put. Several different glass filter and interference filter photometric devices have been successfully utilized with this burner-atomizer system. These include a simple barrier layer photocell-suspension type galvanometer system, vacuum phototube-electronic voltmeter, and a direct current operated photomultiplier tube-suspension galvanometer system.

Comparison of the results of sodium and potassium determinations in urine and plasma by standard chemical methods,[3,4] with the method of flame photometry described herein, are shown in Table I. We do not believe that this comparison reflects the true accuracy of this method of flame photometry, but represents at the present time the only acceptable means of evaluation.

The reproducibility, duplicability and recovery of added sodium and potassium to urine and plasma are of the order ±0.5%. Further work is in progress for the determination of calcium, magnesium and other cations. Further data, as well as information as to the commercial availability of this instrument, will be available in a forthcoming publication.

Summary. A new type of atomizer-burner system is described for use in flame photometry which accomplishes consistently accurate quantitative chemical analysis of sodium, potassium, and other cations.

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[3] Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

[4] Folch, J., and Lauren, M., *J. Biol. Chem.*, 1947, **169**, 539.

17260. Inhibition by Cyanide of Serum Alkaline Phosphatase in Normal Man, Obstructive Jaundice and Skeletal Disorders.

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It has been the general experience that marked increases in serum alkaline phosphatase activity occur consistently in only 2 disease categories in man: 1. in skeletal disorders associated with active, widespread proliferation of bone or cartilage, 2. in diseases of the liver or biliary tract with obstruction of the intra- or extrahepatic biliary channels.[1] There is general agreement that the high serum alkaline phosphatase levels observed in skeletal disorders are the result of augmented production of the enzyme by osteoblasts, which are known to be a prolific source. Opinion is divided as to the origin of the increased serum alkaline phosphatase in biliary tract obstruction. It would seem reasonable to suppose that since alkaline phosphatase is excreted in the bile and (being a large protein molecule) does not escape through intact glomeruli in man, retention of the enzyme in the plasma occurs when the excretory biliary channels are obstructed. There is, however, the possibility that the increased enzyme appearing in the plasma is of hepatic origin, and perhaps a different alkaline phosphatase from that responsible for most of the enzyme activity exhibited by the plasma of normal subjects and of those with skeletal diseases.

Drill and coworkers [2,3] reported that sodium cyanide in concentrations to 0.1M only slightly inhibited the serum alkaline phosphatase activity of normal dogs and man whereas the elevated levels in hepatic damage were markedly reduced, to approximately normal levels but not below. Their data were interpreted as indicating that the alkaline phosphatase appearing in the serum of dogs and man with hepatic damage is, for the most part,

[1] Gutman, A. B., Olson, K. B., Gutman, E. B., and Flood, C. A., *J. Clin. Invest.*, 1940, **19**, 129.

[2] Drill, V. A., Annegers, J. H., and Ivy, A. C., *J. Biol. Chem.*, 1944, **152**, 339.

[3] Drill, V. A., and Riggs, D. S., *J. Biol. Chem.*, 1946, **162**, 21.

TABLE I.
Effect of NaCN on Serum Alkaline Phosphatase Activity.

	Mols NaCN added to substrate					% initial activity remaining
	0	0.001	0.005	0.01	0.05	
	(Alkaline phosphatase, Bodansky units/100 cc serum)					
Normal human subjects						
1. Adult	3.0	2.1	0.8	0.5	—	16.7
2 "	2.7	1.6	0.6	0.3	0.4	11.1
3. "	2.1	1.8	0.8	0.6	0.5	23.8
4. "	2.0	1.5	0.5	0.3	0.4	20.0
5. Infants (pooled)	7.9	5.4	1.1	0.6	0.7	7.6
6. " "	6.0	—	1.0	0.9	1.0	16.7
Obstructive jaundice						
7. Biliary cirrhosis	46.7	28.8	11.9	3.2	—	6.9
8. Choledocholithiasis	27.2	—	7.4	2.0	1.4	5.1
9. Unclassified	22.5	—	4.7	1.4	1.3	5.8
10. ? Ca gall bladder	17.3	10.8	3.9	1.3	—	7.5
11. Cholangiolitic cirrhosis, marked parenchymal damage	16.2	—	2.7	1.2	0.9	5.6
12. Ca head of pancreas, cirrhosis	13.2	—	3.8	1.1	1.0	7.6
Skeletal disorders						
13. Paget's disease	75.9	—	11.3	3.3	2.4	3.2
14. Metastatic prostatic carcinoma	38.6	24.6	9.0	2.0	—	5.2
15. " " "	27.3	18.8	6.3	1.7	0.9	3.3
16. " " "	27.0	17.2	5.5	1.5	—	5.5
17. " " "	25.7	—	5.7	1.8	0.9	3.5
18. " " "	20.3	—	5.4	1.9	0.8	3.9
19. " " "	19.9	—	3.9	1.8	1.4	7.0
20. Paget's disease	9.1	—	2.4	0.6	0.6	6.6
21. Metastatic prostatic carcinoma	7.2	—	2.1	1.0	1.1	13.9

different from that normally present. We have repeated and extended these studies to include the effects of sodium cyanide on increased alkaline phosphatase levels in diseases of bone.

Methods. Serum alkaline phosphatase was determined by the Bodansky method.[4] A stock solution of 0.1M NaCN in buffered β-glycerophosphate solution was prepared and the pH of this solution adjusted to 9.2. Appropriate amounts of this stock solution were added to β-glycerophosphate substrate to make the desired cyanide concentration, the pH again checked, and the cyanide-substrate mixture then added to the serum just prior to incubation. Preliminary tests indicated that these concentrations of cyanide had no significant effect on the development of color by the inorganic phosphate standard.

Results. Four samples of normal adult human sera and 2 of pooled infants sera were examined and in each instance marked inhibition by cyanide was observed (Table I) The degree of inhibition increased with increasing concentrations of cyanide, at least up to 0.01M, although there was always some residual enzyme activity at the end of 1 hour incubation, varying from 0.4-1.0 Bodansky units % or 7.6-23.8% of the initial activity. The data indicate that most of the alkaline phosphatase present in normal human serum is inhibited by cyanide. These results are not in complete disagreement with those of Drill and Riggs[3] since examination of their data reveals inhibition greater than 50% in 2 of their 6 normal sera, and in several normal sera preincubation with NaCN disclosed inhibition of the order we obtained.

The data in 6 cases of obstructive jaundice with elevated serum alkaline phosphatse (Table I) confirm the previous finding[3] of marked inhibition by cyanide, to 5.1-7.6% of the initial activity in our series. In most instances we found the residual enzyme activity somewhat below the normal minimum of 1.5 Bodansky units %; it was higher only in

[4] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

1 serum with very marked initial activity.

In 9 patients with elevated serum alkaline phosphatase due to increased bone formation (in which the excess enzyme appearing in the serum is doubtless of osseous origin) the inhibitory effects of cyanide were indistinguishable from those observed in obstructive jaundice (Table I). The residual activity, 0.6-2.4 Bodansky units % was consistently less than the normal minimum except in the 2 cases with very high initial levels. The % activity persisting, 3.2-13.9% of the original values, was of the same order as observed in obstructive jaundice. A somewhat higher percentage of the original activity, approximately that observed in normal sera after inhibition by cyanide, persisted when initial levels were only moderately elevated.

Our data disclose no essential differences in the inhibiting effect of cyanide in all 3 categories: most of the serum alkaline phosphatase activity present normally, in obstructive jaundice and in skeletal disorders alike was inhibited by cyanide. Moreover, since 100% inhibition of an enzyme is infrequently achieved, the residual enzyme activity, low as it was in most sera, probably represents maximal values for cyanide-insensitive alkaline phosphatases present in serum. That we apparently did not obtain complete inhibition of cyanide-sensitive phosphatases is indicated by the persistence of greater residual activity in sera with higher initial levels.

Discussion. The data obtained appear to throw light upon 2 obscure points, 1. the origin of the alkaline phosphatases in the plasma of normal human adults*, 2. the origin and causal mechanism of the increased serum alkaline phosphatase in obstruction of the extra- or intrahepatic biliary tract.

Cloetens[5] distinguished 2 classes of alkaline phosphatase: Phosphatase I, inactive in the absence of Mg^{++}, markedly activated by appropriate concentrations of Mg^{++}, not inhibited by cyanide when so activated; phosphatase II, active without addition of Mg^{++} and only little affected by addition of Mg^{++} but markedly inhibited by cyanide. Cloetens[5] found that phosphatase II, cyanide-sensitive, accounted for over 90% of the total alkaline phosphatase activity of bone (which we have confirmed in unpublished experiments; see also O. Bodansky[6]), 96-99% of that of the serum of 3 rachitic dogs, and 97% of that of the serum of 1 dog with liver disease. Liver tissue phosphatase, in contrast, was usually more than 60% phosphatase I, cyanide-resistant.[5†] In our experiments,[‡] inhibition by cyanide of 76.2-92.4% of the alkaline phosphatase activity of normal human sera indicates that most of this activity is due to a phosphatase II, like bone phosphatase but unlike most liver phosphatases. Belfanti *et al.*,[7] using oxalate inhibition as a criterion, also found that the alkaline phosphatase of normal serum resembled bone phosphatase but differed from liver phosphatase. The largest proportion of the alkaline phosphatases present in normal human serum would therefore appear from this chemical evidence to be indistinguishable by available methods from bone phosphatase; a small proportion, cyanide-resistant, is probably not of osseous but of other (undetermined) origin. This interpretation is consistent with observations in hepatectomized and eviscerated dogs[8,9] indicating that serum alkaline phosphatase levels

* It is generally accepted that the increased serum alkaline phosphatase of growing children, like that of patients with bone or cartilage proliferation due to skeletal disorders, is due to increased osteoblastic activity.

[5] Cloetens, R., *Enzymologia*, 1939, **6**, 46.

[6] Bodansky, O., *J. Biol. Chem.*, 1949, **179**, 81.

† Presumably, much of the cyanide-sensitive phosphatase II found in liver is serum phosphatase in the blood and bile present in liver tissue preparations.

‡ We did not examine the effects of addition of magnesium ions (see Drill and Riggs[3]) because the presence of magnesium in serum makes interpretation difficult. Moreover, Cloetens worked largely with dialysed tissue extracts and the differences he noted in the effects of addition of Mg^{++} probably reflect differences in the ease with which Mg ion is removed by dialysis from the various alkaline phosphatases of different organs. This factor does not enter into our experiments.

[7] Belfanti, S., Contardi, A., and Ercoli, A., *Biochem. J.*, 1935, **29**, 1491.

[8] Armstrong, A. R., and Banting, F. G., *Can. Med. Assn. J.*, 1935, **33**, 243.

are maintained under these conditions and that the enzyme hence cannot be of hepatic origin.

Our data indicate that the increased serum alkaline phosphatase in obstructive jaundice, as in skeletal diseases, is phosphatase II (cyanide-sensitive) and therefore presumably not of hepatic cell origin. The results are compatible with the view that elevation of serum alkaline phosphatase in extra- or intrahepatic biliary tract obstruction is due to retention of serum alkaline phosphatase, largely of osseous origin. There is a great deal of clinical evidence for this view which was summarized elsewhere.[1]

The marked difference in the effects of cyanide on serum alkaline phosphatase and on liver tissue alkaline phosphatase indicates definite differences between these enzymes. However, the similarity between serum alkaline phosphatase and bone phosphatase with respect to cyanide does not prove their identity; all that can be said is that in this and other respects no significant difference is apparent. Absolute proof of identity of 2 enzymes from different sources is not possible at this time.

It may seem surprising that with so many alkaline phosphatases present in so many organs, the enzyme in the plasma should be so preponderantly of osseous origin. However, not only is the number of osteoblasts in the body very large and their alkaline phosphatase production great, but the secretion of the enzyme is extracellular for production of bone at the cell surface. In most cells, phosphatases operate intracellularly, often apparently within the confines of the nucleus to judge from histochemical evidence, and probably never reach the extracellular fluids.

Summary. Cyanide markedly inhibits the serum alkaline phosphatase of normal human subjects and the increased levels of patients with obstructive jaundice and skeletal diseases; no essenital differences were observed in these 3 categories. The evidence is consistent with the view that in all 3 categories the largest proportion of serum alkaline phosphatase is of osseous origin since bone phosphatase is inhibited by cyanide (Cloetens' phosphatase II) and liver phosphatases are, for the most part, cyanide-insensitive (Cloetens' phosphatase I). The data indicate that the rise in serum alkaline phosphatase in obstructive jaundice cannot be of hepatic origin but they are compatible with retention of serum alkaline phosphatase due to obstruction of the excretory biliary channels.

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[9] Maddock, S., Schmidt, G., and Thannhauser, S. J., *Fed. Proc.*, 1942, **1**, 181.

17261. Intracellular Distribution of Vitamin B_6 in Rat and Mouse Livers and Induced Rat Liver Tumors.*

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In studies[1-4] from this laboratory the intracellular distribution of riboflavin was determined in normal rat livers, in the livers of rats fed various aminoazo dyes, and in liver tumors induced by 4-dimethylaminoazobenzene. Riboflavin is of particular interest since

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† Predoctorate Research Fellow, National Cancer Institute.

[1] Price, J. M., Miller, E. C., and Miller, J. A., *J. Biol. Chem.*, 1948, **173**, 345.

[2] Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., *Cancer Research*, 1949, **9**, in press.

[3] Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., manuscript in preparation.

[4] Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., *Cancer Research*, 1949, **9**, 96.