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DEPHOSPHORYLATION OF ADENOSINETRIPHOSPHATE BY NORMAL AND PATHOLOGICAL HUMAN SERA¹

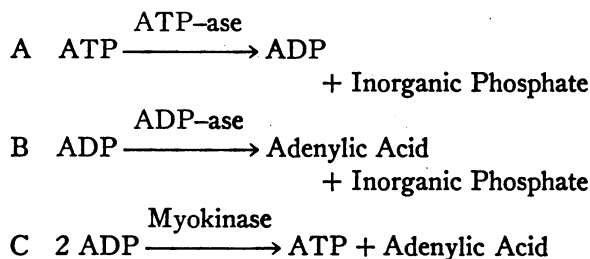
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INTRODUCTION

Recent studies indicate the importance of adenosinetriphosphate (ATP) in cellular metabolism. Adenosinetriphosphatase (ATP-ase), which catalyzes reaction A, has been found in a number of tissues.



Adenosinediphosphate (ADP) may be dephosphorylated to adenylic acid by adenosinediphosphatase (Reaction B). However, evidence for a specific ADP-ase is lacking and it is probable that the two-step dephosphorylation of ATP is catalyzed by the same enzyme (1). In muscle, ADP is converted to adenylic acid by the action of myokinase (1), in which labile phosphate is transferred from one molecule of ADP to another yielding one molecule each of ATP and adenylic acid (Reaction C).

ATP-ase has been found in muscle, liver, kidney, brain, other animal tissues, and in tumors (2), the highest concentrations being present in cardiac and skeletal muscle (3). Optimum ATP-ase activity has been found to occur at about pH 9 (3 to 7). In the course of an investigation of the dephosphorylation of ATP by human serum, a rather definite pH optimum was observed in acid media, as well as the expected optimum at alkaline reaction (8). It appeared that splitting of ATP at acid pH was not due to acid phosphomonoesterase. The dephosphorylation of ATP at acid and alkaline values of pH has been investigated in a small group of human sera.

¹ This study was carried out at Gallinger Municipal Hospital, Washington, D. C.

MATERIALS AND METHODS

Phosphorus was determined by the method of Fiske and Subbarow (9). Nitrogen analyses were carried out as described by Koch and McMeekin (10). Serum magnesium was determined according to a modification of the method of Denis (11). The glass electrode was employed for the measurement of pH.

ATP was prepared from rabbit muscle as the barium salt by the method of Kerr (12). This was converted to the sodium salt by precipitation with the stoichiometric amount of sulfuric acid, the solution then being adjusted to pH 7 with sodium hydroxide. The atomic ratio of nitrogen to phosphorus was 5.07:3, and that of acid-labile² to total phosphorus was 2.01:3. The muscle adenylic acid used in these experiments was prepared and characterized according to Ostern *et al.* (13), and further identified by the rate of color development with orcinol reagent (14).

Serum from patients and apparently healthy individuals was prepared from venous blood. Care was exercised to avoid hemolysis, which was found to increase the activity. In the studies reported, patients are referred to by number and the corresponding diagnoses are listed in Table I. The letter N appears before the numbers assigned to apparently healthy subjects.

Dephosphorylation of ATP was measured in a system designed to make the concentration of enzyme the rate-determining factor. The usual procedure was as follows: All solutions were brought to 37° before use. Five-tenths of a cubic centimeter of serum was added to 2.5 cc. of ATP in 0.1 M veronal-HCl or acetate buffer, and mixed immediately. At the end of a given period of incubation at 37° (usually from 0.5 to 2 hours), the reaction was stopped by the addition of 5 cc. of 8% trichloroacetic acid. The mixture was filtered and a suitable aliquot of the clear filtrate was analyzed for inorganic phosphorus and, in certain experiments, for acid-labile phosphorus. Controls with buffer and ATP alone, and with serum and buffer alone were employed. Determinations were usually carried out in duplicate. In some cases different concentrations of serum or incubation periods were employed in order to check the method. Where the effects of various salts were studied, these compounds were dissolved in the buffer-substrate solution, and controls containing these salts were used. Measurements of pH were carried out on identical mixtures at 37°. The data presented below are corrected

² Determined by hydrolysis with N HCl at 100° for 15 minutes.

Table 1
Dehydrochlorination of ATP by Pathological Sera *

Case No.	Sex	Age	Diagnosis	Remarks	ATP-use pH 6.9 10 cc serum	Phosphatase Alkaline Acid
1	M	67	Carcinoma of prostate (b)	No metastases	0.56	3.2
2	M	81	Carcinoma of prostate (b)	Bony metastases; orchectomy	0.91	10.6
3	M	71	Carcinoma of prostate (b)	Bony metastases; orchectomy	0.43	6.5
4	M	73	Carcinoma of prostate (b)	No metastases; orchectomy	0.19	3.0
5	M	55	Carcinoma of prostate (b)	Bony metastases; orchectomy	1.84	37.6
6	M	72	Carcinoma of prostate (b)	Bony metastases; orchectomy	2.84	31.9
7	M	72	Carcinoma of prostate (b)	Bony metastases; orchectomy†	3.02	34.8
8	M	81	Carcinoma of prostate (b)	Bony metastases; orchectomy	0.51	10.7
9	M	61	Carcinoma of prostate (a)	Metastases to bone and liver; orchectomy†	7.64	84.0
10	M	73	Carcinoma of prostate (b)	Bony metastases; orchectomy	1.00	19.9
11	M	65	Carcinoma of prostate (b)	Bony metastases; orchectomy†	0.44	4.6
12	M	56	Benign prostatic hypertrophy (b)		0.46	0.42
13	M	68	Benign prostatic hypertrophy (b)		0.23	0.40
14	M	61	Benign prostatic hypertrophy (b)		0.93	0.50
15	M	61	Benign prostatic hypertrophy (b)		0.31	0.37
16	M	63	Benign prostatic hypertrophy (b)		0.43	0.35
17	M	48	Acute prostatitis (c)		0.26	0.52
18	M	24	Acute prostatitis (c)		0.38	0.40
19	M	24	Chronic prostatitis (c)		0.58	0.40
20	M	60	Carcinoma of stomach (a)	Liver metastases; jaundice	3.71	1.60
21	M	65	Carcinoma of stomach (c)	Liver metastases	0.67	0.23
22	F	55	Carcinoma of stomach (c)	Liver metastases; jaundice	2.10	0.93
23	F	72	Carcinoma of stomach (c)	Liver metastases; jaundice	2.73	0.74
24	M	42	Carcinoma of stomach (c)	Liver metastases; jaundice	0.42	1.25
25	F	45	Carcinoma of breast (b)	Generalized metastases	1.99	1.94
26	M	78	Carcinoma of bladder (b)	Local extension	1.05	0.40
27	M	68	Carcinoma of colon (a)	Liver metastases	2.18	0.88
28	M	42	Carcinoma of lung (b)	No metastases	0.45	0.47
29	M	53	Carcinoma of lung (b)	Generalized	0.63	1.04
30	M	26	Hodgkin's disease (b)	Anemia, liver damage	0.80	0.62
31	M	85	Possible malignancy	Mild liver damage	3.45	2.42
32	F	49	Girrhosis (c)	Severe liver damage; jaundice	0.51	0.69
33	F	89	Girrhosis (c)	Moderate liver damage	4.82	1.79
34	F	39	Girrhosis (c)	Moderate liver damage	0.86	1.61
35	F	55	Girrhosis (c)	Severe liver damage; jaundice	1.89	0.94
36	F	45	Girrhosis (c)	Mild liver damage	0.50	0.83
37	F	44	Girrhosis (c)	Moderate liver damage	0.53	0.35
38	F	40	Girrhosis (c)	Severe liver damage; jaundice	0.57	0.77
39	M	40	Girrhosis (c)	Moderate liver damage	3.16	0.73
40	M	85	Girrhosis (c)	Severe liver damage; jaundice	0.92	0.47
41	M	37	Girrhosis (c)	Mild liver damage	0.78	0.70
42	M	37	Girrhosis (c)	Mild liver damage	1.80	0.79
43	M	69	Girrhosis (c)	Mild liver damage	0.78	0.78
44	M	87	Girrhosis (c)	Mild liver damage; jaundice	0.65	0.75
45	M	87	Non-specific serum hepatitis (c)	Severe liver damage; jaundice	0.32	0.69
46	M	87	Non-specific serum hepatitis (c)	Mild liver damage	0.32	0.69
47	F	34	Cystitis, ST. SARUM (b)		0.76	12.5

*ATP-use expressed as micromoles P split per cc. serum per hour.

†Acid and alkaline phosphatases are expressed as Galtman and Bodensky units per 100 cc. serum, respectively.

‡Receiving antibiotics

(a)Diagnosis confirmed by autopsy

(b)Diagnosis confirmed by biopsy or operation

(c)Diagnosis based on clinical procedure

Case No.	Sex	Age	Diagnosis	Remarks	ATP-use pH 6.9 10 cc serum	Phosphatase Alkaline Acid
48	M	42	Acute infectious hepatitis (c)	Mild liver damage	0.93	3.3
49	F	25	Acute infectious hepatitis (c)	Mild liver damage	0.50	0.69
50	M	67	Congestive heart failure (c)	Arteriosclerosis	0.50	0.30
51	M	31	Congestive heart failure (c)	Arteriosclerosis	0.31	0.46
52	M	58	Congestive heart failure (c)	Arteriosclerosis	0.65	3.0
53	M	42	Syphilitic heart failure (c)	Arteriosclerosis	0.43	0.35
54	M	65	Congestive heart failure (c)	Arteriosclerosis	0.59	0.80
55	M	30	Congestive heart failure (c)	Arteriosclerosis	0.55	0.64
56	M	59	Congestive heart failure (c)	Arteriosclerosis	0.23	0.98
57	M	53	Rheumatic heart disease; rheumatic mitralis (c)	Arteriosclerosis	0.61	3.3
58	F	14	Subacute rheumatic fever (c)		0.59	7.5
59	M	15	Subacute rheumatic fever (c)		1.30	0.68
60	M	40	Acute rheumatic fever (c)		0.78	0.89
61	M	24	Acute rheumatic fever (c)		0.40	2.6
62	F	45	Labor pneumonia (c)		0.26	3.9
63	M	23	Brucellosis (c)		0.23	2.3
64	M	73	Cerebral infarct (c)	Arteriosclerosis	0.26	2.4
65	M	65	Liver abscess (c)		0.56	0.55
66	M	46	Silico-tuberculosis (c)		1.01	6.6
67	F	17	Peritonitis (c)		0.22	3.5
68	M	22	Tetanus (c)		0.64	0.30
69	M	71	Tertiary syphilis (b)	Gumma of leg	0.53	0.78
70	M	68	Tuberculosis (c)		0.27	0.68
71	F	35	Diabetes mellitus (c)		0.69	0.56
72	F	55	Diabetes mellitus (c)		0.83	0.41
73	M	79	Diabetes mellitus (c)		0.44	0.68
74	F	76	Diabetes mellitus (c)		0.38	3.5
75	F	46	Diabetes mellitus (c)		0.19	2.8
76	F	82	Diabetes mellitus (c)		0.83	6.4
77	M	93	Diabetes mellitus (c)		0.41	3.4
78	M	23	Diabetes mellitus (c)		0.16	3.6
79	M	47	Diabetes mellitus (c)		0.24	4.3
80	M	36	Diabetes mellitus (c)		0.24	4.3
81	F	21	Hypomagnesemia (c)	Receiving propylthiouracil	0.37	0.49
82	F	26	Hypomagnesemia (c)		0.56	0.69
83	M	26	Chronic glomerulonephritis (c)		0.79	1.14
84	M	26	Chronic glomerulonephritis (c)		0.39	3.9
85	M	43	Fracture of humerus (c)		0.90	0.54
86	M	30	Fracture of tibia (c)		0.46	0.82
87	M	48	Chromoblastomycosis (c)		0.53	0.62
88	M	63	Chromoblastomycosis (c)		0.50	0.54
89	F	17	Beck's steroid (b)	Advanced pulmonary fibrosis	0.28	0.39
90	F	38	Neurological arthritis (c)		0.33	0.33
91	M	55	Phlebotomy (c)		0.41	0.48
92	M	40	Thrombolytic purpura (c)	Splenectomy	0.32	0.73
93	M	14	Schist. spl. ganglia (c)		0.64	0.83
94	M	42	Hyalinaceous leukemia (c)		0.51	0.43
95	F	86	Scleroderma, generalized (c)		0.43	0.77
96	F	86	Scleroderma, generalized (c)		0.43	0.77

for serum inorganic phosphorus and nonenzymatic hydrolysis, and are expressed as micromoles of phosphorus split from ATP per cc. serum per hour, unless otherwise stated.

Serum alkaline and acid phosphatases were determined by the procedures of Bodansky (15) and Gutman (16), respectively, and are expressed in the customary units.

RESULTS

pH-activity curves

The liberation of inorganic phosphorus from ATP by human serum was found to exhibit optima in the ranges of pH from 4.6 to 5.1 and 8.7 to 9.1. This was true with normal serum and serum with increased activity (Figures 1 and 2). In normal serum the two optima are of about the same order of magnitude, although frequently the activity at

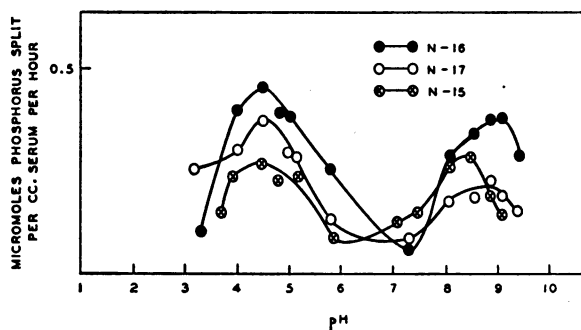


FIG. 1. pH-ACTIVITY CURVES FOR 3 NORMAL HUMAN SERA, CARRIED OUT WITH 9.67 MICROMOLES OF ATP

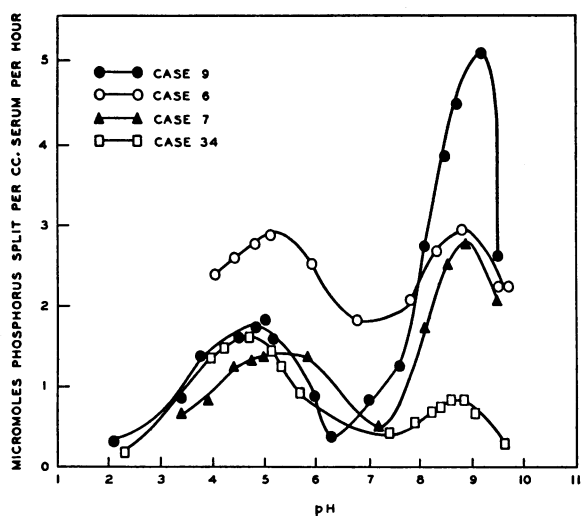


FIG. 2. pH-ACTIVITY CURVES FOR 4 PATHOLOGICAL HUMAN SERA WITH ELEVATED ACTIVITY, CARRIED OUT WITH 9.67 MICROMOLES OF ATP

acid pH was slightly greater. With certain pathological sera, however, the two activities were markedly different and showed no consistent relationship. The acid optimum was a constant finding in all human sera studied and similar data were obtained with 0.01 M citrate buffers and with three different ATP preparations. Identical pH-activity curves were observed with dialyzed serum. Subsequent studies were carried out at the pH optima, *i.e.*, pH 4.8 and 8.9.

Effect of substrate concentration

Greater concentrations of substrate were found necessary for maximum activity at pH 4.8 than at 8.9 (Table II). Usually between two and

TABLE II
Effect of substrate concentration *

ATP conc. micromoles	Micromoles P split per cc. serum per hour	
	pH 4.8	pH 8.9
0.676	1.50	3.42
1.35	1.92	5.54
2.03	2.15	6.70
2.71	2.30	7.64
3.38	2.48	7.84
4.06	2.40	7.68
5.41	2.76	7.27
6.76	2.88	7.37
7.44	2.83	7.15
8.79	2.80	7.80
10.0	2.86	7.18
12.2	2.84	

* These data were obtained with serum of case 9.

three times as much ATP was required at pH 4.8 than at 8.9. In order to insure maximum activity, the studies described below were carried out with 3.38 and 9.67 micromoles of ATP at pH 8.9 and 4.8, respectively, except as noted in certain experiments. The liberation of inorganic phosphorus was directly proportional to serum concentration and duration of incubation. With sera of high activity, shorter incubation periods or lower serum concentrations were employed.

Dephosphorylation of ATP at pH 8.9

On prolonged incubation practically all of the ATP phosphorus was liberated as inorganic phosphorus at pH 8.9 (Figure 3, Table III). In addition the inorganic phosphorus produced was greater than the decrease in acid-labile phosphorus (Table III). The ratio of the decrease in

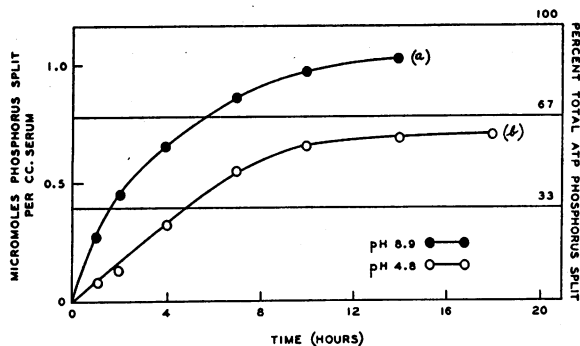


FIG. 3. DEPHOSPHORYLATION OF ATP BY NORMAL HUMAN SERUM (SUBJECT N-16) AT pH 8.9 (a) AND pH 4.8 (b)

Maximum hydrolysis is equivalent to 1.17 micromoles of inorganic P.

TABLE III
Dephosphorylation of ATP at pH 8.9 *

Time	Inorganic P (A)	Decrease in acid-labile P (B)	Ratio B/A	Per cent total P split
hours	micromoles	micromoles		
0.5	1.62	1.07	0.660	24.5
1.0	2.71	1.75	0.646	41.1
2.0	4.19	2.78	0.663	63.5
4.0	5.66	3.68	0.650	85.8
8.0	6.11	3.96	0.648	92.6

* These data were obtained with 1.0 cc. serum (case 5). Maximum hydrolysis \approx 6.60 micromoles inorganic P.

acid-labile phosphorus to inorganic phosphorus was, within experimental error, about two-thirds, suggesting that adenylic acid formed by splitting of labile phosphorus is completely hydrolyzed as soon as it appears. This was confirmed by studies in which serum was added to small amounts of adenylic acid under identical conditions of serum concentration, pH, and duration of incubation. The splitting of adenylic acid by normal and pathological sera was usually complete under these conditions (Table IV). The term "alkaline adenosinepolyphosphatase" (alkaline APP-ase) will be used to represent the enzymatic liberation of phosphorus from ATP at pH 8.9, since several enzymes, including phosphomonoesterase, may be concerned in the splitting of ATP.

Dephosphorylation of ATP at pH 4.8

The hydrolysis of ATP at pH 4.8 appeared to reach completion when about two-thirds of the total ATP phosphorus was split (Figure 3, curve

TABLE IV
Hydrolysis of adenylic acid *

Case No.	Alkaline phosphatase units	Acid phosphatase units	APP-ase		Adenylic acid		
			pH 8.9	pH 4.8	Added	Split	
						pH 8.9	pH 4.8
(1)	(2)	(3)	(4)	(5)	(6)	(7)	
N-15			0.22	0.34	0.17	0.17	0.01
N-16			0.34	0.45	0.17	0.17	0
84	3.4	0.6	0.29	0.27	0.16	0.14	0
92	3.6	1.2	0.41	0.66	0.16	0.16	0.02
35	11.3	2.1	1.89	0.94	0.32 0.64	0.28 0.43	0.05 0.08
15	5.6	2.2	0.31	0.37	0.16	0.18	0
5	37.6	6.5	1.84	0.67	0.16 0.32 0.64	0.16 0.32 0.66	0.03 0.09 0.09
7	36.8	15.3	3.02	1.66	0.71	0.71	0.05
8	10.7	21.6	0.51	0.58	0.16 0.32	0.14 0.32	0.08 0.15
9	58.0	22.4	7.84	2.84	1.20 0.64	1.20	0.34 0.18
10	19.9	22.7	1.00	0.75	0.16 0.32	0.18 0.32	0.09 0.15

* Adenylic acid expressed as micromoles; experimental details given in text. APP-ase expressed as micromoles P split per cc. serum per hour.

b). The liberation of inorganic phosphorus was not appreciably greater than the decrease in acid-labile phosphorus (Table V), indicating that the splitting of ATP at pH 4.8 stops with the formation of adenylic acid. This is confirmed by the finding that negligible to small amounts of adenylic acid were split by most sera (Table IV). Greater, but not complete dephosphorylation of this sub-

TABLE V
Dephosphorylation of ATP at pH 4.8 *

Case No.	Time	Inorganic P	Decrease in acid-labile P
	hours	micromoles	micromoles
7	2	1.66	1.63
7	5	3.40	3.26
39	2	0.77	0.76
35	2	0.96	0.90
6	2	2.47	2.43
25	2	1.94	1.90

* These data were obtained with 0.5 cc. serum.

strate occurred with sera with elevated values of acid phosphatase associated with metastasizing carcinoma of the prostate. The enzymatic liberation of phosphorus from ATP at pH 4.8 will be designated as "acid adenosinepolyphosphatase" (acid APP-ase). In most cases this represents splitting of labile phosphorus and could therefore be termed adenylypyrophosphatase.

Effects of salts and dialysis

The effects of calcium chloride, magnesium sulfate, sodium fluoride, and sodium cyanide on serum APP-ase were studied (Table VI). No significant activation was observed in studies in which the concentration of calcium was varied between 0.1 and 0.0001 M. On the other hand, magnesium sulfate produced activation in some sera at pH 8.9, while there was no effect in acid media. Identical results were obtained with magnesium chloride. Activation by magnesium was quite variable and was usually observed in sera with elevated activity, but not in normal sera. When present,

TABLE VI
Effect of salts *

Salt	Case No.	APP-ase pH 8.9		Case No.	APP-ase pH 4.8	
		Without salt	With salt		Without salt	With salt
CaCl ₂	N-5	0.31	0.36	N-15	0.29	0.29
	N-15	0.20	0.23	N-26	0.55	0.51
	6	1.73	1.80	9	2.48	2.49
	39	0.65	0.68	41	0.47	0.48
	5	2.01	2.05	42	0.78	0.71
	41	0.68	0.64	80	0.49	0.51
MgSO ₄	N-15	0.20	0.23	N-16	0.56	0.53
	N-22	0.26	0.26	N-5	0.32	0.28
	6	1.73	2.92	N-25	0.25	0.23
	39	0.65	1.14	7	1.78	1.70
	45	0.65	0.80	41	0.47	0.46
	7	2.76	3.58	9	2.40	2.36
NaF	N-15	0.29	0.24	N-16	0.57	0.26
	N-5	0.29	0.27	N-5	0.32	0.16
	N-25	0.19	0.21	N-25	0.25	0.04
	33	4.82	4.65	24	1.17	0.51
	5	2.05	2.05	9	2.40	1.50
	45	0.65	0.64	22	0.65	0.25
NaCN	N-15	0.29	0.29	N-15	0.33	0.29
	N-5	0.29	0.28	N-5	0.32	0.32
	N-25	0.19	0.20	N-25	0.19	0.20
	41	0.76	0.61	24	1.17	1.17
	22	2.10	1.96	45	0.75	0.75
	9	4.18	3.48	9	2.10	2.10

* Concentration of added salt was 0.001 M. APP-ase expressed as micromoles P split per cc. serum per hour.

TABLE VII
Effect of dialysis on serum APP-ase *

Case No.	pH 8.9		pH 4.8	
	Undialyzed	Dialyzed	Undialyzed	Dialyzed
N-25	0.19	0.23	0.27	0.28
N-17	0.22	0.21	0.50	0.49
21	0.66	0.67	0.23	0.26
42	0.78	0.60	0.78	0.82
41	0.92	0.99	0.54	0.59
40	3.16	3.17	0.73	0.70
9	5.17	5.17	1.77	1.89

* APP-ase expressed as micromoles P split per cc. serum per hour.

this effect was maximal with magnesium concentrations of 0.001 M. The reason for the variable effect of magnesium is not known. Determinations of serum magnesium revealed the reported normal levels of 1 to 3 mg. per 100 cc. (11), which would correspond to concentrations of 0.0000686 to 0.000206 M under the present conditions. Relatively high concentrations of magnesium (0.01 to 0.1 M) inhibited all sera studied. Sodium fluoride (0.001 M) produced marked inhibition at pH 4.8, while the same concentration of this salt had no appreciable effect at pH 8.9. Inhibition at pH 8.9 was noted, however, with 0.01 M fluoride. Sodium cyanide (0.001 M) inhibited sera of elevated activity but not normal sera at pH 8.9, while 0.01 M cyanide inhibited both normal and pathological sera. On the other hand, at pH 4.8, concentrations of 0.001 to 0.01 M cyanide had no significant effect.

In an attempt to determine the presence in serum of diffusible cofactors, activators, or inactivators, dialyzed sera were studied. Serum was dialyzed in cellophane sacs at 5° for 48 hours against large volumes of distilled water. The small precipitate which forms was resuspended. No appreciable difference in activity was found between the controls (refrigerated at 5°) and the dialyzed sera (Table VII). In addition no loss in activity occurred in sera stored for 48 hours at 5°, nor in sera frozen at -5° for three or four days.

Clinical studies

Studies of 27 apparently normal individuals are summarized in Table VIII. The group was about equally divided between the sexes and the range

TABLE VIII
APP-ase of normal human sera †

	pH 8.9	pH 4.8
Number of subjects	27	24
Range	0.13-0.66	0.20-0.66
Mean	0.31±0.1*	0.40±0.1

$$* \text{Std. dev.} = \sqrt{\frac{\sum d^2}{(n-1)}}$$

† APP-ase expressed as micromoles of P_i split per cc. serum per hour.

of age was 20 to 63, the average age being 36. Significant variation with age or sex was not apparent, although a larger study would be necessary to investigate this point specifically. For the purposes of the present discussion, values greater than 0.70 at pH 8.9, and 0.80 at pH 4.8 are considered to be elevated.³

Of the pathological sera studied at pH 8.9, values above 0.70 were encountered in 31 cases, 23 of which exhibited clinical evidence of disease of liver or bone. All 13 cases with alkaline APP-ase greater than 1.30 had evidence of hepatic impairment or carcinoma of the prostate with bony metastases. Increased alkaline APP-ase was always associated with elevated alkaline phosphomonoesterase activity. All of the 31 cases with elevated alkaline APP-ase were found to have alkaline phosphatase values greater than 4 units, and 21 of these had values between 10 and 60 units. Although there were 29 cases with values of alkaline phosphatase greater than 4 units and normal levels of alkaline APP-ase, none of these exceeded 11 units and more than half were less than 6 units.

Of the pathological sera studied at pH 4.8, 19 were found to have APP-ase exceeding 0.80, and 12 of these (and all of the 8 cases with values greater than 1.50) had clinical evidence of hepatic or bone disease. It is now well established that marked elevations of acid phosphatase occur exclusively in cases of metastasizing carcinoma of the prostate, although slightly increased levels occasionally occur in other diseases (17, 18). Elevated acid phosphatase was present in 6 cases of prostatic carcinoma with bony metastases and in one case where no definite diagnosis could be made (case 31). These values are lower than

³ Values of acid and alkaline phosphatase, greater than 4 units per 100 cc., are considered to be elevated.

those of some of the reported cases probably because no patients with untreated advanced prostatic cancer were available for study. There appeared to be no consistent relationship between phosphomonoesterase and APP-ase at pH 4.8. Elevated acid APP-ase was encountered in cirrhosis and in non-prostatic cancer, where a normal level of acid phosphatase would be expected and was found. Of the 14 cases with elevated acid APP-ase, in which acid phosphatase was also determined, only four exhibited elevated acid phosphatase. In three cases of prostatic cancer with increased acid phosphatase, the acid APP-ase was within the normal range.

Slight variations in the data obtained on several normal individuals were noted over a period of 4 to 6 months. In patients with high values of APP-ase somewhat greater fluctuations with time were noted. Insufficient data are available, however, to relate these changes to the clinical course.

APP-ase of animal sera

The effect of hydrogen ion concentration on the serum APP-ase of three animal species was investigated. The sera of 4 dogs, 5 rabbits, and 4 specimens of pooled rat serum were studied. Representative pH-activity curves are given in Figure 4. Maximum activity occurred in the acid range with dog and rabbit sera, with only a small

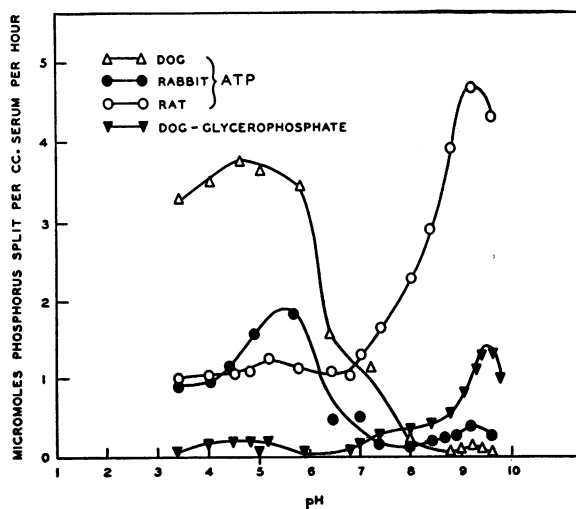


FIG. 4. pH-ACTIVITY CURVES FOR SERUM APP-ASE OF DOG, RABBIT, AND RAT, CARRIED OUT WITH 9.67 MICROMOLES OF ATP

The concentration of glycerophosphate was 0.025 M.

peak at about pH 9. In contrast, very high activity was found at pH 9 with rat serum and only a slight elevation in the rather flat curve in the acid region. As opposed to the results obtained for dog serum APP-ase, the pH-activity curve, with beta-glycerophosphate as the substrate, exhibited maximum activity at alkaline reaction. The latter pH-activity curve is very similar to those reported for normal human serum glycerophosphatase (19). Experiments in which mixtures of dog and human sera were tested for APP-ase activity at pH 4.8 yielded additive results.

DISCUSSION

Although a physical separation of acid and alkaline APP-ase was not attempted, it is probable that these activities represent different enzymes, as has been established for the phosphomonoesterases (20). There is no consistent relationship between the rates of dephosphorylation of ATP at pH 4.8 and 8.9 in various human and animal sera. More substrate is required to saturate acid APP-ase, and added salts (MgSO_4 , NaF, NaCN) have different effects on the two activities. The Russian workers have reported that in addition to the prominent pH optimum at 9, myosin preparations exhibit a second and lower optimum at pH 6.3, the entire pH-activity curve being equally lowered at all points by magnesium. A careful study led these authors to believe that the two optima were related to the dependence of activity upon the ionization of both enzyme and substrate, rather than to two different enzymes (21). This explanation does not appear applicable to the present results.

At pH 8.9, splitting of ATP could be catalyzed by ATP-ase, an ADP-ase or myokinase, and phosphomonoesterase. At this pH, adenylic acid is completely hydrolyzed and the rate-determining factor appears to be the splitting of labile phosphate. The values of adenylypyrophosphatase would therefore be two-thirds of those of APP-ase. Except with sera of increased acid phosphatase content, the values of acid APP-ase are equivalent to adenylypyrophosphatase activity.

The range of values of beta-glycerophosphatase activity reported for normal human serum (15, 19) corresponds to 0–0.35 and 0.48–1.28

micromoles of phosphorus per cc. serum per hour for acid and alkaline phosphatase, respectively. The corresponding normal serum APP-ase values are therefore somewhat less at pH 8.9 and slightly greater at pH 4.8 (Table VIII). With pathological sera, less phosphorus was also split from ATP than from beta-glycerophosphate at alkaline pH, and this difference was quite marked in certain cases. The acid APP-ase values, on the other hand, were higher than those of phenylphosphatase calculated on the same basis, in all but 3 (cases 5, 8, and 10) of the pathological sera studied.

The existence of a fairly specific ATP-ase active at alkaline pH is well established for muscle (6, 21). Purified myosin does not act upon inorganic pyrophosphate or monophosphates (6), but splits ATP, inosine triphosphate (22), and to some extent inorganic triphosphate (23). Jacobsen (24), Hasse (5), Barrenscheen and Lang (4), and Pillai (25) have reported evidence in support of a specific ATP-ase in liver, while Satoh (26) attributed the dephosphorylation of ATP to the combined action of pyrophosphatase and phosphomonoesterase.

At pH 8.9 the dephosphorylation of ATP could be due to several enzymes including alkaline phosphatase. However, in view of the lack of correlation between APP-ase and phosphomonoesterase at pH 4.8, it appears unlikely that acid phosphatase is responsible for ATP hydrolysis. Furthermore, with dog serum (Figure 4), the acid APP-ase was more than 16 times greater than the acid beta-glycerophosphatase activity. These findings are compatible with the recent work of MacLeod and Summerson (27). They report that a partially purified preparation of seminal fluid phosphatase was incapable of acting upon ATP, but they observed complete dephosphorylation of ATP by human seminal fluid. We have confirmed the latter finding, and have determined the pH optimum, which is in the neighborhood of 4, for the APP-ase activity of two specimens of seminal fluid.

An "acid" adenylypyrophosphatase, present in serum and seminal fluid, and possibly other tissues, is suggested by the present data. The question of specificity, however, remains to be investigated. Although acid phosphatase of prostatic origin does not act upon ATP, the possibility has not been ruled out that other phosphomonoesterases,

or even inorganic pyrophosphatase, may split ATP at acid pH.

The absence of activation by calcium is of interest in view of the striking calcium activation reported with myosin (6, 7, 21). Stimulation by calcium of ATP-ase but not beta-glycerophosphatase was reported by DuBois and Potter (3) for liver homogenates. Bailey (6) found that the activation by calcium of liver and electrical tissue ATP-ase was not as marked as with myosin. On the other hand, absence of calcium-activated ATP-ase was reported in rat muscle homogenates (28) and in chick embryos (29). It is possible that the concentration of calcium in serum is sufficient for maximum activity. Determinations of APP-ase of plasma prepared with 0.2% sodium oxalate revealed a slight and variable decrease in activity similar to the results reported for plasma phosphatase (15).

Magnesium increased the activity of certain pathological sera but had no effect with normal sera. Magnesium stimulates ATP hydrolysis by liver and electrical tissue (3, 6), and certain myosin preparations (6, 7), and has been shown to accelerate the conversion of ADP to adenylic acid (1, 6), and to activate alkaline phosphatase (30). Since adenylic acid, under our conditions, is completely split by serum at pH 8.9, in the absence of added magnesium, the most probable effect of magnesium is upon ADP breakdown. The inhibitory effect of higher concentrations of magnesium has also been observed in muscle and liver (3, 6).

Inactivation of ATP-ase by fluoride is well known. It is of interest that fluoride is a more efficient inhibitor at pH 4.8 than at 8.9, similar findings having been noted with acid phosphatase by Gutman (31). Cyanide has no effect on serum acid APP-ase but inhibits at alkaline pH. The latter finding was reported with 0.001 M sodium cyanide for liver ATP-ase (4), but not with chick embryo systems (32) or myosin (21, 33).

The phosphomonoesterases normally present in serum are believed to be derived from at least several body tissues. Additional serum alkaline phosphatase in certain diseases probably arises from the bones and liver, while malignant prostatic tissue is considered to be the source of marked elevations of serum acid phosphatase. In view of the fact that elevated serum APP-ase was associated mainly with liver damage and bony

metastases from prostatic carcinoma, it is quite possible that these tissues may be the source of the increased serum APP-ase. The variable effects of magnesium and cyanide with different sera may be due to qualitative differences in the APP-ase enzymes derived from different tissues, as has been noted for alkaline phosphatase (34, 35, 36).

In view of the striking species differences in serum APP-ase, studies of various human and animal tissues might be expected to exhibit similar variation. Although it is possible that determinations of serum APP-ase may be of some diagnostic value, it is apparent that a study of a larger number of patients, with the above mentioned and other diseases, is necessary for a more complete evaluation of these studies and their relationship to the usual clinical phosphatase determinations.

SUMMARY

The dephosphorylation of ATP by normal and pathological human sera exhibits two pH optima at about 8.9 and 4.8. Hydrolysis of ATP at pH 8.9 is associated with the splitting of all three phosphate bonds of ATP and is probably due to several enzymes including alkaline phosphomonoesterase, although the hydrolysis of labile phosphate is apparently the rate-determining factor. Dephosphorylation of ATP at pH 4.8 usually consists of splitting of the labile phosphate linkages, and the evidence suggests that this activity is not due to acid phosphatase. The existence of an "acid" adenylypyrophosphatase in serum, seminal fluid, and possibly other tissues, is suggested though not established.

The effects of substrate concentration, calcium, magnesium, fluoride, cyanide, and dialysis on the splitting of ATP by human serum are described. The pH-activity curves for the dephosphorylation of ATP by several animal sera are given.

A study of ATP dephosphorylation and phosphomonoesterase in a small group of normal and pathological human sera was made. Elevated adenosinepolyphosphatase activity was frequently associated with liver disease and bony metastasis from prostatic carcinoma.

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