SERUM 5-NUCLEOTIDASE

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Reis (1934, 1940, 1951) has demonstrated the presence of alkaline phosphatase in various tissues specifically hydrolysing 5-nucleotidase such as adenosine and inosine-5-phosphoric acids. The enzyme has its optimum action at pH 7.8 and in all human tissues except intestinal mucosa its activity, at the physiological pH range, is much more pronounced than that of the non-specific alkaline phosphatase. Thus ossifying cartilage, one of the classical sites of high alkaline phosphatase activity, although very active against say phenyl phosphoric acids at pH 9, is more active against adenosine-5-phosphoric at pH 7.5. This finding suggested to Reis that the enzyme might play a part in calcification mechanisms. Studies designed to elucidate the physiological significance of phosphatase are frequently based on measurements of enzyme activity in blood serum or plasma, and there is an enormous literature dealing with such variations caused by different pathological conditions. It seemed, therefore, of interest to determine the activity of both serum-5-nucleotidase and non-specific alkaline phosphatase in a number of disease states.

Reagents

Glycerophosphate Buffer Substrate pH 9.3.—Sodium β -glycerophosphate, 0.05 g., sodium barbitone 0.424 g. made up to 100 ml. with water, adjusted to pH 9.3 and stored in the cold.

Glycerophosphate Buffer Substrate pH 7.5.—As above except that the pH is adjusted to 7.5 by adding approximately 1.2 ml. N HCl.

Adenosine-5-phosphate Buffer Substrate pH 7.5.—Adenosine-5-phosphate, 0.087 g., and sodium barbitone, 0.424 g., made up to 100 ml. with water and the pH adjusted to 7.5 by adding 1.2 ml. N HCl and stored in the cold.

Aminonaphtholsulphonic Acid Reagent.—Fifteen per cent. sodium bisulphite, 195 ml., 1,2,4-aminonaphtholsulphonic acid, 0.5 g., 20% sodium sulphite, 5 ml., stoppered and shaken until dissolved and stored in the cold and used within four weeks.

Molybdate Reagent.—Reagent grade ammonium molybdate, 25 g., dissolved in about 200 ml. water. In a 1-litre volumetric flask place 300 ml. 10 N H₂SO₄,

add the molybdate solution, and dilute with washings to 1 litre with water.

Standard Phosphate Solution.—KH₂PO₄ 0.02195 g., and 50 g. trichloroacetic acid, made up to 1 litre. This solution contains 0.02 mg. P in 4 ml.

Methods

Non-specific alkaline phosphatase activity is high at pH 9 towards phenylphosphate adenosine phosphate and glycerophosphate, in this decreasing order (cf. Reis, 1951). At pH 7.5, however, the total phosphatase activity is equally low towards phenyl- or glycerophosphate and the higher activity towards adenosine-5-phosphate at this reaction is reasonably inferred to be due to the specific 5-nucleotidase. Serum 5-nucleotidase activities were thus measured by subtracting the non-specific phosphatase activity with glycerophosphate as substrate at pH 7.5 from the total activity with adenosine-5-phosphate at 7.5. Serum non-specific phosphatase activities were determined by incubation with glycerophosphate at pH 9.3. Thus for the test solutions 0.2 ml. serum was mixed with 4.5 ml. of the appropriate combined buffer substrate mixture and 0.3 ml. of water and incubated at 37° C. for two and a half hours. Then the tubes were removed from the incubator and immediately mixed with 1 ml. of 30% trichloroacetic acid, allowed to stand for a few minutes, and filtered. In another tube a control is prepared in an exactly similar manner without incubation. To 4 ml. each of the trichloroacetic filtrates and standard phosphate solution are added 0.5 ml. of molybdate reagent, 0.2 ml. aminonaphtholsulphonic acid reagent, and 0.3 ml. of water. After 15 minutes the colours are read in the spectrophotometer at 660 m μ .

Calculation

Adenosine-5-phosphate buffer substrate pH 7.5 = A
Glycerophosphate buffer substrate pH 9.3 = G 9.3
Glycerophosphate buffer substrate pH 7.5 = G 7.5

Alkaline phosphatase (units/100 ml.)=
$$\frac{\text{Reading of G 9.3 (test—control)}}{\text{Reading of standard}} \times 6$$

5-nucleotidase ("units"/100 ml.)=
$$\frac{\text{Reading of A (test—control)—Reading of G 7.5 (test—control)}}{\text{Reading of standard}} \times 6$$

The alkaline phosphatase activities are thus in Bodansky units and the method is essentially that of Shinoware, Jones, and Reinhart (1942). This method of expression, rather than the King-Armstrong phenol unit, was chosen to allow a ready comparison with the 5-nucleotidase activities.

Results

When determinations of the two enzyme activities were made on a number of different sera it became apparent that results fell into three main groups (Table I).

TABLE I

SERUM ADENOSINE-5-PHOSPHATASE AT pH 7.4 COMPARED WITH NON-SPECIFIC GLYCEROPHOSPHATASE AT pH 9.3 ("BODANSKY UNITS")

Group	Adenosine-5-phosphatase at pH 7.4	Alkaline Phosphatase at pH 9.3	Disease State (No. of Cases Examined in Brackets)
I	Normal 0-1.6 units	Normal 2-5 units	Rheumatoid arthritis (17). Tuberculous spine (12). Pulmonary tuberculosis (1). Osteoporosis (1). Myeloma (2). Osteogenic sarcoma, hip* (1). Swollen ankles (1). Gouty toes (1). Kyphosis (2). Muscle injury (1). Osteitis, Osteoarthritis, Iliac condensation (1). Fragilitas ossium (1). Nephrocalcinosis (1). Suspected jaundice (13). Xanthomatosis (1). ? Myxoedema (1). Prolapsed disc (1)
II	Normal 0-1.6 units	Raised 9-19.3 units	Paget's disease (3). Rickets (6). Albright's syndrome (1). Breast cancer (2). Spinal neoplasm (1)
III	Raised 1.7-3.6 units	Raised 4.1-22 units	Tuberculosis with amyloid (4). Jaundice (22). Polycystic kidney with uraemia (1)

First Group.—Both 5-nucleotidase and non-specific alkaline phosphatase values were low in normal men, and a number of miscellaneous conditions.

Second Group.—5-Nucleotidase was low and non-specific alkaline phosphatase values were high in rickets, Paget's disease, osteogenic tumours, Albright's syndrome, breast cancer, and spinal neoplasm.

Third Group.—Both 5-nucleotidase and non-specific alkaline phosphatase values were high in obstructive and infective jaundice, liver haemangioma, lung or bone tuberculosis with amyloid, and polycystic kidney.

Results of other criteria for liver function have not shown precise correlation with the high nucleotidase values in the third group as can be seen in Table II. Sera with low bilirubin contents may have high nucleotidase activities and raised nucleotidase does not exactly parallel raised serum alkaline

TABLE II
DETAILS OF CASES IN GROUP III

Name	Condition	Adenosine Phosphatase, Units	Glycerophosphatase (Bodansky Units/100 ml.)	Bilirubin (mg./100 ml.)	Serum Cholesterol (mg./100 ml.)
B.N.	Tuberculous spine with amyloid	6.6	10.4	0	—
M.H.	" " " "	5.7	4.1	0	—
R.S.	Pulmonary tuberculosis with amyloid	6.0	19.2	0	—
J.F.	" " " "	14	13.8	0	—
5039	Polycystic kidney " "	5.6	20.0	0.6	—
W.F.	Infective hepatitis " "	2.1	12.3	5.9	221
1644	Biliary cirrhosis " "	35.5	18.4	14.7	427
P.O.	Obstructive jaundice " "	3.6	9.9	+	—
875	" " " "	13.9	13.9	8.3	450
5285	" " " "	1.7	12.3	30.5	131
953	" " " "	27.4	33.3	18.0	600
5241	" " " "	16.0	23	2.3	302
5450	" " " "	6.9	13.7	32	70
5429	" " " "	5.3	9.6	30	128
5408	" " " "	4.0	8.8	25	75
E.K.	" " " "	4.5	11.5	+	—
S.	" " " "	8.0	17.6	7.3	—
H.H.	" " " "	4.4	11.4	10.4	255
T.	" " " "	4.6	11.8	4.9	216
N.	Carcinoma of bile duct with obstruction	8.1	25.7	4	—
J.B.	Jaundice, obstructive	4.9	22.5	5.8	640
M.N.	" " " "	7.4	15.4	19.7	234
F.M.	Gallstones " "	20	23	15	515
C.B.	Chronic cholecystitis " "	20.3	22.2	7	375
R.U.	Chronic cholangitis " "	15.7	16.5	19	578
Pr.	Carcinoma of bronchus + jaundice	36	22	15	—
M.M.	Haemangioma of liver probably with obstruction	10.5	20.7	6.6	490

phosphatase. Thus one case (M. H.) showed a low 5-nucleotidase (1.5) value during the peak of his "jaundice" (bilirubin 17 mg./100 ml. and 9.9 units alkaline phosphatase) but two months later a higher 5-nucleotidase (5.7) value with lowered alkaline phosphatase (4.1) and no bilirubinaemia. In general, however, it can be said that serum cholesterol, alkaline phosphatase, and 5-nucleotidase are increased together but not to the same extent.

The serum activity curves at varying pH values from a particular case of jaundice having high 5-nucleotidase activity are shown in Fig. 1 with adenosine-5-phosphate and β -glycerophosphate as substrates. This gives further proof of the occurrence of high concentrations of this specific 5-nucleotidase with an optimum at about pH 7.5 in some sera.

Attempts to induce liver damage in two rabbits by twice weekly carbon tetrachloride (1 ml./kg.) injections for six weeks, although raising the serum cholesterol from 23 to 297 mg./100 ml., did not alter the 5-nucleotidase from 0.2 units although the alkaline phosphatase increased slightly from 0.5 and 0.9 units to 0.85 and 1.9 units per 100 ml. respectively.

Similarly in attempts to produce liver disease in rats, 5% by weight of bromobenzene was added to

FIG. 1.—Hydrolysis of adenosine-5-phosphate and of β -glycerophosphate by blood serum of jaundiced patient (Pr.) at varying pH values. A=adenosine-5-phosphate; B= β -glycerophosphate.

the stock diet of 150 g. rats for 6 weeks as described by Koch-Weser, de la Huerga, Yesinick, and Popper (1953). The serum 5-nucleotidase and alkaline phosphatase of these rats, respectively 2.1 and 14.3, was not appreciably different from the 2.1 and 14.6 of the control group.

Discussion

Although intestine and liver are regarded as contributors to the normal serum alkaline phos-

phatase level there seems general agreement that bone also is the source of at least some of it, and that the hyperactive osteoid tissue in, for example, rickets, produces the high level in that condition. Serum alkaline phosphatase is elevated only in cases where formation of some kind of osseous tissue is proceeding. In hyperparathyroidism, Paget's disease, and rickets, serum alkaline phosphatase values run roughly parallel with the severity. Normal values are found in osteoporosis and in bone tumours unless they are osteoplastic.

The results described above show that a high serum alkaline phosphatase level from liver disease is usually accompanied by a 5-nucleotidase value increased as much as a hundredfold whereas that from bone disease is not. Bone, therefore, is not likely to be the source of serum 5-nucleotidase.

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