estimate, 40–50 molecules of GdmCl were bound per native protein molecule and around 80 GdmCl molecules were bound to the unfolded protein. This suggests that even at submolar concentrations of GdmCl (10^{-2} – 10^{-1} M) similar binding may take place at 0.16 μM protein, used in the present study. The estimated affinity constants at this concentration were 10 M^{-1} for the native and 50 M^{-1} for the denatured LDH[3]. The present study indicated that even millimolar concentrations of the denaturant could perturb the tertiary structure of a protein through a possible direct binding, which is generally assumed to be 'innocent' in many studies of protein folding.

1 Tanford, C , *Adv Protein Chem* , 1968, **23**, 122–282
2 Tanford, C , *Adv Protein Chem* , 1970, **24**, 2–95
3 Zettlmeissl, G., Rudolph, R and Jaenicke, R , *Eur J Biochem* , 1979, **100**, 593–598
4 Jaenicke, R., *Prog Biophys Mol Biol* , 1987, **49**, 117–237
5 Badcoe, I G , Smith, C. J , Wood, S , Halsall, D J., Holbrook, J J , Lund, P and Clarke, A R , *Biochemistry*, 1991, **30**, 9195–9200
6 Jaenicke, R and Knof, S , *Eur. J Biochem* , 1968, **4**, 157–163
7 Lakowicz, J R , *Principles of Fluorescence Spectroscopy*, Plenum Press, New York, 1983
8 Lehrer, S S , *Biochemistry*, 1971, **10**, 3254–3263
9 Kiltz, H -H , Keil, W , Griesbach, M , Petry, K and Meyer, H , *Hoppe Seyler's Z Physiol Chem* , 1977, **358**, 123–127
10 Eftink, M R and Ghiron, C A , *Anal Biochem* , 1981, **114**, 199–227
11 Arakawa, T. and Timasheff, S N , *Biochemistry*, 1984, **23**, 5924–5929
12 Creighton, T E , *Curr Opin Struct Biol* , 1991, **1**, 5–16
13 Makhatadze, G I and Privalov, P L , *J Mol Biol* , 1992, **226**, 491–505

ACKNOWLEDGEMENTS We thank Dr D. DasGupta for helpful discussions CDG acknowledges financial supports from CSIR, DAE and DBT, Government of India SC is a UGC Senior Research Fellow

Received 15 December 1994, revised accepted 21 March 1995

Analysis of fall in serum ferritin after chelation of iron with Deferiprone (L1) in β-thalassaemia and haemoglobin E β-thalassaemia

D. Adhikari*,†, T. Basu Roy*, S. Chandra* and S. K. Adhikari§

*Department of Haematology, Kothari Medical Centre, 8/3 Alipore Road, Calcutta 700 027, India
§Instituto de Fisica Teorica, Rua Pamplona no 145, Sao Paulo, Brazil

Serum ferritin reflects body iron store. It is increased in thalassaemias due to many reasons. The chelation of iron with deferiprone (L1) causes fall of serum ferritin. This is found to be biexponential when chelated by hydrophilic α-hydroxypyridones such as deferiprone *in vivo*.

FERRITIN is a protein which stores iron in the body and is found in the serum as well as inside the cell. It is composed of 24 subunits of at least two types: L (or light 19,700 M_r) and H (or heavy 21,100 M_r)[1]. Those tissues functioning as major iron storage depot (like liver and spleen) have a preponderance of L subunits while other tissues have a higher proportion of H subunits[2].

The amount of serum ferritin is usually proportional to the amount of intracellular ferritin[3]. Serum ferritin is increased due to iron overload in β-thalassaemia (BT) and haemoglobin E β-thalassaemia (EBT) patients. This is partly due to repeated transfusions and partly due to increased intestinal absorption of iron[4]. Effective reduction of serum ferritin was obtained by use of Deferiprone (DFP) or L1 at a dose of 75 mg/kg/day on BT and EBT patients ($n = 20$) in this study over a period of 15 months after signing the informed-consent forms. DFP is a new oral chelator which is undergoing trial in various countries in the world[5].

In BT patients receiving DFP, the mean serum ferritin dropped significantly from initial 3763 ± 1404 ng/ml to 1956 ± 851 ng/ml during the study ($p < 0.005$). In EBT patients receiving DFP, the mean serum ferritin dropped significantly from 2948 ± 1771 ng/ml to 1166 ± 894 ng/ml ($p < 0.005$).

Mean fall of serum ferritin in all the patients was plotted on a semilog paper for the period of study (Figure 1). The fall in serum ferritin fits well in a biexponential curve whose equation can be written as

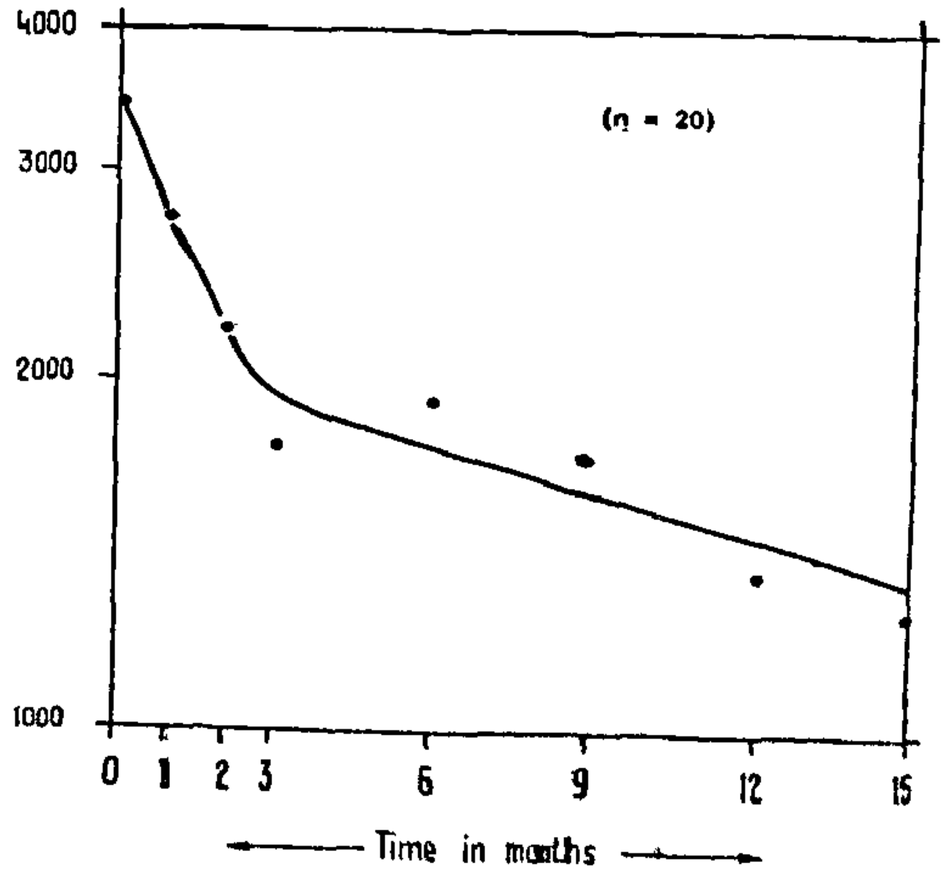

Figure 1. Fall of serum ferritin (ng/ml) over months in thalassaemia patients treated with Deferiprone (L1) at a dose of 75 mg/kg/day

†For correspondence

$$y^t = f(t)$$

or,

$$y^t = \sum_{i=1}^{n-2} A_i \cdot e^{-B_i t}, \quad (1)$$

where y^t is the level of serum ferritin measured in ng/ml at time t, A_i and B_i are the reduction in serum ferritin level constants of the ith exponential term, which may be expressed in terms of the individual intercompartmental transfer rate constant and the degradation rate constant.

The results are shown in Figure 1, where we plot the level of serum ferritin versus time for patients taking DFP. Twenty patients were considered in the study. The present plot can be fitted to eq. (1), where $A_1 = 1400$ ng/ml, $A_2 = 2000$ ng/ml, $B_1 = 0.229$/month and $B_2 = 0.029$/month. Then it can be said that ferritin stays in two compartments in concentrations which are decreasing with time following the exponential law given by eq. (1). The initial decrease is due to the removal of that component of ferritin which is present in plasma and may have some particular nature. The delayed fall may be due to the removal of the intracellular component having a different nature, which comes out in a later phase of treatment.

1 McCaren, G D, *Curr Haematol Oncol*, 1988, **6**, 185.
2 Whitek, Mumro H N., *J. Biol. Chem*, 1988, **263**, 8938
3 Brittenham, G M, in *Haematology, Basic Principles and Practice* (ed Hoffman, R), Churchill Livingstone, New York, 1991, p 329
4 Weatherall, D J. and Clegg, J. B, *The Thalassaemia Syndrome*, Blackwell Scientific Publications, Oxford, 1981, 3rd edn
5 Agarwal, M B, *Indian J Paediatr*, 1993, **60**, 509–516

Received 24 March 1994, revised accepted 20 January 1995

Occurrence of superficial and cutaneous mycotic infections at Rourkela, Orissa

S. Das, P. Swain and B. K. Choudhury

Botany Research Laboratory, P G Department of Botany, Government College, Rourkela 769 004, India

From 250 clinically suspected cases of dermatomycoses, a mycological study was carried out on organisms causing superficial and cutaneous infections. The commonest cutaneous etiological agent detected to be prevalent at Rourkela was *Trichophyton rubrum* in 96 cases of patients. The superficial lesions were formed by the opportunistic fungus *Candida albicans*. The occurrence with respect to age, sex and period of year was also recorded.

TRICHOPHYTON, Microsporum and Epidermophyton generally attack integuments and their appendages like hairs and nails; they involve stratum corneum or deeper layers of the epidermis and hence are called dermatophytes. The superficial infections also occur in human beings due to other fungi, the most common of which are the species of *Candida*[1]. The *Candida* infection may be localized or widespread[2]. Superficial candidiasis may involve the epidermal and mucosal surfaces, including those of oral cavity, pharynx, oesophagus, stomach, intestines, urinary bladder and genital tract. These dermatomycoses are commonly seen in India due to the tropical climate and many other factors like hygiene and socioeconomic status. Greater population *drifts*, fast means of transport and tremendous advancement in industry and technology have resulted in certain pockets called urban areas. Under these circumstances, if these opportunistic fungi accidentally become pathogenic, they continue to spread freely.

In this paper, a detailed study of the incidence of superficial and cutaneous mycotic infections occurring at Rourkela, an industrially important town in Orissa, has been made. Also included were the frequency of occurrence of various species of mycoses and the correlation between the site of involvement and causative sites; and a survey of certain other predisposing factors was also made.

The samples were collected for a period of one year from the suspected patients who visited the Skin and Venereal Diseases Department of Ispat General Hospital, Rourkela. The scrapings were taken aseptically in sterile filter papers after applying 70% alcohol on the affected areas[3]. A part of the scrapings was mounted in 10% KOH and observed directly under the microscope to detect the occurrence of fungal elements (spores and hyphae). The remaining part of the materials was inoculated to Sabouraud's dextrose–agar media containing cycloheximide and chloramphenicol at 30°± 2°C. After an incubation period of 7 days, the fungal species were identified by studying macromorphology and micromorphology of the organisms. Also, some special tests like urease test, hair perforation test and chlamydospore formation tests were done using the appropriate media, depending on the suspected fungal organism. The identifications of the fungal organisms were further confirmed in the Mycology Division of the School of Tropical Medicine, Calcutta.

A record of the patients' habitat, age, sex and the presence of domesticated animals at home was also made.

Out of 250 cases of superficial dermatomycoses, a total number of 195 were observed to be culture-positive. Most of the cases were KOH-positive. Of these, 96, 84 and 15 collections were identified to be cases of dermatophytes, candidiasis and other fungal infections like *Aspergillus* and *Penicillium* (Table 1).