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THE INFLUENCE OF DIET ON THE ACUTE TOXICITY OF INJECTABLE IRON PREPARATIONS IN THE MOUSE

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The influence of dietary factors such as Vitamin A and Vitamin E on the pathological effects of iron overloading in rats has been described (Golberg & Smith, 1958). In addition to these responses in chronic experiments there have also been reports of the effects of Vitamin E on the acute toxicity of iron in piglets and in mice (Lannek, Lindberg & Tollerz, 1962; Lannek & Tollerz, 1962). The results reported below illustrate the very marked change in sensitivity to iron when mice are subjected to an alteration in diet. This alteration was not a major one, merely a change from a stock diet to a breeding diet that might go unnoticed in an animal colony, but it resulted in gross changes in the acute toxicity of several injectable iron preparations. The effect of a dietary factor on the toxicity of iron complexes in rats suffering from hypochromic anaemia has previously been reported by Brownlee (1946).

METHODS

The mice used in these experiments were either derived directly from a commercial breeder (Tuck TT mice) or from breeding pairs of these mice maintained in our own animal unit (Benger TT mice). In most experiments male mice were used. Female mice gave similar results.

The mice in our animal unit were maintained either on Oxoid Diet 41 B or on Oxoid Breeding Diet (Oxo Ltd, London S.E.1).

The acute toxicity of each injectable preparation was determined by injecting into the tail vein at a rate of 0.01 ml./sec. Doses were spaced logarithmically, the LD50 and 95% confidence limits being calculated from the deaths at seven days by the method of Litchfield & Wilcoxon (1949).

The preparations, used in an undiluted form, were the Iron-dextrans Imferon, Imposil 75, and Imposil 200 (Benger Laboratories) containing 50, 75 and 100 mg Fe/ml. respectively, Iron-sorbitol-citrate (Jectofer, Astra-Hewlett) containing 50 mg Fe/ml. and saccharated oxide of iron (Ferrivenin, Benger Laboratories) containing 20 mg Fe/ml.

A freshly prepared solution of ferrous sulphate (A.R.) in distilled water (2 mg Fe/ml.) was also tested.

RESULTS

The effect of a change in stock diet on the acute toxicity of Imferon

The initial observation, which prompted the more detailed investigation reported here was that the sensitivity of Tuck TT mice to intravenous iron-dextran changed

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progressively with the duration of stay of the mice in the animal house. If the intravenous LD50 of Imferon was determined in mice within a few days of their being brought into the animal house a value of about 1,300 mg Fe/kg body-weight was found. Subsequent determinations made on mice from the same batch which had been maintained for up to three weeks in the animal house showed a progressive fall in the LD50 (Table 1) reaching a value of 78 mg Fe/kg after 22 days. The mice showing increased sensitivity to iron were three weeks older and also heavier (24 to 42 g) than the least sensitive mice (18 to 24 g) and it could be argued that age and weight were the significant factors. However, when Tuck TT mice were mated and the progeny tested at a time when they were approximately the same age and weight (14 to 32 g) as the mice showing least sensitivity the LD50 of Imferon was found to be 71 mg Fe/kg. In each case the mice were maintained on Oxoid Diet 41B but it was not possible to establish the precise nature of the diet used by the commercial breeder.

TABLE 1

EFFECT OF DURATION OF STAY IN THE ANIMAL HOUSE ON THE ACUTE INTRAVENOUS LD50 OF IMFERON IN MICE

Source of animals	Time of maintenance in animal house after receipt	Wt. (g)	LD50 (mg Fe/kg)	95% Confidence limits
Tuck (T.T. male mice)	2 days	15–24	1,300	812–2,080
	9 days	22–32	740	430–1,272
	22 days	24–42	78	39–156
Benger (T.T. male mice)	From birth	14–32	71	35.5–142

Effect of a change from stock diet to breeding diet

It seemed possible that mice received from the commercial source could have been maintained on a supplemented diet and therefore mice were bred from Tuck TT stock using Oxoid Breeding Diet and at weaning some were transferred to Oxoid Diet 41B and the remainder were maintained on Oxoid Breeding Diet. LD50 values of Imferon were determined 22 days after weaning, using mice within a similar weight range in each group.

In the Diet 41B group the LD50 was between 50 and 100 mg Fe/kg (28.6% deaths at 50 mg Fe/kg, 100% deaths at 100 mg Fe/kg). In the Breeding Diet group the LD50 was 970 mg Fe/kg (95% confidence limits 524 to 1,794 mg Fe/kg). The latter LD50 does not differ significantly from 1,300 mg Fe/kg, found in Tuck TT mice tested within two days of arrival in the animal house. Dose-response lines for the two groups were parallel. It was obvious that some dietary factor was causing a profound change in the sensitivity of mice to iron-dextran.

Effect of Vitamin E

In view of the previously reported effect of Vitamin E on iron toxicity a preliminary study was made in which Vitamin E was added to Diet 41B. Two groups of Benger-bred TT mice were maintained on Diet 41B from weaning and one group was given a Vitamin E supplement in the diet for three days before the LD50 determination. The

estimated oral intake was 0.5 mg Vitamin E/mouse/day. The LD50 of Imferon in mice on the supplemented diet was greater than 1,000 mg Fe/kg and in those on Diet 41B alone it was less than 100 mg Fe/kg.

Determination of the Vitamin E content of Oxoid diets

Data published by the manufacturers show that the Vitamin E supplement in Oxoid Breeding diet is twenty times that in Diet 41B, this being in addition to the natural and similar tocopherol content of the two diets. Dr S. Y. Thompson of the National Institute for Research in Dairying kindly made an extraction and analysis of the tocopherols. A sample of Breeding Diet contained α-tocopherol 1,800 μg/100 g and β-tocopherol 1,164 μg/100 g whereas Diet 41B contained α-tocopherol 396 μg/100 g and β-tocopherol 393 μg/100 g. Hence the breeding diet contains about four times as much tocopherol as does Diet 41B. From the data reported by Lannek, Lindberg & Tollerz (1962) it would appear likely that the dietary intake of tocopherols from breeding diet, amounting to 7.5 mg/kg/day, would be sufficient to give protection against the acute toxic effects of iron.

TABLE 2

THE EFFECT OF DIET ON THE ACUTE TOXICITY OF FIVE IRON PREPARATIONS

Preparation	Intravenous LD50 (mg Fe/kg) and 95% confidence limits	
	Oxoid breeding diet	Oxo diet 41b
Imposil 75	1,370 (1,096–1,712)	115 (95·8–138)
Imposil 200	3,550 (3,200–3,940)	630 (571–702)
Ferrivenin	233 (197– 275)	76 (61·8–93·4)
Jectofer	44·0 (36·8– 52·6)	15 (11·6–19·3)
Ferrous sulphate	21·7 (20·1– 23·4)	6·7 (5·0– 9·0)

The effect of diets on the toxicity of other iron preparations

That the effect of diet on iron toxicity was not solely applicable to a particular iron-dextran was tested by determining the acute toxicity of other iron-polysaccharide preparations. The results are shown in Table 2. The two iron-dextran preparations Imposil 75 and Imposil 200 are less acutely toxic than Imferon but both show a marked increase in toxicity in mice on Diet 41B. This effect is seen not only with iron-dextran preparations but also with saccharated oxide of iron (Ferrivenin) which is inherently more toxic than iron-dextrans but also shows a marked change in toxicity with a change in diet. The iron-sorbitol-citrate complex (Jectofer) and an aqueous solution of ferrous sulphate show similar changes. It is clear that the enhancement of toxic effect is not dependent upon the nature of the complex but upon the presence of iron in it.

DISCUSSION

Composition of diets and the toxicity of iron complexes

The above results confirm and extend the observations of Lannek, Lindberg & Tollerz (1962) who showed that in animals maintained on Vitamin E-deficient diets the toxicity of the iron-dextran preparation Imposil was increased. The results we have obtained are more striking since they were based on intravenous toxicity figures but we have also

found that the intraperitoneal toxicity of Imposil in mice on a Vitamin E-deficient diet prepared according to the method of Friedman, Weiss, Wherry & Kline (1958) is 860 mg Fe/kg whereas on Diet 41B it is about 2,000 mg Fe/kg, these figures agreeing closely with those published by Lannek, Lindberg & Tollerz (1962). In a subsequent paper Tollerz & Lannek (1964) showed that even the intraperitoneal LD50 of Imposil could be reduced to as low as 100 mg Fe/kg if the mice were maintained on the Vitamin E-deficient diet for up to six weeks rather than for three weeks. Nevertheless it is evident that the intravenous route provides a more sensitive method for the detection of such changes in toxicity. Initially Lannek, Lindberg & Tollerz (1962) protected mice against the toxic effects of iron by pre-treatment with α-tocopherol acetate intramuscularly twice a week during the twenty-one days before the LD50 determination; the dose corresponded to about 7 mg/kg per day. In our experiments the daily intake of α- and β-tocopherols from Oxoid Breeding Diet was approximately 7.5 mg/kg and thus protection can be achieved with a rather lower total dose of Vitamin E administered by injection. Subsequently Tollerz & Lannek (1964) showed that protection can be achieved not only with Vitamin E but with synthetic antitoxidants.

Brownlee (1946) described a very similar situation in rats using ferrous salts. In these experiments the toxic effect of iron was observed in rats on a diet of dried milk and starch supplemented with yeast and salt but without added Vitamin E. This toxic effect was not found in rats fed on varied and mixed full diets but was reproduced in varying degrees by a number of other diets and was attributed by Brownlee (1946) to a variable distribution of some protecting factor in the diets. It was found that cabbage afforded full protection. By chromatographic separation of cabbage extracts a fraction with Vitamin E activity was obtained which was shown to protect against iron toxicity. There was also a fraction without Vitamin E activity but having iron-protective activity and this was taken to indicate that the cabbage factor contained a number of antioxidants of the tocopherol class including α-tocopherol itself. Brownlee (1946) stated that the symptoms in rats treated with iron were comparable to those reported in Vitamin E-deficient animals. However, mice on stock diets such as Diet 41B are apparently not Vitamin E-deficient. They reproduce, though not as satisfactorily as mice on Breeding Diet, and show none of the symptoms of Vitamin E-deficiency and yet there is clearly a marked difference in their sensitivity to iron. However, the α-tocopherol content of Diet 41B barely meets the tentative minimum requirement for growth in mice (see National Academy of Sciences, NRC Publication 999, 1962, for references). Golberg & Smith (1958) suggested that for rats Diet 41 was perhaps adequate with respect to Vitamin E under ordinary circumstances, but not in the presence of tissue siderosis. Our experience would seem to support this view in respect of Diet 41B and mice.

A point of practical importance is that it would be possible for a minor change of diet, supplementing it with cabbage, for example, or changing to a breeding diet, to alter materially the apparent toxicity of iron compounds. Such alterations in diet could occur unintentionally if those responsible for feeding the animals were not aware of the importance of this kind of change.

These implications are not solely restricted to the toxicity of iron-complexes since there is a report of a marked increase in the toxicity of 3-phenyl-3-sulphanilyl-propiophenone in the absence of Vitamin E (Zbinden, 1963).

Mechanism of toxic action, and the protective action of Vitamin E

The work of Brownlee (1946) appears to have been the first to draw attention to the similarity between Vitamin E deficiency and iron toxicity, and at that stage the possible role of tocopherols, or antioxidants of a similar nature, was suggested. Golberg, Smith & Martin (1957) made the suggestion that there was a definite similarity between changes in certain organs of the iron-loaded rat and the pathological manifestations of Vitamin E-deficiency in this species as described by Martin & Moore (1939); these observations were confirmed and extended by Golberg & Smith (1958). There had been previous reports of the biological consequences of the interaction of iron and Vitamin E *in vitro* (Waddell & Steenbock, 1931; King, Chiung Puh Lee & Visscher, 1955) and the possible routes involved in the oxidation of α-tocopherol, and the biological significance of these changes, were discussed by Harrison, Gander, Blakley & Boyer (1956). Lannek and Tollerz (1962) suggested from their experimental results that large amounts of iron were capable of destroying Vitamin E *in vivo*, this concept agreeing in general terms with that previously outlined by Golberg & Smith (1960). Tollerz & Lannek (1964) showed that synthetic antioxidants were capable of protecting Vitamin E-deficient mice against iron toxicity whether given prior to the iron or even simultaneously with the iron.

The relationship between such antioxidants and Vitamin E function was extensively investigated by Krishnamurthy and Bieri (1962). Their findings supported the hypothesis that antioxidants, including α-tocopherol, act primarily by virtue of their ability to prevent cellular damage initiated by the toxic products of the peroxidative process. The occurrence of such lipid peroxides in the liver of iron-loaded rats was demonstrated by Golberg & Smith (1958).

Our findings are in keeping with this concept of a protective effect of tocopherols and suggest that in tests of toxicity of iron compounds the level of antioxidants in the diet may have a significant effect and should be taken into account when comparative studies are made.

SUMMARY

1. The acute intravenous LD50 values of several injectable iron preparations were determined in mice.

2. It was shown that these preparations were more toxic in mice maintained on a normal stock diet than in mice maintained on a vitamin-supplemented breeding diet.

3. Evidence for the role of Vitamin E and other antioxidants in the protection of mice against the toxic effects of iron is discussed.

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