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Studies on a New Antianaemic Preparation— Diaquo-bis (*N*-ethylidene-threoninato) Iron (II) (FT-410)* (I)

K. HIGAKI, S. IMADO and Y. KOWA, *Osaka Research Laboratory of Tanabe Seiyaku Co., Ltd.*

Many known iron preparations for the treatment of anaemia produce gastro-intestinal disturbances as one of the characteristic side effects[1-5] due to the action of salts of heavy metals; ferric ion also causes local irritation. Moreover, they may also give liver damage.[6] Recently, antianaemic agents with lesser side effects have become available.

Important points required for a new iron preparation are lower toxicity, no side effects, easy absorption, higher stability as a pharmaceutical product and easy use for the generation of blood corpuscles and haemoglobin. With these in mind, FT-410 was compared with other iron preparations and found to possess some advantages.

The structure of FT-410 and its pharmacological actions are reported herein.

Methods and Results

Properties and Structure of FT-410

The compound exists as pale yellow needles, stable in air, only slightly soluble in water and most organic solvents but more soluble in basic solvents such as pyridine; no trace of ferrous ion is detectable in aqueous solutions. The amount of Fe^{2+} in the compound is 14·7 per cent.

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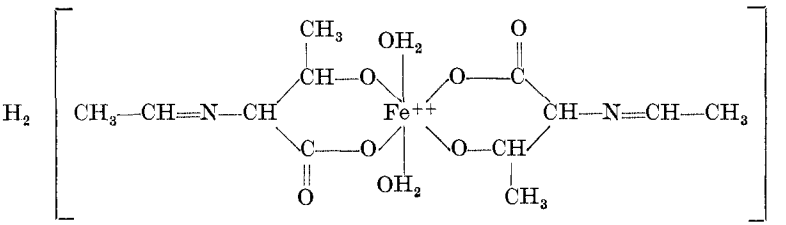
Part of the material of this paper was also presented before the 19th Kinki Regional Meeting of Japanese Pharmacological Society at Kyoto, July 10, 1960.

An approach to the determination of the structure of FT-410 was developed from the study of the structure of *trans*-bis (*N*-ethylidene-DL-threonato) copper (II) dihydrate. Ultraviolet spectra of the Cu-complex compound gave λ_{max} 249 mμ (log ϵ 3·855) and the infrared spectrum showed absorptions between



$C_{12}H_{24}O_8N_2Fe = 380{\cdot}18$

Fig. 1. Structure of FT-410.

3320 and 3430 cm^{-1}, indicative for —OH of crystalline water, 1665, 1685 cm^{-1} (doublet bonds) for $\rangle C{=}N-$ and 1615 cm^{-1} for $-CO_2^-$.

Determination of the crystalline water content by means of the thermobalance method and X-ray structure analysis indicated that hydroxyl and carboxyl groups of *N*-ethylidene-DL-threonine were arranged in such a way as to form a planar square about the Cu atom.

Similar analytical studies indicated the structure shown in Fig. 1 for FT-410.

The full report on the synthesis and structure of FT-410 is to be published elsewhere.[7]

The following iron preparations were also tested: ferrous sulphate, phosphate, lactate, fumarate and orotinate and ferric ammonium citrate.

Pharmacological Methods and Results

1. *Toxicity and general toxic symptoms*

(*a*) *Acute toxicity.* The drugs stated above were given to male na-II strain mice weighing 17 ± 1 g body weight.

Applying Weil's method,[8] the LD_{50} of each iron preparation was calculated from the mortality of the mice after 72 h of dosing, simultaneously observing the toxic symptoms (see Table I).

Table I. Acute toxicities in mice

Drugs	Amount of iron, %	Route	LD_{50}, mg/kg (Confidence limit 95%)
FT-410	14·7	Oral	> 3,000
FT-410	14·7	Intraperitioneal	634 (540–740)
Ferrous sulphate	20·1	Oral	1,750 (1,640–1,860)
Ferrous orotinate	11·8	Oral	> 3,000

Toxic symptoms—Soft faeces or diarrhoea resulted after large doses of all the drugs but especially after ferrous sulphate. Subsequently the animals moved slowly and death occurred in a stand posture. However none of the animals died even after a dose of 3 g/kg of FT-410 on *ferrous orotinate.*

(*b*) *Chronic toxicity.* Young male Wister–King rats, three weeks old and weighing 40–50 g, were divided into three groups of six: Group A served as control, Group B was given 0·3 g/kg of the iron preparations, and Group C 1 g/kg. All drugs were suspended in 0·5 per cent carboxymethylcellulose (CMC) and forcedly fed every day by stomach tube on an empty stomach. The control group received the same amount of 0·5 per cent CMC solution. The animals were offered water and a commercial ration *ad libitum.* The ration had the composition showed in Table II.

Table II. Composition of Oriental FM diet

Composition	%	[a] Ash	%	[b] Vitamin μg/g diet	
Protein	24·8	Fe	0·073	B_1	9
Fat	5·6	Ca	0·924	B_2	4
Carbohydrate	51·4	P	0·883	B_6	5
Ash[a]	5·7	Mg	0·354	Ca-Pantothenate	16
Vitamin[b]	—	Na	0·273	Inositol	700
Water	7·0	K	0·478	Choline·HCl	57
		I	0·052	Biotin	0·18
		SiO_3	0·169	Folic acid	0·5

(*i*) *Effect of the drug on the growth curve of rats*—The growth rate of Group B was 130·7 whereas that of Group C was 113·7 compared with 100 for Group A after 90 days; general symptoms did not differ from those of Group A.

Each group consumed 15–20 g of food per 100 g body weight.

(*ii*) *Urinary findings, haematological examination and liver function tests*—The tests were carried out after 30 and 90 days of daily administration of the iron preparations. Urine-protein of rats was examined by means of the sulphosalicylic acid test[9] and boiling test.[9] Simultaneously, the occult blood in the urine was examined by means of the benzidine test.[9] Erythrocyte count, leukocyte count, haemoglobin value and the leukocyte picture were examined.

The bromosulphalein excretion rate[10] was used to test the liver function. No differences in the rats in Groups A, B and C were revealed.

(*iii*) *Necropsy and histopathological findings*—No observable differences in the groups could be observed.

2. *Stability in synthetic gastric juice and intestinal juice*

An amount of the different iron preparations equivalent to 50 mg as iron was dissolved in a synthetic gastric juice (50 ml) and also in a synthetic intestinal juice (50 ml), prepared according to the Japanese Pharmacopoeia. Each solution was shaken for 2 h in a water bath at 37° and trivalent iron (Fe^{3+}) then determined (see Table III).

Ferrous sulphate, ferrous phosphate and FT-410 were very stable in synthetic gastric juice while ferrous lactate was very

Table III. Formation of trivalent iron in a synthetic gastric juice and a synthetic intestinal juice (%)

Iron preparations	Synthetic gastric juice	Synthetic intestinal juice
FT-410	0·867	1·589
Ferrous fumarate	1·012	5·981
Ferrous orotinate	1·012	33·761
Ferrous lactate	62·152	41·767
Ferrous phosphate	0·723	26·017
Ferrous sulphate	0·254	3·325

unstable. The most stable compound in a synthetic intestinal juice was FT-410.

3. *Absorption of iron*

Doses (12 mg/kg) of each iron preparation were given orally to rabbits weighing approximately 2 kg. After 2, 4, and 6 h, the serum concentrations of iron were determined using Heilmeyer and Ploetner's method.[11] A non-dosed group of rabbits served as a control for the comparison of the variation in serum-iron concentration. The percentage increase in serum-iron concentration was calculated.

As shown in Fig. 2, of the preparations tested FT-410 gave the most favourable absorption of iron; the concentration of iron in

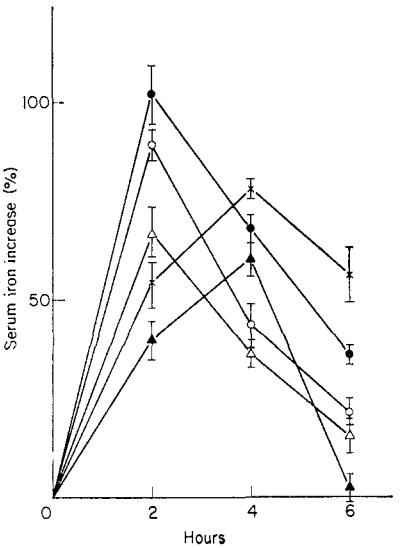


Fig. 2. Percentage increase in serum iron levels of adult male rabbits after oral administration of iron preparations.

●—● FT-410
○—○ Ferrous sulphate
▲—▲ Ferrous phosphate
△—△ Ferric ammonium citrate
×—× Ferrous orotinate

The data were obtained with 5 animals per group.
'I' represents the standard error.

serum 2 h after administration was twice as high as that of normal serum. This value slowly fell but an increase of 30 per cent in the value was observed even 6 h after dosing.

4. *Effect of iron preparations on an anaemic rabbit produced by withdrawing the cardiac blood*

Cardiac puncture for 4 days was carried out each day to withdraw 10 to 20 ml of the blood per 2 kg body weight of male eight-month-old rabbits weighing approximately 2·5 kg (5 animals per group). Normal blood corpuscle, haemoglobin value and serum-iron concentration were determined before withdrawal. When the erythrocyte count and the haemoglobin value had been decreased to half their normal values, FT-410 and other iron preparations equivalent to 2·5 mg of iron per kg of body weight

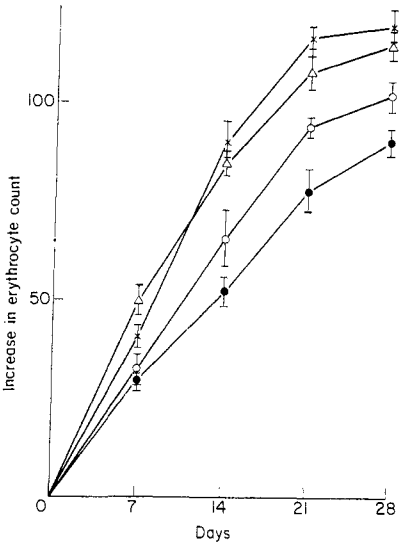


Fig. 3. Effect of iron preparations on the recovery from anaemia produced by withdrawing cardiac blood of adult rabbits.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

The data were obtained using 5 animals per group.

were administered every day. The changes in the erythrocyte count, leukocyte count, haemoglobin value, serum-iron concentration and body weight were examined.

The increase in erythrocyte and leukocyte counts was expressed by taking the values decreased to half to be 0 per cent and the values before drawing the cardiac blood to be 100 per cent. Haemoglobin value was expressed in g/100 ml.

The changes in the serum-iron concentration were calculated from the increase or decrease in the concentration by assuming the value before drawing the blood to be 100 per cent.

(*a*) *Erythrocyte count.* As shown in Fig. 3, all the iron preparations promoted recovery of the erythrocyte count, but particularly FT-410 and ferrous orotinate.

(*b*) *Leukocyte count.* Leukocyte count was not influenced by the administration of FT-410 whereas ferrous orotinate gave an increase.

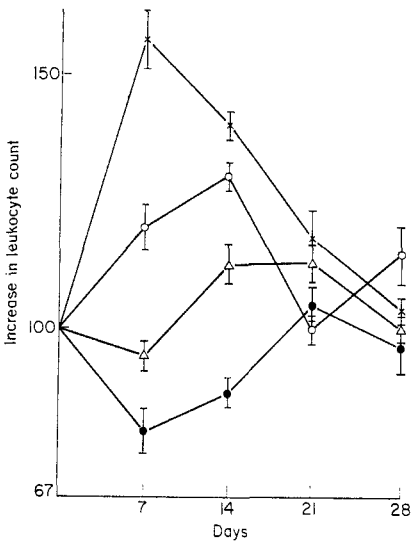


Fig. 4. Effect of orally-administered iron on the leukocyte count of anaemic rabbits.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

(*c*) *Haemoglobin value.* The recovery of haemoglobin value as shown in Fig. 5 was usually faster than that of erythrocyte count; for example, the haemoglobin value returned to normal after 14 days of FT-410 dosing and exceeded the normal value after 28 days.

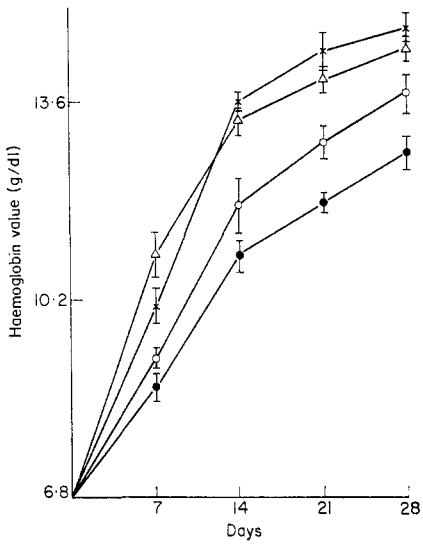


Fig. 5. Effect of orally-administered iron on the recovery of haemoglobin value of anaemic rabbits.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

(*d*) *Serum-iron.* Serum-iron is generally low during the first week after withdrawal of blood, but increases gradually with complete recovery to normal after 28 days. On the other hand, the decreased serum-iron concentration was increased to 1·5 and 1·9 times that of the normal value 3 and 7 days later, respectively, by the administration of FT-410 and ferrous sulphate; the values

then returned to normal with the recovery from anaemia (see Fig. 6).

Although the ration given to the rabbits contained slight amounts of iron, this iron alone could not give swift recovery from anaemia.

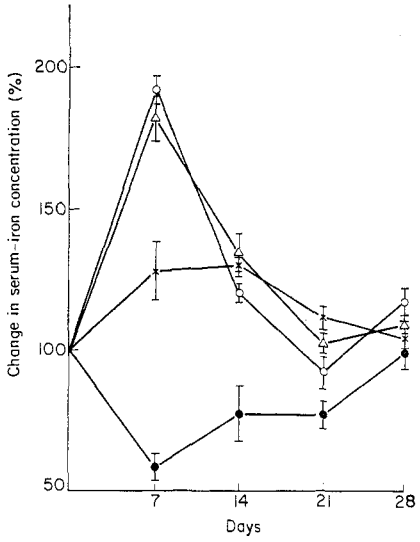


Fig. 6. Effect of orally-administered iron on serum-iron concentration of anaemic rabbits.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

On the other hand, the iron preparations are considered to maintain a high serum-iron level and subsequently to promote the haematopoietic function. The serum-iron response to ferrous orotinate was weaker than the response to ferrous sulphate and FT-410, a result consistent with the results on absorption of iron experiments.

(*e*) *Body weight.* A slight body-weight loss was observed during the first few days after withdrawal of blood. Faster recovery was obtained with the FT-410 group, while the recovery of other groups was less pronounced (see Fig. 7).

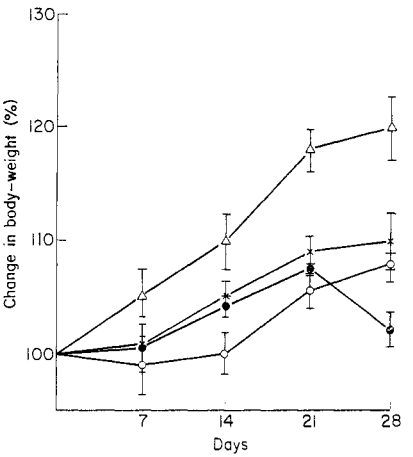


Fig. 7. Effect of orally-administered iron on the body weight of anaemic rabbits.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

5. *Influence of various iron preparations on anaemia in rabbits produced by the administration of phenylhydrazine hydrochloride*

Anaemic rabbits were obtained by injecting into the auricular vein a single dose of 20 mg of 1 per cent phenylhydrazine hydrochloride solution per kg body weight of rabbits each weighing approximately 2·5 kg. Next day, oral doses of FT-410, ferrous sulphate and ferrous orotinate equivalent to 2·5 mg of iron per kg of body weight were commenced and daily dosage continued.

Determination of the erythrocyte count, leukocyte count, haemoglobin value, serum-iron concentration and body weight were made during a period of 3 weeks.

(*a*) *Erythrocyte.* Erythrocyte count was lowered to approximately half the normal value 3 days after dosing with phenylhydrazine, followed by recovery about 3 weeks later. The

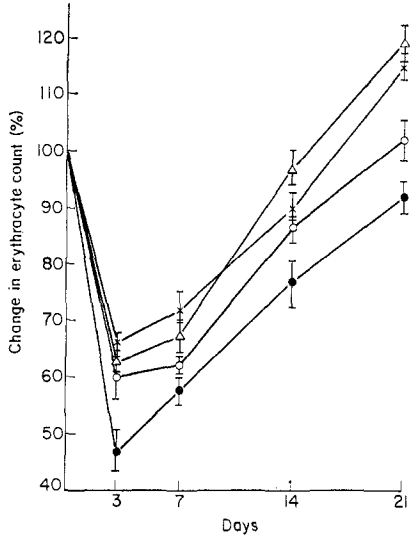


Fig. 8. Changes in erythrocyte count of phenylhydrazine-induced anaemic rabbits produced by orally-administered iron.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

recovery was accelerated by the administration of iron preparations. FT-410 gave recovery by about 2 weeks and further increased the value to approximately 20 per cent above that before dosing (see Fig. 8).

(*b*) *Leukocyte count.* Approximately twice the normal count of leukocyte was manifested 7 days after the administration of phenylhydrazine, followed by recovery to normal about 3 weeks later. Those groups receiving FT-410, ferrous sulphate and ferrous orotinate gave results nearly similar to the control group.

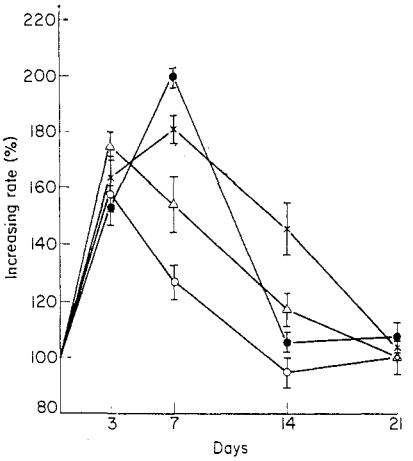


Fig. 9. Changes in leukocyte count of phenylhydrazine-induced anaemic rabbits produced by orally-administered iron.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

(c) *Haemoglobin value.* As shown in Fig. 10, the recovery in the haemoglobin value showed almost the same characteristics as that of the erythrocyte count.

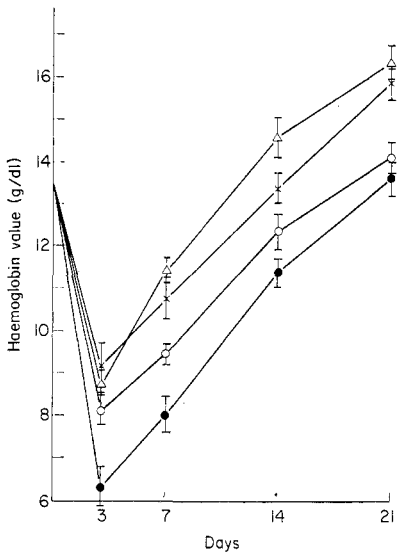


Fig. 10. Changes in haemoglobin value of phenylhydrazine-induced anaemic rabbits produced by orally-administered iron.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

(*d*) *Serum-iron concentration.* The serum-iron concentration of normal rabbits was lowered by administration of phenylhydrazine. The concentration became approximately 60 per cent of the initial value after 7 days, followed by gradual recovery to the normal value 21 days later. On the other hand, the serum-iron concentration was increased to about twice the normal value during the first 3 to 7 days of administration of FT-410 or ferrous sulphate, and subsequently returned to normal approximately 3 weeks later (see Fig. 11).

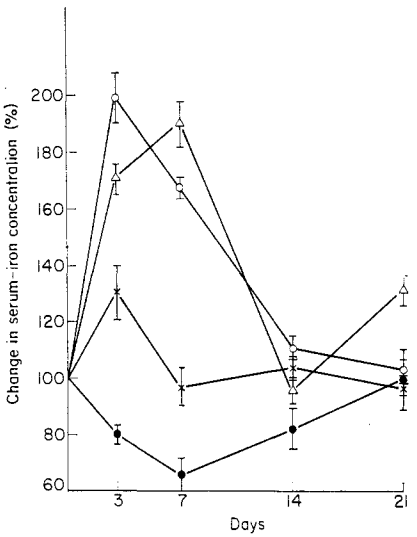


Fig. 11. Changes in serum-iron concentration of phenylhydrazine-induced anaemic rabbits produced by orally-administered iron.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

(*e*) *Body weight.* The body weight of the rabbits was decreased to approximately 80 per cent of the initial weight on the 7th day by the administration of phenylhydrazine, whereas subsequent administration of FT-410 increased the body weight (see Fig. 12). All the groups showed almost the same food consumption during the experiments.

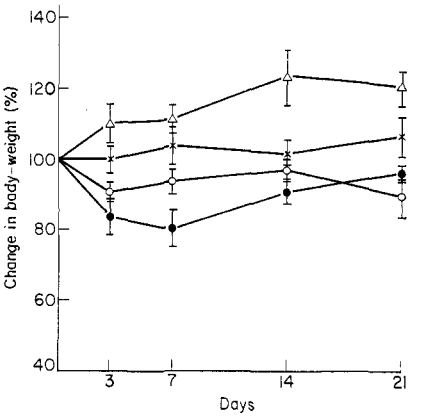


Fig. 12. Changes in the body weight of phenylhydrazine-induced anaemic rabbits produced by orally-administered iron.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

6. *Prophylactic effect of various iron preparations on anaemic rabbits produced by administration of phenylhydrazine hydrochloride*

Doses of iron preparations equivalent to 2·5 mg as iron per 1 kg of body weight were administered to rabbits for 3 weeks before administration of phenylhydrazine as described in the previous section.

(*a*) *Erythrocyte count.* Previous administration of the iron preparations reduced the effect of the injection of phenylhydrazine on the erythrocyte count (see Fig. 13).

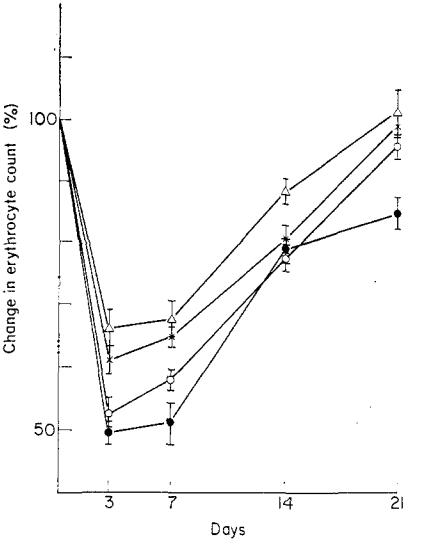


Fig. 13. Changes in the erythrocyte count of phenylhydrazine-induced anaemic rabbits following previous administration of iron preparations.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

(*b*) *Haemoglobin value.* FT-410 and ferrous orotinate partially inhibited the decrease in the haemoglobin value upon injection of phenylhydrazine hydrochloride (see Fig. 14).

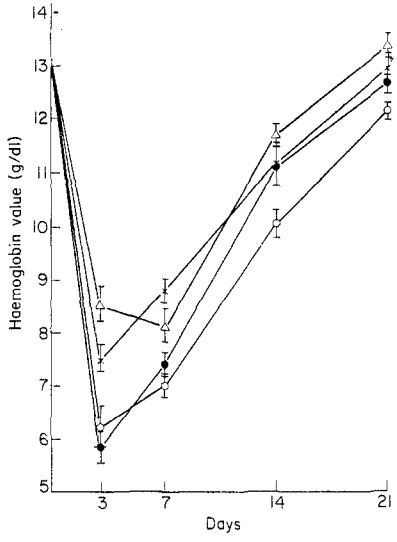


Fig. 14. Changes in the haemoglobin values of phenylhydrazine-induced anaemic rabbits following previous administration of iron preparations.

●—● Control
△—△ FT-410
○—○ Ferrous sulphate
×—× Ferrous orotinate

Discussion

The results of the acute toxicity tests on FT-410 and other iron preparations indicate that the former may be safely administered orally to humans.

Since chronic acute toxicity tests proved that FT-410 promotes the growth of rats, it is considered that continuous administration of FT-410 should give a favourable influence rather than untoward effects.

In the chronic toxicity experiments, ferrous orotinate showed no effect on the growth of rats different to that of the control group,

but because after dosing for 3 successive months some histopathological findings in the liver and kidney were observed, continuous and large-dose administration of ferrous orotinate is probably undesirable.

The stability to synthetic gastric and intestinal juices indicates that the amount of divalent iron of FT-410 to be absorbed and utilized is higher than with other ferrous compounds tested. It was also superior to the other iron preparations tested with respect to absorption, and its absorption is in agreement with the stability of FT-410 in synthetic gastric juice and synthetic intestinal juice.

All preparations were found to be effective against the anaemia produced by blood withdrawal or phenylhydrazine injection, but especially FT-410 and ferrous orotinate.

Prophylactic activity of FT-410 above that preserved by other iron compounds is indicated.

FT-410 provides not only haematopoietic activity but also increases in body weight. The results suggest that the compound might prove to be clinically useful in cases of hypochromic anaemia.

Summary. The structure of FT-410, a new antianaemic compound, is presented. A comparison of the toxicities, and antianaemic properties of this and other iron compounds is made.

(*Revised MS received 5 January, 1961*)

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