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# THE MECHANISMS OF TOXICITY OF SOME IRON PREPARATIONS

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Post-mortem and histological studies after the intravenous injection of toxic doses of iron preparations revealed extensive haemorrhages in the lungs (Nissim, 1949, 1953a, b). With some compounds the haemorrhages were present in the complete absence of precipitation, whereas with others their extent was much greater than would have been expected from the degree of precipitation produced. It seemed possible that the compounds concerned might possess an anticoagulant action which would account for this toxic manifestation. Coagulation tests were accordingly carried out on some iron compounds. The part played by capillary injury in producing haemorrhages was also investigated, as was the effect of iron preparations in the production of haemolysis *in vitro*.

## METHODS

In vitro *Testing of the Anticoagulant Effect of Iron Compounds.*—In each of a series of 10 4-ml. test-tubes there was placed 0.32 ml. of a dilution, with physiological saline, of the iron solution to be tested: the range was from 2.0 to 0.004% Fe. A control tube was set up with 0.32 ml. of physiological saline only. A similar series of tubes containing 0.32 ml. of heparin in descending serial dilutions of 1.0 to 0.004% (100 i.u./mg.) was used for comparison. Fresh blood, obtained from the ear of a rabbit, was received into a small beaker and 1 ml. transferred quickly to each of the test-tubes. These were then shaken to ensure thorough mixing. The tubes, kept at room temperature, were observed at intervals for three hours and the clotting time in each tube was noted.

In vitro *Haemolysing Effect of Iron Compounds.*—A series of 10 test-tubes was set up and 1 ml. of the serially diluted iron solutions was pipetted into each (2–0.004% Fe). Rabbit blood was diluted a hundred times with physiological saline; 0.2 ml. of this diluted blood was added to each test-tube and the degree of haemolysis observed under the microscope.

## RESULTS

*Anticoagulant Effect.*—"Ferrous chloride ascorbate" proved the most active of all the iron compounds studied (Table I), which is in striking agreement with the incidence of haemoptysis and massive pulmonary haemorrhages produced by it. This iron preparation had an anticoagulant activity which, by weight of its iron content, was almost equal to that of heparin, but the majority of compounds had an activity about 1/10 that of heparin. The least effective of all was saccharated iron oxide, which had only about 1/100 the activity of heparin.

TABLE I

THE ANTICOAGULANT EFFECT OF DIFFERENT IRON PREPARATIONS COMPARED WITH HEPARIN

1 ml. blood added to 0·32 ml. of solution of iron or heparin. Numerals represent coagulation time in min.

| Conc. of Fe or Heparin % | Saccharated Iron Oxide. Sample $G_4$ | Ferrivenin | Colloidal Ferric Hydroxide | Ferronascine<br>Ferric Chloride Caramelate<br>Ferrous Ascorbate (Alk. or Neutral) | Ferrous Chloride Pyruvate<br>Iron and Ammonium Citrate<br>Ferric Tartrate | Ferric Hydroxide Ferrous Ascorbate<br>Ferric Chloride Lactate | Ferric Glucosate | Ferrous Chloride Ascorbate | Heparin |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 90 | 120 | — | — | — | — | — | — | — |
| 0·5 | 30 | 30 | 120 | — | — | — | — | — | — |
| 0·25 | 6 | 10 | 30 | — | — | — | — | — | — |
| 0·125 | 5 | 6 | 15 | 90 | 120 | — | — | — | — |
| 0·0625 | 5 | 5 | 10 | 15–60 | 30–60 | 120 | — | — | — |
| 0·0312 | 5 | 5 | 5 | 5–30 | 5–30 | 30 | 120 | — | — |
| 0·0160 | 5 | 5 | 5 | 5–10 | 5–10 | 15 | 30 | 120 | 180 |
| 0·0080 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 30 | 90 |

*Haemolysing Effect.*—The haemolysing effect of four iron compounds was studied—saccharated iron oxide, ferric glucosate, "ferric hydroxide ferrous ascorbate" and iron and ammonium citrate. The results are shown in Table II, and reveal no striking differences amongst the compounds. The haemolysing effect disappeared quickly with dilutions below 1–0.5% Fe, and must therefore be negligible *in vivo*, as iron injected intravenously is very rapidly diluted in the blood stream.

TABLE II

THE HAEMOLYSING EFFECT OF IRON PREPARATIONS

0.2 ml. of 1% blood added to 1 ml. of serially diluted iron solution

| Percentage Concentration | Saccharated Iron Oxide | Ferric Glucosate | Ferric Hydroxide Ferrous Ascorbate | Iron and Ammonium Citrate |
|---|---|---|---|---|
| 2·0 | Completely haemolysed | Completely haemolysed | 90% haemolysed | 90% haemolysed |
| 1·0 | 90% haemolysed | ,, | Severely crenated cells | Severely crenated cells |
| 0·5 | 10% haemolysed | 90% haemolysed | ,, | ,, |
| 0·25 | Severely crenated cells | 10% haemolysed | ,, | ,, |
| 0·125 | ,, ,, | Severely crenated cells | ,, | ,, |
| 0·0625 | ,, ,, | ,, | Some crenation | Some crenation |
| 0·03125 | ,, ,, | ,, | ,, | ,, |
| 0·016 | Some crenation | ,, | ,, | ,, |
| 0·008 | ,, ,, | Some crenation | ,, | ,, |
| 0·004 | ,, ,, | ,, | ,, | ,, |

Iron compounds were not found to have any agglutinating effect on the red cells.

*Capillary Damaging Effect.*—(*a*) *Saccharated Iron Oxide and Heparin.* To find to what extent this anticoagulant effect is responsible for the pulmonary haemorrhages, saccharated iron oxide was given together with heparin, to see whether it would produce massive haemorrhages comparable with those seen after ferric glucosate. The anticoagulant activity of ferric glucosate is about half that of heparin.

Two rabbits were therefore injected intravenously with 22.5 and 45 mg. Fe/kg. of saccharated iron oxide ($G_4$), mixed with 11.25 and 22.5 mg. heparin/kg. respectively. It is generally accepted (Wilson and Schild, 1952) that the action of heparin is maintained for 3–4 hours, during which time the haemorrhagic manifestations of ferric glucosate become evident. The two rabbits were killed 24 hours after the injection; the first showed no haemorrhages whatever, whereas the second revealed only a few punctate haemorrhages in the lungs—in contrast with the massive lesions seen with ferric glucosate, and which cause death in 2–6 hours. Hence, the anticoagulant effect presumably cannot, by itself, account for the pulmonary haemorrhages which ferric glucosate causes.

(*b*) *Pulmonary Oedema after Different Iron Preparations.* Pulmonary oedema and pleural effusion followed the administration of some iron preparations. They were the predominant toxic features after intravenous "ferric chloride lactate," some of the rabbits spouting oedema fluid even as the iron preparation was being injected. Pulmonary oedema was also marked after "ferric hydroxide ferrous ascorbate" and ferric glucosate, and to a lesser extent after some other iron compounds, such as ferric tartrate, iron and ammonium citrate, and "Ferronascine" (Roche). The injury to the capillary endothelium, as well as to the epithelial lining of the alveoli, occurred in three stages. In the first stage there was shortening and thickening of the cells of the alveolar walls, resulting in semi-collapse of the alveolar air sacs. In the second stage oedema fluid collected in the alveoli, distending them again, and giving the lungs a more solid consistency. In the third stage oedema fluid appeared in the pleural cavities.

## DISCUSSION

The experiments reported in this paper have uncovered two major factors in the toxicity of some iron compounds. A definite and measurable anticoagulant activity has been demonstrated for each of the compounds studied. This activity is particularly striking with "ferrous chloride ascorbate" and ferric glucosate, the two iron preparations responsible for the severest haemorrhages in the lungs. By administering saccharated iron oxide with heparin, however, it was shown that anticoagulant activity alone was not sufficient to explain massive haemorrhages in the lungs. The considerable pulmonary oedema associated with the haemorrhages of ferric glucosate indicated a severe degree of capillary damage as well. These experiments lead to the conclusion that capillary damage plays an important role in the production of haemorrhage by such iron compounds. When capillary injury is not associated with marked anticoagulant effect, pulmonary oedema and pleural effusion without haemorrhage result. The present experiments provide a further explanation for the very low toxicity of saccharated iron oxide, which appears to be devoid of any capillary damaging effect in addition to being the most feebly anticoagulant of the iron preparations investigated.

SUMMARY

1. Pulmonary haemorrhages produced by iron compounds without evidence of iron precipitation led to a study of their anticoagulant activity.

2. One compound, " ferrous chloride ascorbate," shows an anticoagulant activity (by weight of iron) almost as high as that of heparin.

3. Pulmonary oedema produced by some iron preparations suggests severe capillary damage; when this is associated with a high anticoagulant activity of the compound the effects are much more severe.

REFERENCES

Nissim, J. A. (1949). M.D. thesis, University of London.
—— (1953a). *Brit. J. Pharmacol.*, **8**, 197.
—— (1953b). Ibid., **8**, 371.
Wilson, A., and Schild, H. O. (1952). *Clark's Applied Pharmacology*, 8th ed., p. 480. London: Churchill.