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Activation of serum 5′ nucleotidase by magnesium ions and its diagnostic applications

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SYNOPSIS Magnesium ions increase the hydrolysis of adenosine 5′ monophosphate (5′ AMP) by human serum at *p*H 7·9 but not at *p*H 9·3. The additional hydrolysis at *p*H 7·9 is predominantly due to increased activity of the specific phosphatase 5′ nucleotidase (5Nase). This increase is proportional to enzyme concentration and has been employed as a measure of 5Nase activity in a sensitive micro-estimation.

The normal range for serum 5Nase activity by this technique was 0 to 20 mIU/ml. In a series of over 200 patients, raised values were found frequently in hepatobiliary disease and infrequently in bone disease. Assay of 5Nase activity gave a more reliable indication of the source of raised serum alkaline phosphatase than isoenzyme electrophoresis in agar gel. The correlation between activities of the two enzymes was low in bone disease generally, and fairly good in hepatobiliary disease. The closest correlation was found in patients with parenchymal liver disease.

There exists in human tissues a widely distributed enzyme, 5′ nucleotidase (5Nase, EC 3·1.3·5), which specifically catalyses the hydrolysis of the 5′ monophosphates of purine and pyrimidine ribosides (Reis, 1951), adenosine 5′ monophosphate (5′ AMP) being the substrate generally used in the measurement of its activity.

Clinical interest in this enzyme was aroused by the finding that its activity in human serum was frequently raised in liver disease, and seldom raised in bone disease (Dixon and Purdom, 1954; Wachstein and Sigismondi, 1958). This is surprising, since bone has a high, and liver a poor concentration of this enzyme (Reis, 1951). Whereas the measurement of serum 5Nase did not seem to be of value in its own right, its use was advocated in clarifying the source of a raised serum alkaline phosphatase (APase, EC 3·1.3·1) since this enzyme is raised physiologically in infancy and pregnancy, and pathologically in diseases of bone and the hepatobiliary system. Only in the last-mentioned group are raised levels of serum 5Nase generally encountered.

Others have been more enthusiastic, claiming an important place for the estimation in the differential diagnosis of jaundice (Young, 1958); in the diagnosis of biliary obstruction (Kowlessar, Haeffner, Riley, and Sleisenger, 1961; Bardawill and Chang, 1963); in the diagnosis of pericholangitis (Vinnik, Kern, and Corley, 1963) and of metastatic liver disease (Schwartz and Bodansky, 1965); and as a general liver function test (Hill and Sammons, 1965; 1966; 1967). Davidge and Philpot (1966), on the other hand, found the test of no value in liver disease.

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The estimation of 5Nase is complicated by the fact that APase also hydrolyses 5′ AMP, though at higher optimal *p*H. Different methods, none of them entirely satisfactory, have been used to compute or to eliminate the hydrolysis of 5′ AMP by APase. These differences, together with variability in the clinical material and methods of diagnosis, may partially explain the divergent results. Although the frequent discrepancy between results for APase and 5Nase in individual patients has received comment from previous authors, the extent of these discrepancies have not been examined or correlated with the disease process. We report such a study aimed at clarifying these relationships, and the development of a sensitive micro-assay for 5Nase which has advantages over existing procedures.

MATERIALS AND METHODS

Sera were collected from adult patients with jaundice, previously raised APase, conditions associated with raised APase, or from patients where 5Nase estimation was specifically requested. Sera were stored at 4°C for up to four days or at −20°C for up to 10 weeks where assays were not carried out promptly. No activity is lost under these conditions. The patients were thus selected, and fell into the following categories.

1 EXTRAHEPATIC OBSTRUCTION (55 CASES) All were jaundiced at the time of examination. Sixteen were diagnosed on clinical and radiological grounds as cholelithiasis, and in a further seven cases the diagnosis of stone or stricture was substantiated at operation. Ten subjects had clinical evidence of cholecystitis associated with fever, leucocytosis, and raised ESR; in all but two, subsequent operation revealed a chronically inflamed gallbladder. Twenty-two patients had a tumour obstructing the biliary tree; this was diagnosed at laparotomy, and in all, the liver was free of metastases at the time of operation.

2 PARENCHYMATOUS LIVER DISEASE (64 CASES) Twenty-two patients with hepatitis were included. Two had the clinical, immunological, and histological features of lupoid hepatitis. The remaining 20 had the clinical and biochemical features of viral hepatitis, and the diagnosis was confirmed by needle biopsy in seven. Six patients with portal cirrhosis were diagnosed on clinical and biochemical criteria alone, while 19 had histological confirmation of the diagnosis. Eleven patients with primary biliary cirrhosis were diagnosed on clinical and histological criteria, and in all nine patients in whom it was carried out, the antimitochondrial antibody test was positive. Miscellaneous conditions accounted for six cases: drug jaundice (two), hepatic venous congestion diagnosed at necropsy (three), sarcoidosis of liver (one).

3 BONE DISEASE (49 CASES) No patient in this group had clinical or biochemical evidence of hepatobiliary disease. Twenty-two patients had clinical and radiological features of Paget's disease. Fourteen had an identified cancer (prostate nine, bronchus two, breast two, thyroid one) and osteoblastic secondaries diagnosed radiologically. Thirteen patients with miscellaneous bone diseases were examined (secondary hyperparathyroidism four, thyrotoxic bone disease four, degenerative bone disease three, bone cyst one, tuberculosis one).

4 HEPATIC METASTASES (31 CASES) All patients had a known primary tumour, hepatomegaly, and abnormal liver function tests, and the diagnosis was confirmed at laparotomy, needle biopsy, or postmortem examination in 21. None of the patients had known bone involvement, but in few was a thorough skeletal survey carried out.

5 RETICULOSIS (17 CASES) One patient with lymphosarcoma and 14 with Hodgkin's disease were studied, the diagnosis being made histologically in all cases. Two cases shown at necropsy to have chronic lymphatic leukaemia with infiltration of the liver were included as they were considered to fit best with this group. Six subjects were jaundiced; five had hepatomegaly without jaundice; six had no clinical or biochemical evidence of hepatic involvement.

6 OTHERS Estimations were carried out on six subjects in whom a final diagnosis could not be made. They are not considered further.

5NASE ESTIMATION The procedure finally adopted involved measurement of the phosphorus liberated by hydrolysis of 5′ AMP at 37°C under conditions of zero-order kinetics. Two assays were carried out, in one of which Mg^{++} ions were present in a final concentration of 10 mM, while in the other no Mg^{++} ions were added. The difference was a measure of 5Nase activity.

To 1·7 ml 0·1M Michaelis veronal buffer *p*H 7·9 (King, 1965) was added 50 μl water or 50 μl 400 mM $MgCl_2$ prepared by dissolving the requisite amount of MgO in HCl of appropriate strength. To each, 50 μl serum was added, and after equilibrating at 37°C both reactions were started by the addition of 200 μl 10 mM 5′ AMP. After 100 minutes, 2 ml 10% trichloroacetic acid was added, and the tube vigorously rotated on a Vortex Jr mixer (Scientific Industries International Inc Ltd). The mixture was allowed to stand for 10 minutes, centrifuged at 3,000 rpm for 10 minutes, and the phosphorus content of 2 ml supernatant determined (Delsal and Manhouri, 1958). Activity was expressed in terms of the difference in phosphorus content of the two tubes as mμM phosphorus liberated/min/ml (mIU/ml).

APASE ESTIMATION APase was determined routinely by the AutoAnalyzer (Technicon Instruments Co. Ltd), the normal adult range in our laboratory being 3 to 15 Kind-King Units/100 ml. In preliminary experiments, APase was measured by the liberation of inorganic phosphate from disodium phenyl phosphate in a final concentration of 1 mM under conditions identical with those described for assay of 5Nase.

ISOENZYME ELECTROPHORESIS Separation of APase isoenzymes on Agar gel was attempted on 20 sera at *p*H 8·6 in 0·05 M veronal buffer (Haije and de Jong, 1963) at 200 v for one hour. The silver stain of Stevenson (1961), and the azo-dye coupling technique of Barka (1961) were applied and the Rf values of the bands calculated with respect to the midpoint of the albumin band as indicated by bromophenol blue staining (Dymling, 1966).

RESULTS

DEVELOPMENT OF METHOD The effect of Mg^{++} ions upon hydrolysis of 5′ AMP and phenyl phosphate was studied in relation to *p*H using pooled sera of high APase activity. One pool was composed of sera from patients with liver disease and another from patients with bone disease. In the latter (Fig. 1) 10 mM Mg^{++} ions increased the hydrolysis of phenyl phosphate, but not of 5′ AMP, over the *p*H range 6·8 to 9·5. In the former (Fig. 2), Mg^{++} ions had no consistent effect upon hydrolysis of phenyl phosphate, but dramatically increased hydrolysis of 5′ AMP. This effect was strictly *p*H-dependent, being maximal at *p*H 7·9. The profile of 5′ AMP hydrolysis in the absence of Mg^{++} ions was remarkably similar to that of disodium phenyl phosphate hydrolysis, indicating both to be the function of a single enzyme, APase. The profile of 5′ AMP hydrolysis in the presence of Mg^{++} ions was quite different, and is due

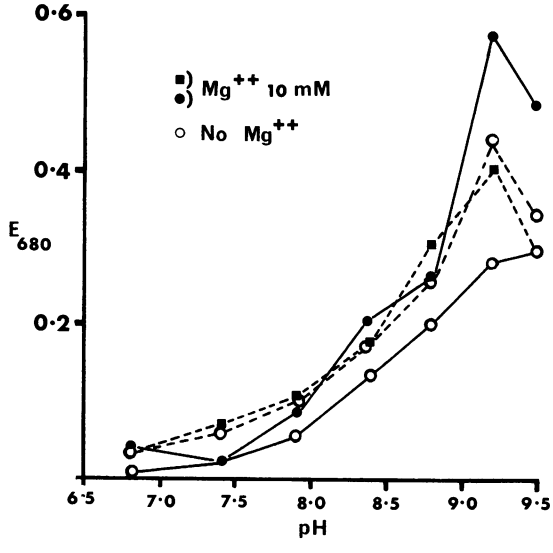

FIG. 1. *Effect of Mg^{++} ions on hydrolysis of disodium phenyl phosphate (solid line) and 5′ AMP (broken line) 1 mM in Michaelis veronal buffer by pooled sera from patients with bone disease, APase 135 Kind-King units/100 ml.*

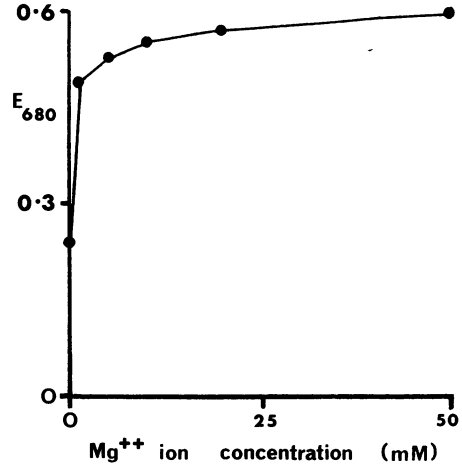

FIG. 3. *Effect of Mg^{++} ion concentration on hydrolysis of 5′ AMP (1 mM) at pH 7·9 by pooled serum (hepatobiliary disease).*

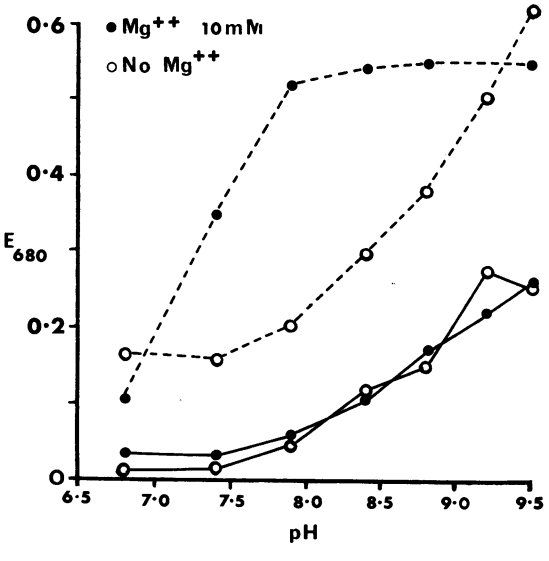

FIG. 2. *Effect of Mg^{++} ions on hydrolysis of disodium phenyl phosphate (solid line) and 5′ AMP (broken line) 1 mM in Michaelis veronal buffer by pooled sera from patients with hepatobiliary disease, APase 70 Kind-King units/100 ml.*

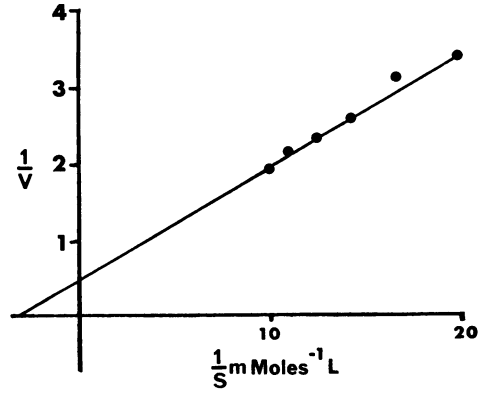

FIG. 4. *Lineweaver-Burk plot showing effect of substrate concentration on rate of hydrolysis of 5′ AMP by pooled human serum (hepatobiliary disease) at pH 7·9 in the presence of 10 mM Mg^{++} ions.*

to summation of the action of APase which is not activated by Mg^{++} ions, with that of another enzyme, 5Nase, which is. The difference between the hydrolysis of 5′ AMP in the presence and absence of Mg^{++} ions is thus a measure of 5Nase activity, and this difference is maximal at *p*H 7·9 which is the optimal *p*H of 5Nase under our conditions.

The extent of activation of 5′ AMP hydrolysis with varying concentrations of Mg^{++} ions was studied (Fig. 3). A rapid increase took place up to a final concentration of 10 mM. Raising the concentration beyond this point gave small increments in activity, but the reaction no longer followed zero-order kinetics. At 10 mM, the Mg^{++} ion concentration is 200-fold that provided by normal serum when no other source of Mg^{++} ions is added. The relationship of activity to 5′ AMP concentration was studied, and the results are presented in Fig. 4 in the form of the reciprocal plot of Lineweaver and Burk (1934). The Michaelis constant was 0·33 mM. A final substrate concentration of 1 mM is sufficient to give a velocity 75% of the theoretical maximum. That the Mg^{++}-activated hydrolysis of 5′ AMP at *p*H 7·9 was a measure of the nucleotidase content of serum was verified by adding increasing amounts of serum to a buffer-substrate mixture while maintaining the volume with 0·15 M NaCl. The difference between the hydrolysis of 5′ AMP in the presence and absence of 10 mM Mg^{++} ions was linearly related to the amount of enzyme present (Fig. 5). The serum

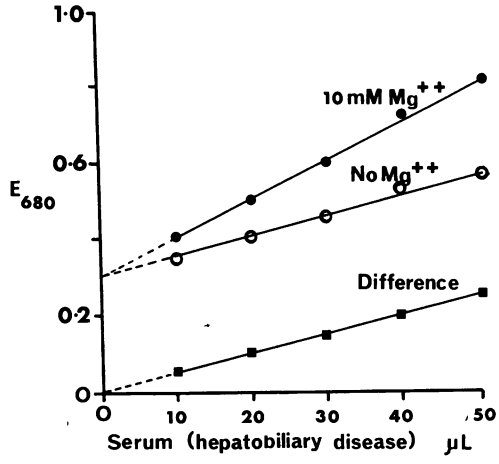

FIG. 5. *Relationship between amount of serum and hydrolysis of 5′ AMP (1 mM) at* p*H 7·9 with and without the addition of Mg^{++} ions.*

tested had an activity of 80 mIU/ml which is four-fold the upper limit found in our normal population. The values for the points in assays with and without Mg^{++} ions lay on straight lines which met where they intersected the ordinate. The intercept corresponded to the reagent blank which was not deducted from individual assays.

The reproducibility of the method was assessed on 88 sera assayed in duplicate. The mean difference

TABLE I

SUMMARY OF APase AND 5Nase RESULTS ON 216 PATIENTS

	No. of Cases	*APase*		*5Nase*			
		Raised	*Mean*	*Raised*	*Mean*	*r*	*Slope*
Extrahepatic Obstruction	55	55	66	40	52	0·344[3]	0·468
Simple (clinical)	16	16	58	9	41	0·388	0·514
Simple (operation)	7	7	70	6	75	0·243	0·642
Inflammatory	10	10	68	9	52	0·263	0·196
Malignant	22	22	71	17	53	0·376	0·496
Liver Disease	64	55	42	31	30	0·724[4]	0·518
Hepatitis	22	17	26	8	20	0·612[2]	0·608
Portal cirrhosis (clinical)	6	5	61	2	39	0·998[4]	0·619
Portal cirrhosis (biopsy)	19	16	34	9	23	0·553[1]	0·555
Primary biliary cirrhosis	11	11	72	9	53	−0·037	−0·028
Miscellaneous	6	6	56	4	34	0·818[1]	0·409
Bone Disease	49	47	82	10	13	0·051	0·013
Paget's disease	22	21	88	6	11	0·458[1]	0·052
Metastatic	14	14	105	3	13	−0·087	−0·009
Miscellaneous	13	12	43	1	17	0·150	0·478
Hepatic Metastases	31	31	93	27	50	0·191	0·112
Reticulosis	17	17	59	10	31	0·176	0·170
Non-hepatic	6	6	38	3	34	0·922[2]	1·671
Hepatic (anicteric)	5	5	79	1	16	0·267	0·092
Jaundiced	6	6	67	6	42	0·440	0·687

[1]$P < 0·05$ [2]$P < 0·02$ [3]$P < 0·01$ [4]$P < 0·001$

was ±10·2%, being greater with sera of low activity and smaller with sera of high activity.

NORMAL RANGE An adult range was determined using laboratory staff and patients free of liver and bone disease and other serious organic illness. Five males and five females were taken from each decade between 20 and 81 years, 60 in all. A range for children was also determined, five males and five females being taken from each of the following age groups making 50 in all: 0 to 4 weeks, 5 to 11 weeks, 3 months to 9 years, 10 to 15 years, and 15 to 20 years. No differences attributable to age or sex were apparent. Consequently the entire population was taken for calculation of the distribution, giving a mean of 12 mIU/ml and SD 4 mIU/ml corresponding with the observed range 0 to 20 mIU/ml.

ABNORMAL SUBJECTS The data are summarized in Table I which also gives the results of linear regression functions relating activities of APase and 5Nase in the various clinical groups and subgroups. The correlation coefficient (r) measures the closeness of the points to the regression line, while the slope (5Nase on APase) estimates the increase in 5Nase with increasing levels of APase.

Extrahepatic obstruction Whereas raised APase was found in all cases, 15 had normal 5Nase levels (Fig. 6). The mean activities of both enzymes in simple extrahepatic obstruction diagnosed clinically were lower than in other subgroups, and 40% of the subjects had normal 5Nase levels. Twenty per cen of the patients with malignant extrahepatic obstruction had normal 5Nase levels. One patient with cholecystitis, and one diagnosed surgically as simple extrahepatic obstruction had normal 5Nase activity. Only six patients in the entire group had APase activity less than 35 units/100 ml, and thus failed to meet the criterion of Maclagan (1947) for the diagnosis of obstructive jaundice. The overall correlation between APase and nucleotidase for the group gave a value of $r = 0{\cdot}344$ ($P < 0{\cdot}02$). Within the subgroups, simple extrahepatic obstruction (clinical) gave the best correlation ($r = 0{\cdot}388$) and simple extrahepatic obstruction (surgical) the poorest ($r = 0{\cdot}243$).

Liver disease Of the 64 subjects in this group, 55 had raised APase activity, and 31 had raised 5Nase (Fig. 7). Only 36 had APase values less than 35 units/100 ml, and thus met the criterion of Maclagan (1947) for the diagnosis of parenchymal liver disease. The mean for the entire group was substantially above this value. The correlation between the activities of both enzymes was good in the group as a whole, and in all the subgroups with the exception of primary biliary cirrhosis where, despite high mean values for both enzymes, the correlation between the two in individuals was poor.

Bone disease One patient with Paget's disease and one with degenerative bone disease had normal APase activity (Fig. 8). In the remaining subjects APase was raised. Of 22 patients with Paget's

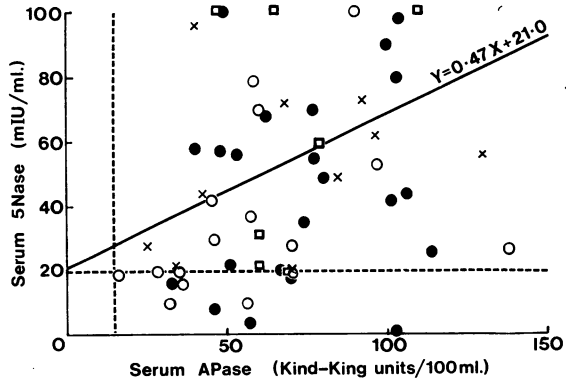

FIG. 6.

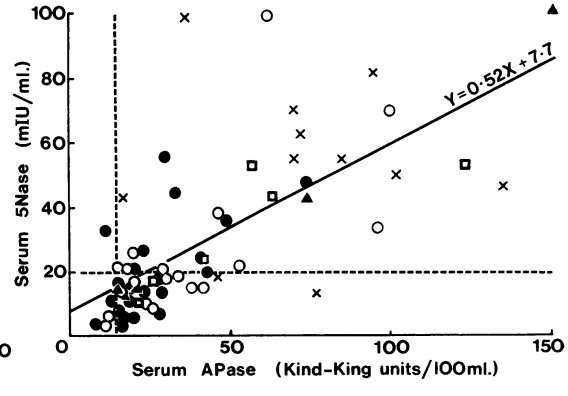

FIG. 7.

FIG. 6. *Correlation of serum 5Nase and APase activities in patients with extrahepatic obstruction. Malignant obstruction (solid circles); simple obstruction diagnosed clinically (open circles) and at operation (open squares); inflammatory obstruction (crosses). The dotted lines in this Figure, and in Figs. 7, 8, and 9 indicate the upper normal limits.*

FIG. 7. *Correlation of serum 5Nase and APase activities in patients with parenchymal liver disease. Viral hepatitis (solid circles); portal cirrhosis diagnosed histologically (open circles) and clinically (solid triangles); primary biliary cirrhosis (crosses); miscellaneous (open squares).*

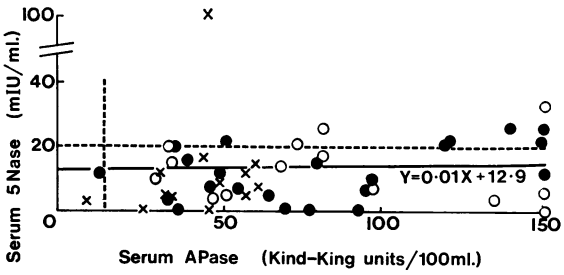

FIG. 8. *Correlation of serum 5Nase and APase activities in patients with bone disease: Paget's disease of bone (solid circles); bone metastases (open circles); miscellaneous (crosses).*

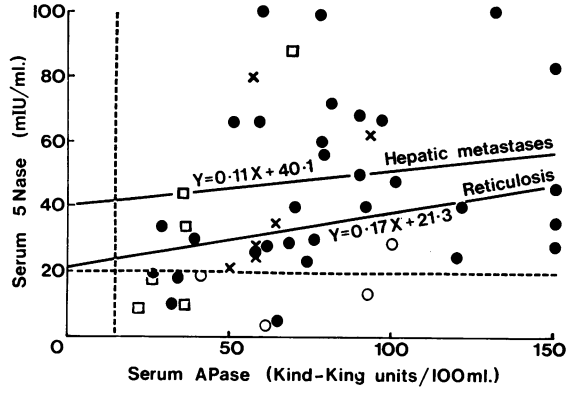

FIG. 9. *Correlation of serum 5Nase and APase activities in patients with hepatic metastases (solid circles) and in patients with reticulosis classified as anicteric hepatic (open circles), jaundiced (crosses), and non-hepatic (open squares).*

disease, six had slightly raised 5Nase (up to 26 mIU/ml). Five of these had APase activities well above 100 units/100 ml, and in the sixth the value was 52 units/100 ml. Three of 14 patients with metastatic bone disease gave 5Nase activities outside the normal range, the highest value (38 mIU/ml) being in a subject whose APase was 182 units/100 ml. One patient in the miscellaneous subgroup, whose APase was 46 units/100 ml had 5Nase activity of 130 mIU/ml. Radiologically-diagnosed degenerative bone disease was the only abnormality found in this subject. All other patients in this subgroup had normal 5Nase levels.

The correlation between the activities of the two enzymes in individual patients was poor in the group as a whole, and in metastatic and miscellaneous bone disease. In Paget's disease, however, the correlation was moderately good ($r = 0{\cdot}458$; $P < 0{\cdot}05$).

Hepatic metastases All subjects had raised APase activity, and in only four did this fall below 35 units/100 ml (Fig. 9). Four subjects had normal 5Nase activity. The correlation between the two enzyme levels in individual subjects was not good ($r = 0{\cdot}191$).

Reticulosis All 17 subjects had raised APase activity (Fig. 9). High 5Nase levels were found in three patients without hepatic involvement, in only one of the five non-jaundiced subjects with hepatic involvement, and in all the jaundiced patients. The correlation between the two enzymes was not good for the group as a whole, but a surprisingly high correlation was given by the patients without hepatic involvement ($r = 0{\cdot}922$; $P < 0{\cdot}01$), all six of whom were cases of Hodgkin's disease.

Isoenzyme electrophoresis Where present, the slow intestinal band ran with R_f values 0·24 to 0·28. Only one fast band was seen. In nine patients with bone disease this fast band gave R_f values 0·49 to 0·58. In 11 patients with hepatobiliary disease the R_f values were 0·44 to 0·72, and in only four was the value greater than 0·58. We conclude that APase specific to bone disease cannot be identified with certainly by the techniques used, and the unequivocal identification of APase specific to liver disease can be made in less than half the subjects with this condition.

DISCUSSION

The fact that Mg^{++} ions activate 5Nase of human serum without substantially affecting its APase activity has previously been noted (Young, 1958; Kowlessar *et al*, 1961). While this work was in progress, Hardonk and Koudstaal (1968) reported that 5Nase could be distinguished histochemically from non-specific phosphatases since only the former is stimulated by Mg^{++} ions; the concentration of Mg^{++} in their medium was 10 mM, as in our procedure. This concentration was found to be optimal for serum 5Nase by Schwartz and Bodansky (1964). Their data, in common with our own (Fig. 2), indicate a 2·5-fold activation by 10 mM Mg^{++} ions at *p*H 7·5 whereas we find activation at *p*H 7·9 to be almost three-fold. The percentage increase in activity is of course dependent upon the relative proportions of APase and 5Nase in the serum. *p*H 7·9 is optimal for 5Nase under our conditions, and accords with estimates of 7·8 by Reis (1951) for 5Nase of human tissues. At this *p*H, the effect of Mg^{++} ions upon hydrolysis of disodium phenyl phosphate is not impressive, activity being unchanged or slightly increased. The increased hydrolysis of 5′ AMP in the presence of 10 mM Mg^{++} ions is therefore due to 5Nase and is linearly related to the enzyme concentration (Fig. 5). The requirements of a specific quantitative assay for

5Nase are satisfied, and only 100 μl of serum is needed.

The reproducibility of the method ($\pm 10{\cdot}2\%$) is not entirely satisfactory, though in our hands the methods of Young (1958) and Campbell (1962) are less satisfactory. This information is not given by the authors of any previous manual method, though Hill and Sammons (1966) claim better results for their automated assay. We are examining the possibility of a continuous kinetic method, and have had encouraging preliminary results (Belfield and Goldberg, 1968).

The coexistence of high APase activity in the same sample is unlikely to augment the activity attributed to 5Nase. At *p*H 9·5, where hydrolysis of 5′ AMP is predominantly due to APase, Mg^{++} ions are inhibitory (Figs. 1 and 2). If this effect also occurs at *p*H 7·9 a somewhat lower estimate of 5Nase activation may result when APase activity is very high, though it is improbable that this error would be serious. Our normal range (0 to 20 mIU/l) is higher than that given by other methods. This may reflect the sensitivity of the technique and the fact that maximum activation is obtained.

Alternative methods suffer various disadvantages. Those involving the use of one substrate to measure the sum of APase and 5Nase and another to measure APase alone (Dixon and Purdom, 1954; Wachstein and Sigismondi, 1958) ignore the different affinities of APase for the two substrates. Young (1958) employed ethylene diamino tetraacetate to inhibit APase, but the requisite degree of inhibition of APase requires a concentration which partially inhibits 5Nase, and the extent of neither inhibition can be predicted (Walker and Bayliss, 1966). Our own experience confirms these observations. Bardawill and Chang (1963) measured the hydrolysis of 5′ AMP at *p*H 7·3, making no allowance for non-specific phosphatase activity. The method of Campbell (1962) relies on measurement of 5′ AMP hydrolysis in the presence and absence of Ni^{++} ions, the difference being ascribed to 5Nase which is inhibited by Ni^{++} ions. Other workers have reported results by this method (Hill and Sammons, 1965; Schwartz and Bodansky, 1965; Davidge and Philpot, 1966) but difficulties arising from formation of insoluble metallo-phosphate-protein complexes frequently arise, as we have experienced, and led to modifications in the *p*H at which the reaction is carried out (Hill and Sammons, 1966; 1967). A more serious objection is that the inhibition of 5Nase by Ni^{++} ions is not complete (Ahmed and Reis, 1958), while, as pointed out by Schwartz and Bodansky (1964), APase of bone is inhibited by up to 90% under the conditions described by Campbell (1962).

The activation of 5Nase by L-histidine has also been advocated as a measure of its activity (Schwartz and Bodansky, 1964) but the differences between activated and non-activated assays are small compared with those of the present procedure.

The substitution of 5Nase for APase as a liver function test, as implied by Hill and Sammons (1967), does not receive support from our data, and we are in agreement with Davidge and Philpot (1966). The level was not raised as frequently as that of APase in hepatobiliary disease, although the element of selection in the case material would limit our likelihood of finding high levels of 5Nase in association with normal APase, and there was no such example in this series. The distinction between extrahepatic obstruction and parenchymatous liver disease is not assisted, since 40 of 55 patients with extrahepatic obstruction had raised 5Nase, as did 31 of 64 cases with parenchymatous disease. These figures do not support the conclusions of Young (1958) whose paper does not give clinical details of his case material. This is important in explaining discrepancies between the results of different series. Hill and Sammons (1967) give details for fewer than half their patients, and the criteria for diagnosis are not always indicated, leading to uncertainty regarding, for example, cases classified as cholecystitis and cholelithiasis with normal liver function tests. The fact that only two of their 10 patients with Paget's disease had APase values > 50 units/100 ml, whereas 18 of our 22 cases were in this category, emphasizes the wide differences in our clinical material.

On the other hand the estimation is valuable when due weight is given to the level concerned and the clinical problem is clearly defined. In distinguishing hepatitis or portal cirrhosis from jaundice due to extrahepatic obstruction, a 5Nase level of 40 mIU/ml, twice our upper normal limit, may be helpful, since seven out of 47 cases in the first category, as opposed to 28 out of 55 in the second, exceeded this level; so did 17 of 31 cases with hepatic metastases. The incidence of raised levels in hepatobiliary disease (66%), as opposed to bone disease (20%), may not seem promising as a means of separating the two diseases, but a level 50% above our normal limit (30 mIU/ml) is useful, since only two of 49 patients with bone disease exceeded this value which was surpassed in 76 of 149 cases of hepatobiliary disease. This has given better discrimination than isoenzyme electrophoresis; our disappointment with this technique is shared by Dymling (1966) and by Fishman and Ghosh (1967).

The correlation of APase with 5Nase activity in relation to the disease process has produced unexpected results, and we have for purposes of comparison applied this technique to such data as are available in the literature. In Paget's disease, the data

of Schwartz and Bodansky (1965) and of Hill and Sammons (1967) give values for $r = 0{\cdot}626$ ($P < 0{\cdot}05$) and 0·690 ($P < 0{\cdot}05$) respectively, while in other forms of bone disease the data of the latter authors give $r = -0{\cdot}393$ thus corroborating our findings. No significant correlation in cancer or biliary obstruction emerges from the data of Bardawill and Chang (1963), but in their hepatitis cases $r = 0{\cdot}490$ ($P < 0{\cdot}05$); in contrast with our findings, their data for portal cirrhosis give a low value for $r = 0{\cdot}098$. No significant correlation in obstructive jaundice exists in the data of Dixon and Purdom (1954), while, in this condition, a value of $r = 0{\cdot}662$ ($P < 0{\cdot}02$) was calculated from Hill and Sammons (1967); their data for viral hepatitis gives $r = 0{\cdot}863$ ($P < 0{\cdot}001$). In metastatic liver disease, the data of Schwartz and Bodansky (1964; 1965) gives $r = 0{\cdot}720$ ($P < 0{\cdot}001$) by the histidine-activation method of 5Nase assay, and $r = 0{\cdot}414$ ($P < 0{\cdot}02$) by the nickel-inhibition method, illustrating the divergence of results by the same authors employing different methods. Our findings of better correlation between APase and 5Nase in parenchymal liver disease, especially viral hepatitis, than in extrahepatic obstruction or metastatic liver disease thus receives partial support. We have no explanation at present for the high correlation found in Hodgkin's disease without hepatic involvement and propose to study this matter further.

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