pids were found to be much lower than those reported earlier<sup>12</sup>. This may be due to either extraction procedure or composition of the growth medium<sup>13</sup>. The most abundant phospholipids found in fungi are phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine<sup>14</sup> as has been shown in M. gypseum in this study and other dermatophytes viz Trichophyton rubrum<sup>5</sup> and Arthroderma

Table 1. Phospholipid composition of Microsporum gypseum

Phospholipids	% of total phospholipid*		
Lysophosphatidyl choline	11.0±0.9		
Phosphatidyl choline	$23.1 \pm 1.6$		
Phosphatidyl inositol	$3.7 \pm 1.5$		
Phosphatidyl serine	$19.4 \pm 1.0$		
Phosphatidyl ethanolamine	$29.8 \pm 2.8$		
Unknown phospholipids	$13.0 \pm 2.0$		

<sup>\*</sup> Values are mean  $\pm$  SD of 4 different batches analyzed.

Table 2. Neutral lipid composition of Microsporum gypseum

Lipids (mg/g dry wt)	
Triglycerides	$15.0 \pm 2.6$
Diglycerides	$2.6 \pm 0.20$
Free cholesterol	$0.7 \pm 0.1$
Esterified cholesterol	$1.3 \pm 0.1$

Values are mean  $\pm$  SD of 4 different batches analyzed.

uncinatum<sup>6</sup>. Triglycerides were the major components within the neutral lipids in M. gypseum as has been reported for other fungi<sup>15</sup>. Thus, the results of this study, together with the previous work<sup>5,6</sup>, indicate no striking differences in lipid composition amongst various genera of dermatophytes.

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## Serum iron level of the common Indian frog Rana tigrina Daud.

## K. Singh1

Department of Zoology, Bareilly College, Bareilly-243001 (India), 15 August 1977

Summary. A study extending over a period of 2 years has been made on serum iron level of common Indian frog R. tigrina. Serum iron averages 99.4 µg/100 ml in female and 92.60 µg/100 ml in males. The serum iron concentration is relatively high from May to October. Starvation has been found to decrease the serum iron level from the 16th day onwards.

Among the indispensable trace elements, iron occupies a primary place in the metabolism of higher animals. It plays a central role in life processes as a constituent of 2 main groups of iron-containing compounds. The total iron of the blood is present almost entirely in the form of hemoglobin. Serum iron gives a picture of iron transport and its metabolism. Although a considerable amount of work has been done<sup>2-4</sup>, on serum iron level of other vertebrates, nothing is known about this element in any species of Rana. The present study is a contribution in that direction.

The methods of selection, feeding and maintenance of experimental animals and collection of blood samples were as reported earlier<sup>5</sup>. Serum iron was determined colorimetrically by the method of Peters et al.<sup>6</sup>.

The values of serum iron determined in R. tigrina of both the sexes, throughout the year are compiled in table 1. In females, serum iron ranges from 84.58 to 108.09 with an average of 99.4 µg/100 ml and in males it ranges from 77.9 to 103.10 with an average of 92.60 µg/100 ml in different months. A marked variation is observed in serum iron level in respect to sex, the females having a relatively high concentration. The serum iron concentration is comparatively high from May to October (103.10-118.09 µg/100 ml in females and 96.42-104.20 µg/100 ml in males).

The effect of starvation on serum iron concentration was studied up to 28 days and the values are shown in table 2. The iron content was found to decrease progressively from the 16th day onwards.

Table 1. Iron concentration in serum

Months	Female	Male	
January	84.58 ± 1.285	$77.90 \pm 1.559$	
•	(70.5-90.5)	(60.7-78.4)	
February	$90.65 \pm 1.269$	$85.05 \pm 1.690$	
•	(80.4–100.5)	(80.2-90.2)	
March	$91.01 \pm 0.881$	$88.04 \pm 1.431$	
	(87.3-100.4)	(80.1-100.2)	
April	$94.52 \pm 0.849$	$90.63 \pm 0.897$	
_	(89.3-99.3)	(82.4-97.4)	
May	$109.76 \pm 2.437$	$96.42 \pm 0.837$	
	(93.4-131.3)	(88.4–104.3)	
June	$112.89 \pm 2.287$	$103.10 \pm 3.427$	
	(97.3-130.4)	(81.2-116.2)	
July	$104.20 \pm 0.947$	$96.53 \pm 1.783$	
	(99.1-109.3)	(89.3–101.2)	
August	$105.13 \pm 0.910$	$99.20 \pm 1.919$	
_	(100.2-112.3)	(81.2-110.3)	
September	$108.09 \pm 1.464$	$98.20 \pm 1.310$	
	(101.1-123.3)	(91.1-106.2)	
October	$106.37 \pm 3.843$	$96.54 \pm 1.442$	
	(87.3-113.2)	(90.1-105.3)	
November	$95.02 \pm 1.463$	$90.63 \pm 3.997$	
	(90.3–111.0)	(88.0-99.4)	
December	$92.01 \pm 1.272$	$83.05 \pm 1.000$	
	(85.0-98.1)	(79.1 - 89.3)	

Values expressed in  $\mu g/100$  ml, are mean  $\pm$  SEM of 16 observations made for each sex. Range values are given in parentheses. Average value for the whole year: female = 99.4, male = 92.0.

Table 2. The effect of starvation on serum iron concentration

Period of starvation (days)	December Female	Male	January Female	Male	June Female	Male	July Female	Male
starvation (days)	remaie	Maie	remale	Male	Temale	Male	remale	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  $\mu$ g/100 ml in females and 92.0  $\mu$ g/100 ml in males are quite high as compared to the values of 25  $\mu$ g/100 ml reported in fish<sup>2</sup> and lower than the serum iron concentration of laying hens, laying goose (1260  $\mu$ g/100 ml) and laying duck (1065  $\mu$ g/100 ml) reported by Planas et al.<sup>3</sup>. It compares favourably with the serum iron concentration of 80–180  $\mu$ g/100 ml recorded for humans<sup>4</sup>.

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<sup>7</sup>. 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<sup>8</sup>.

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.

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## Nature of inhibition of rat testicular alkaline phosphatase by isatin

P. Kumar<sup>1</sup>, J.P. Nagpaul, B. Singh, R.C. Bansal and R. Sharma

Department of Biochemistry, Panjab University, Chandigarh-160014 (India), 22 August 1977

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<sup>2</sup> and kidney alkaline phosphatase<sup>3</sup> from rat. It has also been used for studying the organ and species specificity of acid phosphatase<sup>4,5</sup>. 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<sup>6</sup>. 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<sup>7</sup>. Thermodynamic parameters were calculated according to the method of Taketa and Pogell<sup>8</sup>.

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<sup>9</sup>. 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.  $\Delta H = -1.038 \text{ kcal/mole}$ 

Temperature (°C)	K*	Ki (mM)**	△F (kcal/ mole)	△S (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.