATPases of patients with various diseases and normal subjects are statistically tested by Student's *t*-test.

Fig. 29. Effect of cations on human serum acid ATPase activity.

Effect of Cations on Serum Acid ATPase Activity

Human sera were dialysed against deionized water overnight. Sodium free ATP solution neutralized with tris was used as a substrate solution. The total volume of the reaction mixture was 4 ml in which Na^+ and K^+ were interchanged maintaining a constant concentration of 155 mM. Mg^{++} (0.5 or 1.0 mM) or ouabain (10 mM) was put into the reaction mixture in addition to Na^+ and K^+ . Serum acid ATPase was not activated by any of them (Fig. 28, 29 and 30). No remarkable inhibition of the activity by quabain was found (Fig. 30). Addition of Ca^{++} did not activate the enzyme (Fig. 31).

Representative Examples of Patients with Various Diseases

Case 1. A fifty-nine-year-old woman with high blood pressure of 204/126 mmHg was admitted because of diplopia. As shown in Fig. 32 her blood pressure was slowly reduced due to low salt diet and medication. The electrocardiogram showed a strain pattern. Result of the percutaneous renal biopsy was malignant nephrosclerosis associated with malignant phase of essential hypertension. Ophthalmological findings were fundus hypertonicus o. utr. Keith-Wagener III, Scheie H_3S_2 . Retinal bleeding was found after hospitalization. There was no strict correlation between the levels of blood pressure and serum acid ATPase, although correlation between the two has been statistically

Fig. 30. Effect of Na^+ , K^+ and quabaine on human serum acid ATPase activity.

○—○ no quabaine
△---△ 10mM quabaine

found.

Case 2. A fifty-four-year-old man was admitted because of retrosternal pain. Twelve years previously, he began to experience retrosternal pain on effort. Two years before he was attacked by severe pain in the anterior portion of the chest, shoulders and abdomen. Sweating, vomiting and incontinencia alvi et urinae took place. He recovered after four weeks of absolute rest. During the year before entry he experienced frequent chest pains of short duration, several times in the daytime and a few in the night. Oxygen, nitroglycerin, an anticoagulant etc. were given. He felt well although the frequency of attacks did not significantly diminish. Four weeks after admission, in an emergency, his oxygen tent was passed to a patient with congestive heart failure (Class IV). On the very night when he slept without oxygen supply, severe chest pain attacked him which was hardly remedied with morphine. He was again placed in an oxygen tent. Electrocardiogram demonstrated no typical pattern of myocardial infarction (Fig. 33). He recovered gradually. As shown in Fig. 34, acid ATPase as well as the acid phosphatase showed very high values during the early hospital days. Urological examinations disclosed no abnormal finding. The level of acid ATPase was gradually decreased during hospital days. Lactic acid dehydrogenase (LDH) isozyme pattern determined by T. NOSAWA M. D. was heart type with a normal

Fig. 31. Effect of Ca^{++} on human serum acid ATPase activity.

value in total activity.

Case 3. A seventy-two-year-old man was admitted because of left chest pain. The day before the admission, he experienced epigastralgia. On the day of admission after epigastralgia, followed by nausea and vomiting, there was the onset of a severe pain in the left chest with radiation to the inner aspect of left arm. He became pale and bathed in cold sweat, feeling

that he was dying. Electrocardiogram and determination of serum enzymes revealed a pattern of anterolateral myocardial infarction with right-bundle-branch block (Fig. 35). However the levels of acid ATPase were almost within normal range (Fig. 36).

Case 4. A forty-two-year-old woman with mitral stenosis (Class II) and auricular fibrillation was admitted because of sudden pain in the left flank. The sediment of her urine contained many white cells but no red cells. Intravenous phyllography, I^{131} -Hippuran renogram (Fig. 37) and renal scintiscanning (Fig. 38) revealed an infarction of the upper part of the left kidney, although no portion of the lesion was obtained by renal biopsy. The level of serum acid ATPase was very high on the seventh day after the attack and decreased after three weeks to normal range (Fig. 39). Other serum enzymes examined were within normal values during the course. However, LDH isozyme pattern on the sixth day after the infarction was heart type which became normal type later, supporting the diagnosis as renal infarction.

Case 5. A fifty-eight-year-old woman was admitted because of orthopnea. She was immediately treated by the intravenous adminis-

Fig. 32. Case 1, A fifty-nine-year-old woman with essential hypertension.

tration of lanatoside C and oxygen supply and well responded to the therapy. The diagnoses were mitral insufficiency, right-bundle-branch block and arteriosclerosis. Cardiac catheterization was not performed because of her rejection. The levels of acid and alkaline ATPase as well as alkaline phosphatase were high during the early hospital days and decreased in the course of recovery from congestive heart failure. There was no remarkable elevation of the level of acid phosphatase (Fig. 40).

Case 6. A twenty-nine-year-old male patient was admitted. His chief complaints were anorexia and dull pain in the epigastric region.

About eight years before he was operated for nephrolithiasis and received blood transfusion. Two weeks after the operation he began to suffer from anorexia and nausea and became icteric. He got better during hospitalization for a month and had been fairly well until this relapse. Laboratory data as follows: serum bilirubin, total 1.4mg/dl, direct 1.0mg/dl; thymol turbidity, 1-2; zinc sulfate turbidity, 6-8; bromsulphalein, 30 per cent retention at end of 30 min.; alkaline phosphatase, 12.5 units (K. A.); acid phosphatase, 2.5 units; S-GOT, 103.5 units; S-GTP, 42.5 units; total protein, 6.9g/dl; albumin, 3.57g/dl; α -globulin, 0.83g/dl;

Fig. 33. Electrocardiograms of Case 2.

Fig. 34. Case 2, A fifty-four-year-old man with intermediate type of cardiac ischemia.

Fig. 35. Electrocardiogram of Case 3 on the 1st hospital day.

Fig. 36. Case 3, A seventy-two-year-old man with acute myocardial infarction.

Fig. 37. ^{131}I -Hippuran renogram of Case 4 by Dr. A. Hirakawa.

Fig. 38. Renal scintiscanning of Case 4 by Dr. Nakao.

β -globulin, 0.95 g/dl; γ -globulin, 1.55 g/dl; A/G, 1.07; liver biopsy, chronic hepatitis with fatty liver; laparoscopy, large white liver.

The values of serum acid and alkaline ATPases were 23.1 units and 9.8 units, respectively. The increased level of serum acid ATPase remained unchanged as well as transaminases during four months in the hospital. The levels of alkaline phosphatase were slightly increased or at the upper limit of the normal value. The levels of alkaline ATPase and acid phosphatase

were almost within normal values.

Two other cases of post transfusion hepatitis are found in Fig. 41 in which change in the level of acid and alkaline ATPase is somewhat accordant with that of transaminases.

DISCUSSION

In the previous publication³, the occurrence of acid ATPase as a lysosomal enzyme has been suggested. Optimal pH of acid ATPase activity of rat liver lysosomal fraction is at about 5 in

Fig. 39. Case 4, A forty-two-year-old woman with renal embolism.

Fig. 40. Case 5, A fifty-eight-year-old woman with congestive heart failure.

accordance with that of human serum acid ATPase¹. ATPase activity of lysosomal fraction at pH 9 could be explained by the contamination of mitochondria. Similarly, the activity of mitochondrial fraction at pH 5 would be due to the contamination of lysosomes, although MAYERS and SLATER¹⁵ have suggested the occurrence of a ATP phosphohydrolase with an optimal pH of 6.3 in the purified mitochondria from rat liver which is activated by 2:4-dinitrophenol. The microsomal ATPase activity at pH 5 may be attributed to not only lysosomal enzyme but also Mg⁺⁺-activated ATPase. Beta-glycerophosphatase activity of kidney lysosomal fraction was abolished by heating at

70°C for 20 minutes, whereas ATPase remained somewhat active (Fig. 42). This fact suggests that acid ATPase is a different enzyme from acid β -glycerophosphate. The Km of acid ATPase of rat kidney lysosomal fraction is 0.14 mM (Fig. 43) and not so different from that of human serum (0.5 mM).

The estimated molecular weight¹⁶ of acid ATPase of rat kidney lysosomal extract was between 9 and 10 $\times 10^4$ and that of the major acid ATPase of human serum was approximately 9 $\times 10^4$ by gel-filtration technique. The dissociation of acid ATPase and acid *p*-nitrophenyl phosphatase in the elution diagrams of DEAE-cellulose column chromatography and Sephadex gel-filtration also indicate the independency of the two activities. These facts, in addition to the previous data, demonstrate the occurrence of lysosomal acid ATPase which is distinct from acid β -glycerophosphatase or acid *p*-nitrophenyl phosphatase.

Recently, CHERSI, BERNARDI and BERNARDI¹⁷ have isolated acid phosphomonoesterase which has no hydrolating activity against ATP. This report strongly supports the author's view.

Acid ATPase activity of rat liver lysosomal fraction was not activated by the addition of Mg⁺⁺ or Na⁺ and K⁺, suggesting that these cations do not activate the enzyme, although the possibility is not yet excluded that a very small amount of metal ions in the enzyme preparation had been sufficient for full enzyme activation. Acid ATPase activity of human

Fig. 41. Two patient with post-transfusion hepatitis.

Fig. 42. Heat stability after incubation for 20 min at 37 to 70 $^{\circ}\text{C}$ enzyme assay was carried out.

Fig. 43. Influence of substrate concentration.

ATP : acid ATPase

BGP : acid β -glycerophosphatase

serum was not enhanced by the addition of these metal ions, instead, the activity was rather reduced probably due to the increased ionic strength of the reaction mixture; this was true in rat liver enzyme also. Serum acid ATPase could not be such a complex ATPase as membrane ATPase system proposed by Skou¹⁸. It

does not seem to be any of the mitochondrial ATPase which have been considered to be implicated in oxidative phosphorylation.

SECCI et al.^{2,19} have suggested in a preliminary communication that serum ATPase of a hypertensive subject is derived from vascular smooth muscle i.e. serum ATPase of a hypertensive subject is similar to tonomyosin ATPase of cow carotids which has been investigated by GASPAR-GODFROID²⁰. As the author has not carried out any investigation concerning this possibility, no data to comment the validity of Secchi's view can be presented. However at least, it may be said that the enzyme handled by him seems to be different from acid ATPase in the present studies. The author considers that the possibility of the release of myosin ATPase from vascular smooth muscle is small and it, if any, would hold only a small portion of serum acid ATPase activity.

The author has crucial interest in the works of BIAVA²¹ who delineated the nonspecific granules in juxtaglomerular and vascular smooth muscle cells of human kidney which are essentially similar to lysosomes in many types of cells. A considerable enlargement of the granules in patients with diabetes mellitus and a marked increase in their number in patients with benign essential hypertension have been reported. If it is permitted to state a speculation, the author suggests the possibility that serum acid ATPase of patients with essential hypertension might be in part derived from the lysosomes of the vascular smooth muscle cells.

Many lysosomal enzymes have been detected in serum, such as acid phosphatase⁹, β -glucuronidase²², acid deoxyribonuclease, acid ribonuclease, cathepsin-like acid proteinase, lysozyme, hyaluronidase etc. Recently, MATSUNAGA and the author et al.¹⁶ have found acid angiotensinase activity in rat kidney lysosomes which is related to acid angiotensinase activity in human serum. Therefore, it is reasonable to expect further discovery of many other undetermined lysosomal enzymes in the circulation. Acid ATPase would be one of them. The significance of the lysosomal enzymes in the pathogenesis of vascular injuries has been discussed elsewhere²³.

Clinical study on serum acid ATPase has

offered interesting problems. In various diseases such as essential hypertension, hepatitis, neoplasm, chronic coronary insufficiency, congestive heart failure, hyperthyroidism etc., the levels of acid ATPase were increased.

The patient with chronic coronary insufficiency suffering from frequent anginal attacks (Case 2) showed remarkable high levels of acid ATPase and the level of the enzyme diminished gradually during the course of hospitalization. Although parallel changes in the levels of acid ATPase and transaminases appeared, the decrease of the enzyme level was not accompanied with significant improvement of clinical symptom or laboratory and electrocardiographical findings. This case is considered to be an intermediate type of coronary ischemia. On the other hand, the patient with definite myocardial infarction showed no increase in serum acid ATPase level in its acute phase. NOHARA, NOZAWA and the author et al. investigated acute coronary insufficiency in dogs. In this study, serum acid ATPase activity was never increased by ligation of a coronary artery. Moreover, cardiac muscles showed very low activity of the enzyme as well as acid phosphatase (unpublished data). From these facts, the author assumes that in the heart of the patient with advanced chronic coronary insufficiency there was an increase in number of lysosomes because of chronic ischemia and subsequent metabolic disturbance. This consideration is supported by the increased levels of serum acid phosphatase during the early hospital days. Furthermore, consistent with present studies, patients with clinically evident coronary artery disease have been shown to have increased activity of serum β -glucuronidase²². Normal values of serum acid ATPase in the acute phase of myocardial infarction (Case 3) may be explained as follows. Normal cardiac muscle cells contain a comparatively smaller number of lysosomes than other organs such as the liver and kidney. If the normal heart suffers from ischemia, the amount of enzymes released from injured lysosomes into the circulation is small.

On the contrary, the patient with renal infarction (Case 4) showed a high level of the enzyme on the seventh day after the beginning of pain in flank and the enzyme level declined

rapidly thereafter. It is of considerable interest that the values of serum acid phosphatase were within normal range. These observations offer two possibilities as explanation. First, acid phosphatase activity had been already decreased on the first determination, if it was high during the early days after the embolism. Second, as shown in the previous paper³, acid ATPase is much more easily released from the particles, suggesting that acid ATPase containing particles are more labile than those containing acid phosphatase. Therefore, it is possible that only acid ATPase had been released into the circulation in this case. In regard to the cases with post-transfusion hepatitis, it seems very likely that acid ATPase is released from the inflamed liver into the blood stream. The levels of acid ATPase were high in patients with obstructive jaundice also.

In patients with congestive heart failure serum ATPase levels were high. The high level may in part result from impaired disposition. However, because all the known enzymes have not been found to be increased under these conditions, the high levels of acid ATPase would result from increased rate of release from tissues. Disturbance of cellular metabolism due to hypoxia in congestive heart failure would be the cause of lysosomal labilization. From the data in the present studies, acid ATPase seems to be a more sensitive indicator of metabolic disturbance than acid phosphatase.

Studies on the enzyme with DEAE-cellulose and Sephadex gel-filtration technique elucidated the presence of acid ATPase isozymes in not only various organs but also in serum. Occurrence of some enzyme peaks in various human organs which are absent from human serum indicates that not all the acid ATPases usually leak into the circulation. Because a limited number of examinations on human sera were carried out, it is not clear whether damage of a specific organ corresponds to a constant pattern of acid ATPase isozymes like LDH isozyme or not.

SUMMARY

The origin of serum acid ATPase was investigated. Lysosomes were the most likely origins of the enzyme, although direct evidence is not

yet obtained. Acid ATPase levels in sera from patients with various diseases were high. Several interesting cases in regard to the enzyme were presented and discussed from the lysosomal point of view.

Presence of acid ATPase isozyme in various organs and serum was evident. Human serum acid ATPase activity was electrophoretically associated with α and β -globulins and showed a minor peak with larger molecule and a major peak with smaller molecule (approx. 9×10^4) on Sephadex G-200. Human serum acid ATPase activity was resolved into at least three peaks by DEAE-cellulose column chromatography.

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