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PREVENTION OF INDIUM INTOXICATION BY FERRIC DEXTRAN

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Experiments on the rat indicate that intravenous administration of indium chloride produces severe hepatic necroses with fatal icterus within a few days. These actions can be prevented by the prophylactic administration of ferric dextran. This protective effect of the iron compound must be largely specific since it could not be duplicated by pretreatment with any of a large series of other agents. The possible mechanism of the protective effect is briefly discussed.

Earlier observations have shown that treatment with ferric dextran can prevent the generalized soft-tissue calcinosis that is normally elicited by chronic overdosage with dihydrotachysterol in the rat (Selye & Strebel, 1962). Various metallic salts and in particular indium chloride act as " direct calcifiers " in that they cause topical calcification at the site of injection wherever they are introduced into connective tissue (Selye, 1962); this effect is likewise inhibited by ferric dextran pretreatment (Selye, Tuchweber & Gabbiani, 1962).

The question arose therefore whether ferric dextran would also antagonize the toxic effects of such direct calcifiers that are not connected with manifest calcium deposition. Indium chloride was chosen for the study of this point, first because in the event of intravenous injection indium chloride produces acute fatal hepatic necroses in the rat without any histochemically detectable calcium deposition, and second because, despite the ever-increasing industrial utilization of indium compounds, their pharmacology and toxicology are still very incompletely known (McCord, Meek, Harrold & Heussner, 1942).

As we shall see from the experiments to be reported here, prophylactic treatment with ferric dextran not only protects the liver against the induction of hepatic necroses by indium chloride, but also prevents mortality in conditions under which all the unprotected control animals die from acute indium intoxication.

METHODS

120 female rats of the Holtzman strain, with an initial body weight of 90 to 110 g, were subdivided into 8 groups as indicated in Table 1.

All animals received 2 mg of indium chloride in 1 ml. water into the jugular vein under light ether anaesthesia on the sixth day. The controls were otherwise untreated, while the principal experimental group received 1 ml. ferric dextran (Imposil®) equivalent to 50 mg of metallic iron intraperitoneally on the first and sixth day.

Additional groups were treated with other agents in order to establish the specificity of the ferric dextran effect. For this purpose, we used methyltestosterone because it shares with ferric dextran the ability to antagonize dihydrotachysterol (Selye & Bois, 1956; Selye & Renaud, 1958), various histamine liberators (compound 48/80, polymyxin, dextran) since pretreatment with these can antagonize certain calciphylactic tissue reactions (Selye, 1962); and non-specific stressors (restraint, formaldehyde injections) to ascertain whether ferric dextran acts merely by eliciting non-specific stress. These latter agents were injected as follows: *Methyltestosterone* (Oreton-M®, Shering), 2 mg in 0.2 ml. water subcutaneously daily, beginning on the first day. *Compound 48/80* (Burroughs-Wellcome), 300 μg in 0.5 ml. water subcutaneously, beginning on the first day. *Polymyxin B sulphate* (Burroughs-Wellcome), 1 mg in 0.5 ml. water subcutaneously, beginning on the first day. *Dextran* (Abbott), 1 ml. of a 6% solution intraperitoneally on the sixth day, just before the administration of indium chloride. *Formaldehyde*, 0.3 ml. of the 4% solution subcutaneously on the sixth day, just before the administration of indium chloride.

Restraint was accomplished by immobilizing the animals under light ether anaesthesia on a board according to a previously described technique (Selye, 1961).

During the period of observation the animals were maintained exclusively on Purina Laboratory Chow (Purina Company of Canada) and tap water. All surviving rats were killed with chloroform on the eleventh day and their tissues fixed in alcohol-formol (4 parts of 80% alcohol and 1 part of 10% formalin) for subsequent embedding in paraffin. They were stained with the von Kóssa technique and haematoxylin-phloxine as a counterstain for general orientation and the detection of possible traces of calcium.

RESULTS

Immediately following the intravenous injection of indium chloride, the control animals showed no obvious sign of damage (Group 1), but after two or three days they became ill and exhibited a gradually increasing icterus with yellow discoloration of the skin, mucous membranes and urine. Mortality began on the third day after the injection of indium and reached 100% by the time the experiment was terminated.

TABLE 1

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In addition to the treatments listed in the third column, the rats of all groups received indium chloride as indicated in the text

Group	No. of rats	Treatment	Hepatic necroses (scale 0–3)	Mortality (%)
1	30	None	2·8	100
2	10	Ferric dextran	0	0
3	10	Methyltestosterone	2·6	100
4	10	Compound 48/80	2·8	100
5	10	Polymyxin	2·8	80
6	20	Dextran	3·0	100
7	10	Restraint	1·4	100
8	20	Formaldehyde	3·0	80

At autopsy the liver was peppered with small yellow or reddish patches which on histological examination proved to be foci of necrosis and haemorrhage. There was also some connective-tissue proliferation and oedema around the intrahepatic bile ducts, and the hepatic veins contained many desquamated reticulo-endothelial and even hepatic parenchymal cells. However, no trace of calcification could be demonstrated by the von Kóssa method.

By contrast, the ferric-dextran-protected animals (Group 2) showed no sign of illness at any time during the period of observation and at autopsy their livers were of essentially normal appearance. Even histologically, we saw no sign of necrosis or calcification although occasionally traces of oedema and some dilatation of the sinusoids could be detected. Of course, in this group the Kupffer cells were laden

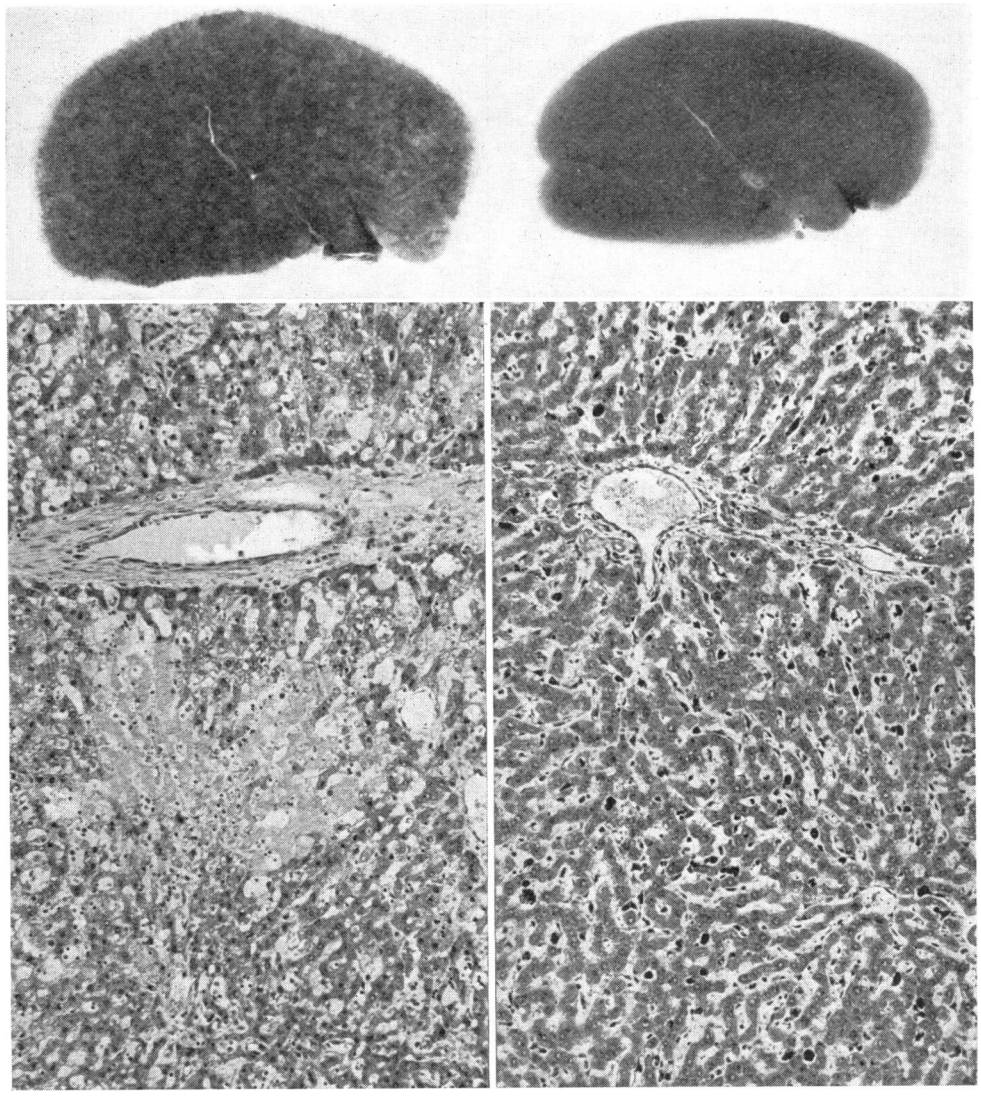

Fig. 1. Prevention of indium-induced hepatic lesions by ferric dextran. *Top:* Hepatic lobe of a rat treated with indium chloride alone is enlarged and shows multiple haemorrhagic and necrotic foci (left),while in the rat pretreated with ferric dextran the corresponding hepatic lobe is macroscopically normal. *Bottom:* Microscopic aspect of the same two hepatic lobes. In the control there is a focus of hepatic necrosis and the perivascular tissue is oedematous, but there is no trace of histochemically demonstrable calcification. In the ferric-dextran-protected rat there is no necrosis, but the Kupffer cells are laden with dark iron granules. (von Kóssa, × 120.)

with iron particles derived from the injected ferric dextran ; similar granules were seen in the spleen and to a lesser extent throughout the connective tissue in other organs (Fig. 1).

None of the other agents (Groups 3 to 8) showed any definite protective effect ; indeed, treatment with dextran, restraint and formaldehyde actually aggravated indium intoxication in that mortality began earlier and virtually all animals in these groups were dead within three days after indium administration.

DISCUSSION

As shown by the data summarized in Table 1, it is evident that ferric dextran is extraordinarily efficacious in preventing the manifestations of acute indium intoxication. Not only did it protect the liver against the induction of the usual necroses conducive to severe jaundice but it also kept the animals alive and in good condition despite the administration of indium chloride at a dose which normally induces 100% mortality. Apparently ferric dextran protects not only against the local calcifying action of various direct calcifiers as previously demonstrated (Selye *et al.*, 1962) but also against the systemic damage induced by the intravenous administration of indium chloride, a compound of this group. This fact is especially noteworthy since, when so given, indium causes no histochemically demonstrable calcification.

Through what mechanism ferric dextran exerts its protective effect remains to be seen. We have already mentioned that this iron compound is particularly efficacious in preventing both the systemic calcinosis induced by dihydrotachysterol and the topical calcinosis elicited by the local administration of direct calcifiers. In addition many earlier histochemical observations show a close relationship between tissue calcification and ferrification (Selye, 1962). Possibly some competitive interaction may exist between iron and calcium. However, under the condition of the present experiments the intravenously injected indium chloride produced no calcification and yet it was " detoxified " by ferric dextran ; hence, the protective action of the latter does not appear to be limited to the simple prevention of gross calcium deposition.

It is well known that the first step in both physiological calcification (osteogenesis) and pathological calcification (soft-tissue calcinosis) is the formation of a calcifiable, usually PAS-positive, " organic matrix " (McLean & Urist, 1961). It is conceivable, therefore, that heavy systemic intoxication with a direct calcifier, such as indium chloride, causes hepatic damage by placing too great a strain upon the liver as an organ possibly involved in the metabolism of this calcifiable organic material. If this were the case, ferric dextran might act by rectifying this disturbance since it stimulates the formation of PAS-stainable material wherever it is deposited in the tissues. However, these consideration are purely speculative ; they are mentioned here only to point out possible avenues for further research.

In any event, it is clear that, among all the agents investigated, only ferric dextran exerted a protective effect against indium intoxication, and hence the prophylactic action of this iron compound must be fairly specific.

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