APPENDIX V

ESTIMATION OF ALKALINE PHOSPHATASE

(King and Armstrong, 1980)

Principle

In the presence of alkaline oxidizing agents aminoantipyrine gives a red or purple colour with compounds containing a phenolic group which can be measured at 520 nm. This reaction has been used to determine the phenol produced by the action of phosphatases on disodium phenyl phosphate.

Reagents

1. Sodium carbonate - Sodium bicarbonate buffer, 0.1 M pH 10.0
2. Disodium phenyl phosphate substrate (2.18 g in 1 liter distilled water) 
3. Buffered substrate was prepared by mixing equal volumes of solutions (1) and (2)
4. Stock phenol standard (1 mg/ml in 0.1 N HCl) 
5. Working phenol standard (0.01 mg/ml in distilled water)
6. 0.5 N Sodium hydroxide 
7. 0.5 N sodium bicarbonate 
8. 0.6 per cent 4 - aminoantipyrine 
9. 2.4 per cent potassium ferricyanide.
Procedure

Buffered substrate (2 ml) was added to the test and control tubes and the tubes were placed in a water bath at 37°C for 3 minutes. Then 0.1 ml of 20 per cent liver homogenate was added to the 'test' and the tubes were incubated at 37°C for 15 minutes. After incubation the tubes were removed from the water bath and 0.8 ml NaOH (0.5 N) and 1.2 ml of sodium bicarbonate (0.5 N) were added to both tubes and 0.1 ml of sample was added to the control tube. 4-Aminoantipyrine (1 ml) and potassium ferricyanide (1 ml) were then added. After mixing the colour developed was measured at 520 nm. Similarly the colour developed using phenol standard (0.01 mg) was also measured. The result was expressed in mg of phenol liberated by one gram tissue homogenate in 15 minutes at 37°C using the following equation.

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\text{ALP U/g liver} = \frac{\text{O.D of sample} - \text{O.D of control}}{\text{10 x dilution Factor}} \times \frac{\text{O.D of Standard}}{0.1 \times 94 \times 15}
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