G-6PD uptake by G-6PD-deficient erythrocytes after hypotonic hemolysis

NaCl (%)	0.100	0.150	0.175	0.200	0.225	0.250	0.300	0.400	0.900
G-6PD activity (IU/ml)									
Added	33	33	33	33	33	33	33	33	33
Recovered	4.45 (14%)	5.67 (17%)	6.72 (20%)	7.28 (22%)	8.15 (25%)	7.35 (22%)	3.30 (10%)	1.10 (3%)	0 0
Hemolysis (%)	95	92	90	87	85	82	78	27	0

Some experimental data support the hypothesis that the added enzyme is really trapped into the cells. There is no enzyme activity when the loading procedure is carried out at NaCl concentrations between 0.40 and 0.90% where negligible or no hemolysis occurs. The loaded enzyme is not washed out by 5 centrifugations of the cells, but is released only when erythrocytes are lysed.

Additional evidence of enzyme trapping comes from the behavior of GSH in loaded red cells after incubation with acetylphenylhydrazine. The percent fall of GSH in deficient cells after incubation with acetylphenylhydrazine amounted to 64%, while the value for G-6PD loaded erythrocytes was 44%.

Thus we conclude that, in accordance with what was shown in previous reports with other enzymes[1-7], G-6PD also enters through the red cell membrane pores during hypotonic hemolysis, remains inside when tonicity is corrected to normal value, and is functionally active in reducing glutathione.

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Lipid composition of *Microsporum gypseum*

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Summary. The lipid composition of *Microsporum gypseum* has been studied. The lipids amounted to 10.1% and phospholipids to 1.1% of the mycelial dry weight. Phosphatidyl choline, phosphatidyl serine and phosphatidyl ethanolamine were the major components, while lysophosphatidyl choline, and phosphatidyl inositol were present in smaller quantities. Neutral lipids consisted of monoglycerides, diglycerides, triglycerides, free and esterified cholesterol.

Lipids are vital constituents of biological membranes and have been involved in allergic responses[2]. The fatty acids of some dermatophytes, a group of fungi causing superficial infections, have been investigated[3,4], whereas other lipid classes have received limited attention. Among the classes of lipids, phospholipids of the genus *Trichophyton* have recently been examined[5,6] while no such report is available on the genus *Microsporum*. Therefore, it was of interest to conduct a quantitative study on the lipid classes of *M. gypseum. Materials and methods. M. gypseum* isolated from a human case of dermatophytosis were grown at room temperature in Sabouraud's dextrose broth, pH 5.4–5.6. Cells were grown as stationary cultures for 4 weeks. Mycelia were harvested after autoclaving by filtration. The washed cells were dried at 50 °C to constant weight. Extraction and purification of lipids were done as described previously[7]. Total lipids were quantitated gravimetrically. The purified lipids were fractionated into phospholipids and neutral lipids by silicic acid column chromatography. The phospholipid classes were analysed by 2 dimensional TLC[8] and quantitated as described[9,10]. The separation, isolation, characterization and quantitation of neutral lipids were done as detailed earlier[11]. Alkaline hydrolysis was carried out as reported previously[7].

Results and discussion. The total chloroform-methanol soluble fraction of *M. gypseum* amounted to 10.1% and phospholipids to 1.1% of the mycelial dry weight. The total phospholipids of *M. gypseum* gave 8 components on TLC with iodine vapors. The 5 major components were identified as lysophosphatidyl choline, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine and phosphatidyl ethanolamine. The presence of these phospholipids was confirmed by cochromatography of the isolated lipids in different solvent systems. Identification was further substantiated by paper chromatography of the water-soluble products obtained after mild alkaline hydrolysis of these components along with authentic standards. In addition, 3 more phospholipid fractions were present only in minor amounts and were not characterized. It is evident from table 1 that phosphatidyl choline, phosphatidyl serine and phosphatidyl ethanolamine are the major phosphatides of *M. gypseum.* Chromatography of the neutral lipids revealed the presence of free and esterified cholesterol, triglycerides, diglycerides and monoglycerides. It is clear from table 2 that triglycerides represent a major fraction of the quantitated neutral lipids.

Almost all of the major classes of lipids were present in *M. gypseum,* but the values for total lipids and phospholi-

pids were found to be much lower than those reported earlier[12]. This may be due to either extraction procedure or composition of the growth medium[13]. The most abundant phospholipids found in fungi are phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine[14] as has been shown in *M. gypseum* in this study and other dermatophytes viz *Trichophyton rubrum*[5] and *Arthroderma uncinatum*[6]. Triglycerides were the major components within the neutral lipids in *M. gypseum* as has been reported for other fungi[15]. Thus, the results of this study, together with the previous work[5,6], indicate no striking differences in lipid composition amongst various genera of dermatophytes.

Table 1. Phospholipid composition of Microsporum gypseum

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

* Values are mean ± SD of 4 different batches analyzed.

Table 2. Neutral lipid composition of Microsporum gypseum

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

Values are mean ± SD of 4 different batches analyzed.

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

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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[2-4], 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[5]. Serum iron was determined colorimetrically by the method of Peters et al.[6].

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

Values expressed in µg/100 ml, are mean ± 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.