EFFECT OF CYANIDE, FLUORIDE, AND MAGNESIUM ON SERUM PHOSPHATASE ACTIVITY DURING HEPATIC DAMAGE

BY VICTOR A. DRILL,* J. H. ANNEGERS, AND A. C. IVY

(From the Department of Physiology, Northwestern University Medical School, Chicago)

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Alkaline phosphatase, normally present in the serum, is known to be raised above normal in various types of liver damage (1-5). Although the effects of various inhibitors, such as cyanide and fluoride, have been studied on phosphatases extracted from tissue ((6-8) and others), no observations have been made on the effect of such ions on serum phosphatase activity during hepatic damage. In this paper the effect of cyanide, fluoride, and magnesium ions, alone and in various combinations, was studied to ascertain their effect on the serum phosphatase activity of normal dogs and dogs with hepatic damage. It was found that the increase in serum phosphatase obtained during liver damage was due to a phosphatase that was normally present in the blood in only small amounts.

Methods

Inorganic serum phosphate and alkaline serum phosphatase were determined according to the method of Bodansky, β-glycerophosphate being used as substrate (9). Substrate was also prepared with the following concentration of salts: MgSO₄ 0.01 M, NaF 0.01 M, NaCN 0.0001 to 0.1 M. Hepatic damage with a consequent rise in serum phosphatase activity was produced by the following procedures: administration of CCl₄ (10, 11), cholecystectomy (12), production of a bile fistula (13), and in bile fistula dogs in which the biliary tree had become obstructed. The dogs were fed a stock diet consisting of bread, meat scraps, bones, cod liver oil, and yeast. On this diet the serum phosphatase values of normal dogs varied between 4.0 and 6.5 units per 100 cc. of serum.

Results

NaCN, in concentrations from 0.0001 to 0.1 M, had only a slight effect on the activity of serum phosphatase from normal dogs (Tables I and II). In dogs with hepatic damage produced by various procedures the serum phosphatase was elevated above normal, the rise being most marked in cases of biliary obstruction. In these dogs the addition of NaCN pro-
duced an inhibition of phosphatase activity, and as the molarity of the NaCN was increased the loss of activity became progressively greater (Table I). However, as the concentration of NaCN was increased, the serum phosphatase activity decreased to normal values, but did not go below normal. This can be seen with NaCN concentrations of 0.01, 0.05, and 0.1 M, when the serum phosphatase activity has decreased to fairly constant values. This effect of NaCN on elevated serum phosphatase was obtained

<table>
<thead>
<tr>
<th>Phosphatase units per 100 cc. serum</th>
<th>0.005 M</th>
<th>0.01 M</th>
<th>0.05 M</th>
<th>0.075 M</th>
<th>0.1 M</th>
<th>0.15 M</th>
<th>0.175 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dogs</td>
<td>4.72</td>
<td>5.67</td>
<td>6.32</td>
<td>5.73</td>
<td>5.30</td>
<td>5.20</td>
<td>4.90</td>
</tr>
<tr>
<td>Dogs with hepatic damage</td>
<td>3.20</td>
<td>4.08</td>
<td>4.72</td>
<td>5.46</td>
<td>4.56</td>
<td>4.72</td>
<td>4.59</td>
</tr>
</tbody>
</table>

**Table I**

Effect of Various Concentrations of NaCN on Serum Phosphatase Activity of Normal Dogs and Dogs with Hepatic Damage

in the three types of liver damage studied, and suggests that the increased serum phosphatase during hepatic damage is due to an alkaline phosphatase that is normally present in the blood in only small amounts.

Magnesium sulfate (0.01 M) had only a slight activating effect on serum phosphatase in normal dogs, the average increase being 0.91 unit per 100 cc. of serum (Table II). The addition of NaF also produced a slight increase in phosphatase activity (Table II).
NaCN (0.01 M) inhibited serum phosphatase activity in normal dogs by an average of 1.92 units (29.9 per cent). The NaCN was also effective in the presence of Mg, inhibiting the slight stimulation produced by Mg, and lowering the value to that produced by NaCN alone (Table II). In a few trials with 0.01 M NaF and 0.01 M NaCN, the NaCN again abolished any stimulating effect of the NaF, lowering the value to that obtained with NaCN alone.

These studies were then repeated on dogs with liver damage. In dogs with high serum phosphatase values Mg had only the small stimulating action seen in normal dogs, suggesting that it had no effect on the increased serum phosphatase produced by the liver damage. The effect with NaCN and Mg plus NaF was slight, and also similar to that observed in normal dogs. NaCN again inhibited the increased phosphatase activity, even in the presence of Mg.

### Table II

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Operation</th>
<th>Inorganic PO₄ (mg per 100 cc.)</th>
<th>Phosphatase units per 100 cc. serum</th>
<th>Additions to phosphatase substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>0.01 M Mg</td>
</tr>
<tr>
<td>93</td>
<td>Normal</td>
<td></td>
<td>4.90</td>
<td>6.32</td>
</tr>
<tr>
<td>94</td>
<td>&quot;</td>
<td></td>
<td>4.10</td>
<td>4.62</td>
</tr>
<tr>
<td>109</td>
<td>&quot;</td>
<td></td>
<td>5.21</td>
<td>6.00</td>
</tr>
<tr>
<td>110</td>
<td>&quot;</td>
<td></td>
<td>3.92</td>
<td>6.24</td>
</tr>
<tr>
<td>95</td>
<td>CCl₄ administered</td>
<td></td>
<td>6.87</td>
<td>11.3</td>
</tr>
<tr>
<td>G-8</td>
<td>Obstruction</td>
<td></td>
<td>3.80</td>
<td>30.2</td>
</tr>
<tr>
<td>81</td>
<td>&quot;</td>
<td></td>
<td>3.92</td>
<td>106.2</td>
</tr>
<tr>
<td>91</td>
<td>Bile fistula</td>
<td></td>
<td>4.94</td>
<td>22.8</td>
</tr>
<tr>
<td>84</td>
<td>Cholecystectomy</td>
<td></td>
<td>4.76</td>
<td>8.99</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Extracts of non-specific alkaline phosphatase from various tissues, generally characterized as a phosphomonoesterase, are not inhibited by 0.01 M fluoride, while 0.01 M cyanide will produce about 90 per cent inhibition of activity (6–8). Recently Cloetens prepared phosphatase extracts from liver tissue which he fractionated into alkaline phosphatase I and alkaline phosphatase II (8). The activity of alkaline phosphatase I was not in-
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Inhibited by 0.01 m KCN but was reduced by 0.01 m KF. Alkaline phosphatase II, however, was completely inhibited by 0.01 m KCN, and a similar concentration of KF was without effect. Gomori (14) recently prepared an alkaline phosphatase from various tissues which had a specific effect on hexose diphosphatase. This hexosediphosphatase was inhibited by fluoride and activated by cyanide.

The alkaline phosphatases of Cloetens and the hexosediphosphatase of Gomori are present in liver. The rise in alkaline serum phosphatase activity observed in dogs with hepatic damage is not due to hexosediphosphatase, as this enzyme will not act on β-glycerophosphate. Neither does the increased serum phosphatase activity correspond to Cloetens' alkaline phosphatase I, for the effects of CN and F are opposite in either case. The effect of CN and F on the increased serum phosphatase activity during hepatic damage does correspond to the effect of these ions on Cloetens' alkaline phosphatase II. However, 0.01 m Mg will activate alkaline phosphatase II 200 to 300 per cent, but has only a very slight effect on serum phosphatase activity of dogs with liver damage (Table II). It does not seem likely that the lack of effect of Mg on serum phosphatase activity is due to the presence of the small amount of Mg in the blood serum. Serum is reported to contain 1 to 3 mg. of Mg per 100 cc. (15), which would correspond to only 0.0008 m MgSO₄. In the determination of phosphatase activity 1 cc. of serum is added to 10 cc. of substrate, so that the final concentration of Mg is very low.

SUMMARY

1. Sodium cyanide, in concentrations of 0.0001 to 0.1 m, had only a slight inhibitory effect on serum phosphatase activity of normal dogs. When the serum phosphatase values increased as a result of liver damage, the addition of NaCN inhibited phosphatase activity. However, as the concentration of NaCN was increased the serum phosphatase activity decreased to normal values, but did not go below normal. As this result was obtained in three types of liver damage, it suggests that the increased serum phosphatase activity during hepatic damage is due to an alkaline phosphatase normally present in the blood in only small amounts.

2. Magnesium sulfate (0.01 m) and NaF (0.01 m) each had a slight activating effect on serum phosphatase activity of normal dogs. In dogs with high serum phosphatase values Mg and F had only the slight effect noted in normal dogs. A combination of Mg and F did not further increase activity in either case. The addition of NaCN (0.01 m) still produced the characteristic inhibition in the presence of Mg or F.
BIBLIOGRAPHY

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