Table 2. The effect of starvation on serum iron concentration

Period of starvation (days)	December		January		June		July	
	Female	Male	Female	Male	Female	Male	Female	Male
Control	92.01	83.05	84.58	77.90	112.09	103.10	96.53	104.20
4	93.12	84.00	83.10	76.34	109.30	99.34	97.34	99.34
8	95.10	83.00	84.20	77.10	110.34	100.12	95.21	98.12
12	91.30	84.10	80.13	78.20	106.10	98.34	96.12	94.10
16	90.31	80.00	78.34	72.10	99.34	93.12	93.10	90.20
20	87.10	76.30	72.12	70.34	97.12	90.10	90.13	87.12
24	80.20	72.10	71.34	69.10	95.31	89.34	85.34	82.10
28	79.10	69.34	71.20	66.31	94.13	89.00	83.10	80.34

The values are mean of 4 replicates.

The values of 99.4 µg/100 ml in females and 92.0 µg/100 ml in males are quite high as compared to the values of 25 µg/100 ml reported in fish[2] and lower than the serum iron concentration of laying hens, laying goose (1260 µg/100 ml) and laying duck (1065 µg/100 ml) reported by Planas et al.[3]. It compares favourably with the serum iron concentration of 80–180 µg/100 ml recorded for humans[4].

The serum iron concentration in the present experiments has been observed to be related to sex, season and spawning habits of the frogs, which is in agreement with the report on fishes[7]. The high concentration of serum iron in females observed in the present experiments appears to be in direct correlation with high hemoglobin concentration reported in females[8].

During May to October, which roughly corresponds to the spawning period, the serum iron concentration is relatively high indicating that the iron transport and metabolism are at a higher pitch during the spawning period both in males and females. The figures are a little higher for females than males indicating that egg production entails a greater mobilisation of iron than sperm production.

Starvation has been found to decrease the serum iron level but not until about the 16th day. It may be because of the mobilisation of iron from reserves which continues until such time that the reserves begin to get depleted and then only serum iron level is decreased.

1 Acknowledgment. Thanks are due to Professor Dr M.M. Goil, Head of the Zoology Department for guidance and help in various ways and to Professor Dr R.K. Sharma for valuable suggestions.

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Nature of inhibition of rat testicular alkaline phosphatase by isatin

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Summary. Isatin has been found to inhibit rat testicular alkaline phosphatase (EC 3.1.3.1). That the inhibition is non-competitive as well as non-allosteric is evident from a) the hyperbolic curve relating inhibition as a function of inhibitor concentration; b) the small change in enthalpy, free energy and entropy; c) the number of isatin molecules associating with 1 molecule of the enzyme (n = 1.29); and, d) the decrease in the values of both K_m and V_{max} in the presence of isatin.

Isatin (2,3-dioxo-indoline) is a known inhibitor of liver xanthine oxidase[2] and kidney alkaline phosphatase[3] from rat. It has also been used for studying the organ and species specificity of acid phosphatase[4,5]. The present communication describes the nature of inhibition of rat testicular alkaline phosphatase by isatin.

Materials and methods. Male albino rats, weighing 200–250 g, were used. Alkaline phosphatase from rat testes was purified by the method of Morton[6]. Each enzyme preparation comprised pooled tissues from 2 animals. In all, 6 such preparations were made. All standard experiments in the absence and presence of isatin were performed using 10 mM disodium phenyl phosphate as substrate and carbonatebicarbonate buffer of pH 9.4 at 37 °C as described earlier[7]. Thermodynamic parameters were calculated according to the method of Taketa and Pogell[8].

Results and discussion. Figure 1 depicts isatin inhibition of rat testicular alkaline phosphatase which varied from 8.9 to 56.2% with isatin concentration ranging from 1.0 to 10.0 mM. The non-allosteric nature of this inhibition is suggested by the hyperbolic profile of the curve. A family of parallel lines obtained in the line-weaver Burk plot (figure 2) of I/S versus I/V implied that isatin inhibition of the enzyme was of non-competitive type[9]. The K_m value in the presence of 10 mM isatin (0.37 mM) was less than that in the absence of isatin (0.83 mM). V_{max} for the inhibited enzyme (0.005 units) was also lower than that of the uninhibited enzyme (0.011 units).

Kinetic and thermodynamic parameters for the binding reaction of testicular alkaline phosphatase with 10 mM isatin. $\Delta H = -1.038$ kcal/mole

Temperature (°C)	K*	Ki (mM)**	ΔF (kcal/mole)	ΔS (e.u./mole)***
17	457.1	8.67	−3.52	+8.65
28	431.5	9.08	−3.62	+8.67
37	407.4	9.48	−3.69	+8.65

*K, Association constant. **Ki, inhibitor constant; concentration necessary for 50% inhibition. ***e.u./mole, expressed as calories/mole/degree.

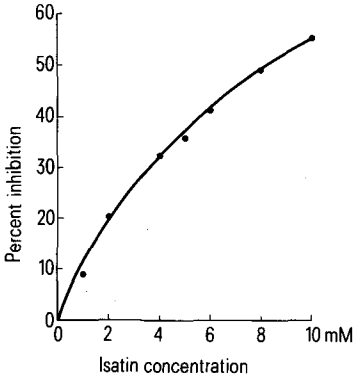

Fig. 1. Inhibition of rat testicular alkaline phosphatase by isatin at different concentrations.

The data on thermodynamic parameters for the enzyme-inhibitor interaction is presented in the table. The value of the apparent association constant, K, was obtained at log (isatin) = -2.0 from figure 3. The molar enthalpy change, ΔH, was found to be -1.038 kcal/mole (figure 3, inset). The entropy change, ΔS, varied from +8.65 to +8.67 e.u./mole with the temperature ranging from 17° to 37 °C. This positive entropy change may be attributed to the unfolding of the protein structure to fit the substrate.

The number of inhibitor molecules combining with 1 molecule of the enzyme was found to be 1.29. Also, the values for the change in entropy and enthalpy were characteristic of non-allosteric inhibition of the enzyme. From these findings, it may be inferred that inhibition of rat testicular alkaline phosphatase by isatin is non-allosteric and non-competitive in nature.

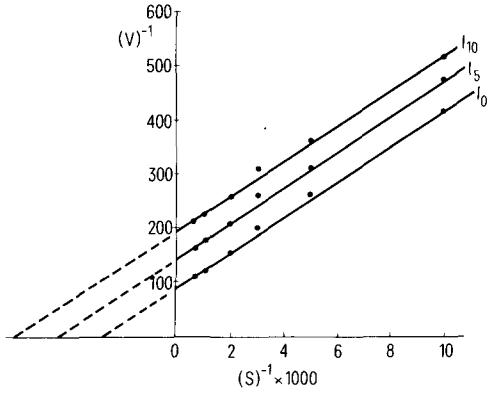

Fig. 2. Line-weaver Burk plot of the effect of substrate concentration. I_0, without isatin; I_5, 5 mM isatin; I_{10}, 10 mM isatin.

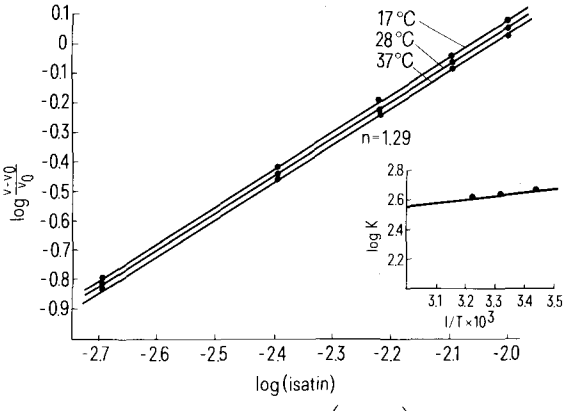

Fig. 3. Plot of log (isatin) versus log $\left(\frac{v-v_0}{v_0}\right)$ at different temperatures.

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Effect of prenatal and neonatal pantothenic acid deficiency on rat intestinal phosphatases

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Summary. Alkaline phosphatase activity was increased in the distal part of the small intestine of pantothenic acid deficient neonatal rats, while acid phosphatase activity was slightly increased and protein concentration was decreased throughout the small intestine. The growth and maturation of the distal part of the small intestine were retarded more severely than in the proximal part.

Pantothenic acid deficiency during gestation results in deranged lipid metabolism of foetuses in guinea-pigs[2]. The acetylating capacity of tissues is markedly reduced in pantothenic acid deficients rats[3]. Deprivation of the vitamin in pigs reduces coenzyme A activity in the mucosa of the intestine[4].

Acute deficiency of the vitamin produces a reduction in flux rate of sodium and mild reduction in the net glucose and potassium transport[4]. Pantothenic acid is also essential to maintain the structural integrity of intestinal mucosa[5,6]. It is also critically required for normal foetal development[7]. The involvement of intestinal alkaline phosphatase in the