Table 2. Changes in the weight of testis, prostate and seminal vesicle after 15 mg/kg of Cimetidine ingestion.

	Control	1 Week	2 Weeks
Feeding phase			
Testis wt (g)	0.606 ± 0.021	0.450 ± 0.025	0.380 ± 0.020
Prostate wt (g)	0.125 ± 0.015	0.110 ± 0.014	0.098 ± 0.015
Seminal vesicle wt (g)	0.055 ± 0.001	0.052 ± 0.001	0.054 ± 0.002
Recovery phase			
Testis wt (g)	0.597 ± 0.020	0.551 ± 0.010	0.400 ± 0.015
Prostate wt (g)	0.130 ± 0.020	0.120 ± 0.015	0.100 ± 0.012
Seminal vesicle wt (g)	0.060 ± 0.002	0.058 ± 0.003	0.062 ± 0.002

Values are mean ± SD ($n = 5$), Statistically significant difference from controls, $P < 0.001$ (Student's t test).

sacrificed via cervical dislocation. The uterine horns, Fallopian tubes and ovaries were exposed and freed from surrounding tissues. Haemostats were clamped on each Fallopian tube and the lower end of the vagina. The entire reproductive tract was removed and transferred to a 10 cm petri dish containing 0.9% saline at 37°C. The clamps were removed and the vagina and uterine walls were scraped gently to remove any embedded spermatozoa. The number of spermatozoa present was determined using an improved Neubauer haemocytometer (white cell dilution). Counts were performed in duplicate and were expressed as sperm count ($\times 10^6$).

Ejaculate sperm count, as determined during both phases of the investigation, is presented in Table 1. Treated mice showed a significant reduction in ejaculate sperm content ($P < 0.001$) compared to the control mice. Analysis of male ejaculate content after recovery phase also showed significant reduction in sperm content after 30 days.

Table 2 shows a significant decrease in the weights of testis and prostate although seminal vesicle appeared to be normal after Cimetidine ingestion.

Histopathological analysis of the testes revealed significant alterations. Spermatogenesis was observed in some central tubules of 1 week-treated mice (Figure 1) but there was high incidence of multinucleated giant cells in most of the tubules (Figure 3). After 2 weeks of treatment the effect was more deleterious as there were vacuolations and displacements of the germinal elements (Figures 2 and 4). When observed after the recovery periods of 15 and 30 days the damage to the testes appeared to be reversible after 1-week treatment (Figures 6 and 7). However, after 2-week treatment, various forms of architectural disarrangements (vacuolization, karyolysis, pycnosis) were very common in most of the tubules even after the recovery phase (Figures 5 and 8).

The present investigations were undertaken to evaluate the effect of Cimetidine on the testes of male albino mice. In addition to the lowered sperm counts and altered testicular weights following Cimetidine ingestion, histopathological studies revealed irreversible damage to the testes after 2 weeks of treatment. In concurrence with the present results the male rat pups exposed to Cimetidine during late gestation have been reported to appear phenotypically 'feminized' at birth and having smaller testes with lower testosterone level. They even demonstrated reduced sexual performance when studied in later adult life distant from any exposure to the drug[9].

It has also been observed that Cimetidine interacts with androgen receptors but fails to initiate a post-receptor message[2], which is the cause for the significant decrease in the weights of testes and prostate. Moreover, the clinical usefulness of Cimetidine is occasionally inhibited as a result of such actions, which account, at least in part, for the impotence, loss of libido, reduction in sperm counts and gynaecomastia seen in some men who use the drug[5,9].

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Serum ferritin and cholesterol levels in B and EB thalassaemia

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In multitransfused B and EB thalassaemic children, level of serum ferritin, an index of depot iron, is inversely related to serum cholesterol level. The observation suggests that excess body iron has an inhibitory action on the biosynthesis of cholesterol.

IRON overload is a serious problem with multitransfused individuals like B and EB thalassaemics because each unit of 250 ml blood that is transfused adds approximately 150 mg of iron to the body stores[1]. The body's limited ability to get rid of the excess iron results in a

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gradual increase in storage iron levels and eventually tissue damage. The extent of iron overload can be measured by assaying for levels of ferritin in the serum which is a direct reflection of the amount of storage iron in the body[2]. Besides causing changes in the rates of reactions that require iron, high iron levels have been reported to inhibit the enzyme HMG CoA reductase[3] which catalyses the conversion of HMG CoA to mevalonate, the committed step in the biosynthesis of cholesterol[4]. It therefore stands to reason that excess of iron will result in diminished synthesis of cholesterol. The present report confirms that high levels of serum ferritin are correlated with low serum cholesterol values in B and EB thalassaemia.

Venous blood was collected in glass test tubes from B thalassaemic ($n=72$) and EB thalassaemic ($n=53$) children as well as age matched controls ($n=10$). The consent was obtained from informed parents. The diagnosis of B and EB thalassaemia was based on clinical and haemoglobin electrophoresis[5,6]. Serum ferritin was assayed by the enzyme-linked immunosorbent assay (Behring Diagnostics FRG with Mini II Reader). Serum cholesterol was assayed by the method of Henley[7], using reagents obtained from Ortho Diagnostics, USA.

The results are shown in Tables 1 and 2. It can be seen from Table 1 that B-thalassaemic children with less than 50 units transfusions had an average ferritin level of 1854.02 ± 211.23 ng/ml and a cholesterol value of 132.62 ± 6.03 mg/dl whereas the normal children who had never received blood transfusions had ferritin levels of 16.07 ± 5.17 ng/ml and 203 ± 8.45 mg/dl of cholesterol. Similarly, with B thalassaemic children who had received between 50 and 100 units transfusions, the ferritin level was found to be 2276.78 ± 331.70 ng/ml while the cholesterol was 120.94 ± 6.66 mg/dl. With EB thalassaemic children a similar picture was observed. Those receiving less than 50 units transfusions had 1067.92 ± 191.35 ng/ml ferritin and 125.96 ± 8.26 mg/dl cholesterol. The ferritin level increased to 1690.59 ± 301.52 ng/ml in the group who received between 50 and 100 units transfusions. The cholesterol level remained at 124.37 ± 9.98 mg/dl. Thus it was evident that extremely high ferritin values correlated with the very low cholesterol values in these multitransfused subjects. Since the low cholesterol values may be a reflection of the small age of the subjects we have presented the ferritin and cholesterol values of these multi-transfused children in relation to their age and compared them with the normal children. The results are shown in Table 2. It can be seen that the group of B thalassaemic children above the age of 10 had ferritin value of 2198.11 ± 230.13 ng/ml and 106.33 ± 5.32 mg/dl of cholesterol. The EB thalassaemic children of the same age group had ferritin values of 1555.28 ± 261.33 ng/ml and 123.21 ± 8.05 mg/dl of cholesterol. However, normal children below the age of ten, had a cholesterol value of 203.00 ± 8.45 mg/dl which is much greater than the cholesterol values obtained for the multi-transfused B and EB thalassaemic children although they were older than the normal children. It is evident that the low cholesterol values seen in multi-transfused subjects are not related to their age, but are related to high serum ferritin levels, i.e. high iron overload suppresses the synthesis of cholesterol in the multitransfused children.

Table 1. Levels of serum ferritin and serum cholesterol of B and EB thalassaemic children (related to number of unit transfusions).

State	No. of transfusions	Ferritin (ng/ml*)	Cholesterol (mg/dl*)
B thalassaemia ($n=72$)	<50 ($n=35$)	1854.02 ± 211.23 $P<0.005$	132.62 ± 6.03 $P<0.005$
	50–100 ($n=37$)	2276.78 ± 331.70 $P<0.005$	120.94 ± 6.66 $P<0.005$
EB thalassaemia ($n=53$)	<50 ($n=26$)	1067.92 ± 191.35 $P<0.005$	125.96 ± 8.26 $P<0.005$
	50–100 ($n=27$)	1690.59 ± 301.52 $P<0.005$	124.37 ± 9.98 $P<0.005$
Control ($n=10$)		16.07 ± 5.17	203.00 ± 8.45

*Mean ± S.E.

Table 2. Levels of serum ferritin and serum cholesterol in B and EB thalassaemic children in different age groups.

State	Age (yrs)	Ferritin (ng/ml*)	Cholesterol (mg/dl*)
B thalassaemia ($n=72$)	<10 ($n=25$)	2051.17 ± 173.11 $P<0.005$	127.03 ± 6.15 $P<0.005$
	>10 ($n=9$)	2198.11 ± 230.13 $P<0.005$	106.33 ± 5.32 $P<0.005$
EB thalassaemia ($n=53$)	<10 ($n=25$)	1234.56 ± 231.90 $P<0.005$	127.20 ± 9.15 $P<0.005$
	>10 ($n=28$)	1555.28 ± 261.33 $P<0.005$	123.21 ± 8.05 $P<0.005$
Control ($n=10$)	(5.2)	16.07 ± 5.17	203.00 ± 8.45

*Mean ± S.E.

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