# COMPARATIVE STUDY OF THE CALCIPHYLACTIC CHALLENGING POTENCY OF VARIOUS IRON COMPOUNDS

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Systematic experiments in rats sensitised for calciphylaxis by pretreatment with dihydrotachysterol were made using fourteen different iron compounds as challengers. It was noted that the selective localisation of soft-tissue calcinosis in various organs depends largely upon the carrier to which the iron atom is attached; hence, under otherwise identical conditions, essentially distinct calciphylactic syndromes can be produced in rats given the same amounts of iron attached to different carriers.

CALCIPHYLAXIS is a condition of hypersensitivity in which—especially during a "critical period" after sensitisation by a systemic calcifying factor, for example vitamin-D compounds, parathyroid hormone—topical treatment with certain challengers such as egg white, egg yolk or metallic salts, causes an acute local calcinosis (calcification of soft tissues) followed by inflammation and sclerosis. In rats suitably sensitised, for example with dihydrotachysterol, such calciphylactic reactions can also be elicited selectively at predetermined sites (in the skin, joints, pancreas, bile ducts, uterus, spleen, lung, trachea, thyroid, parathyroid, carotid body, Brunner's glands, cells of the reticulo-endothial system) by the intravenous administration of challenging agents that exhibit a particular affinity for certain organs. The techniques for the production of the various calciphylactic syndromes as well as their structural characteristics have been described in detail elsewhere (Selye, 1962).

Among the challengers that can elicit both topical and systemic calciphylactic reactions, iron salts are of special interest for various reasons:

1. Iron-containing complexes are widely distributed in the body and since they often occur in the pathological calcium deposits of man, iron may play a rôle as an endogenous elicitor of calcinosis.
2. When administered intravenously to appropriately sensitised animals, various iron compounds elicit calcification in different parts of the body, depending presumably upon their special affinity for one or the other tissue.
3. Iron preparations are commonly used in clinical medicine, especially in the treatment of anaemias.
4. Under certain circumstances, calciphylaxis can prevent the cardiovascular and renal calcinosis, normally produced by vitamin-D compounds and parathyroid hormone. This is due to a kind of "reverse calciphylaxis" obtainable only through very diffuse iron impreganation

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of the tissues throughout the body. Here, apparently, innumerable minute foci of iron in various forms cause a widespread and diffuse distribution of the mobilised calcium and thus impede the formation of large circumscribed lime deposits in vital organs (Selye and Strebel, 1962).

In view of these considerations, we felt that it would be rewarding to compare the calciphylactic challenging potency of various iron compounds given under strictly comparable conditions in doses containing equiatomic amounts of metallic iron.

## Material and methods

145 female Sprague-Dawley rats of the Holtzman farm, with a mean initial body weight of 99 g. (range 95–105 g.) were subdivided into 15 groups as shown in Table I.

Dihydrotachysterol (Calcamin, Dr. A. Wander, S.A., Basel, Switzerland) was administered at the dose of 1 mg. in 0·5 ml. of corn oil by stomach tube to all animals on the first day of the experiment.

On the second day 14 iron compounds were injected intravenously (jugular) in amounts containing 1 mg. of metallic iron in 1 ml. of water. Since the chemical composition of many among these preparations has not been completely clarified, available information with the name of the suppliers is listed.

*Ferrous carbonate saccharated* (City Chemical Co., New York): 20 per cent ferrous carbonate, 70 per cent sugar, 10 per cent lactose (N.F. = not less than 15 per cent $FeCO_3$ = 7·23 per cent Fe).

*Iron nucleate* (National Biochemical Co., Cleveland, Ohio): a complex of iron and nucleic acid said to correspond to 1 ml. of nucleic acid and 2 atoms Fe. The preparation contains 4·6 per cent of metallic iron.

*Iron dialysed* (British Drug Houses, Poole, England): contains about 3·5 per cent Fe $\equiv$ 5 per cent $Fe_2O_3$, or 6·7 per cent $Fe(OH)_3$.

*Ferric phosphate soluble* (Fisher Scientific Co., Fair Lawn, N.J.): a complex salt of sodium ferricitrophosphate, containing 12–15 per cent Fe, 15 per cent $P_2O_5$, 45 per cent citric acid.

*Ferric potassium tartrate or tartrated iron* (City Chemical Co., New York): approximate composition $K(FeO)$ $(C_4H_4O_6)$ + water of crystallisation. It contains about 18 per cent Fe, 65 per cent tartaric acid.

*Ferric potassium citrate* (City Chemical Co., New York): a complex salt containing about 16 per cent Fe and 65 per cent citric acid.

*Ferric potassium oxalate* (City Chemical Co., New York): $K_3Fe(C_2O_4)_3.3H_2O$. Anhydrous salt 89·00 per cent, $H_2O$ 11·00 per cent, $K_2C_2O_4$ 50·75 per cent, Fe 11·37 per cent, anhydrous oxalic acid 54·98 per cent.

*Iron peptonised* (City Chemical Co., New York): contains 16–18 per cent Fe.

*Iron oxide saccharated or "Fe-OS"* (*Proferrin*, Merck Sharp & Dohme, West Point, Pa.):a mixture of ferric saccharate, approximate composition $C_{12}H_{22}O_{11}(Fe_2O_3)_2$ and some sodium saccharate plus free sugar.

*Ferric hydroxide dextran complex or "Fe–Dex"* (*Imferon, Imposil*, Benger Laboratories, England): each ml. is equivalent to 50 mg. Fe.

*Ferric hydroxide dextrin complex or "Fe–Din"* (*Ferrigen*, Astra Soedertälje, Sweden): contains 2 per cent Fe.

*Ferric chloride* (Fisher Scientific Co., Fair Lawn, N.J.): $FeCl_3.6H_2O$.

*Ferrous chloride* (Fisher Scientific Co., Fair Lawn, N.J.): $FeCl_2.4H_2O$.

*Ferrous sulphate* (Fisher Scientific Co., Fair Lawn, N.J.): $FeSO_4.7H_2O$.

The animals were maintained exclusively on Purina Laboratory Chow (Purina Co. of Canada) and tap water. On the sixth day the experiment was terminated by killing all surviving animals with chloroform. At autopsy the organs were macroscopically inspected with a stereoscopic loupe and representative specimens from various organs were fixed in ethanol-formol (4 parts absolute ethanol, 1 part 10 per cent formalin) for the subsequent histochemical demonstration of calcium with the von Kóssa or celestine blue techniques as previously described (Selye, 1962). The former technique depends upon formation of a black precipitate between calcium phosphate and silver nitrate, while the latter results from staining the calcified organic matrix with celestine blue under standard conditions.

## Results

As can be seen from Table I, the 14 iron compounds used in this study produced very different calciphylactic syndromes (which consist of diverse patterns of organic calcification) although all of them were administered at a dose containing equiatomic amounts of metallic iron. To comment on each of the tabulated organ lesions here would hardly be warranted, but a few of them deserve special mention.

In the dihydrotachysterol-pretreated controls which received no iron compounds (Group 1), calcium deposition was only of moderate intensity. This "nonspecific calcinosis," produced by systemic calcifiers alone, is virtually limited to the naturally predisposed organs namely the heart, kidney and stomach; being independent of challenge, it should not be confused with true calciphylactic reactions.

The most widespread calciphylactic responses were obtained by solubilised ferric phosphate (Group 5) and ferric potassium tartrate (Group 6), which produced calcium deposition in most of the target organs examined. The action of some other compounds is much more organ-specific. For example, Fe–Din (Group 12), which elicits a very obvious anaphylactoid type of swelling of the snout, induced calcification of the lips in the dihydrotachysterol-sensitised animal. This change was extremely pronounced in our experiment although the other organs showed little calcinosis at the low dose level at which Fe–Din was administered here.

It is also noteworthy that the acute toxicity of the iron compounds tested varied between wide limits, some of them being tolerated without causing any immediate disturbance (Groups 3, 4, 9, 10, 11 and 12), while others could be tolerated only if the injections were performed slowly, taking several minutes for the administration of the required

## TABLE I

### COMPARATIVE STUDY OF THE CALCIPHYLACTIC CHALLENGING POTENCY OF VARIOUS IRON COMPOUNDS

| Group | Number of rats | Treatment* | Skin | Salivary gland | Thyroid | Parathyroid | Heart | | Oesophagus | Stomach | Duodenum | Pancreas | Thymus | Kidney | Carotid body | Articulation | Lips | Mortality per cent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Auricle | Ventricle | | | | | | | | | | |
| 1 | 10 | None | 0 | 0 | 0 | 0 | 0 | 1·0 | 0 | 0·2 | 0 | 0 | 0 | 1·3 | 0 | 0 | 0 | 0 |
| 2 | 10 | $FeCO_3$ saccharated | 0·4 | 0·1 | 0·1 | 0 | 0·2 | 1·5 | 0 | 0·6 | 0·2 | 0 | 0·2 | 1·5 | 0 | 0 | 0 | 10 |
| 3 | 10 | Iron nucleate | 0·1 | 0 | 0 | 0 | 0 | 0·7 | 0 | 0·5 | 0 | 0 | 0 | 0·65 | 0 | 0·1 | 0 | 0 |
| 4 | 10 | Iron dialysed | 0 | 0 | 0·1 | 0 | 0 | 0·7 | 0 | 1·1 | 0 | 0 | 0 | 0·5 | 0 | 0 | 0 | 0 |
| 5 | 10 | Ferric phosphate soluble | 0·7 | 1·6 | 1·8 | 2·1 | 0 | 0·3 | 0·4 | 2·2 | 2·35 | 1·15 | 0·2 | 2·2 | 0·5 | 0·1 | 0 | 10 |
| 6 | 15 | Ferric potassium tartrate | 0 | 1·2 | 1·4 | 1·5 | 0 | 1·0 | 0 | 2·0 | 1·8 | 1·5 | 0·4 | 1·6 | 0·3 | 0 | 0 | 30 |
| 7 | 10 | Ferric potassium citrate | 1·2 | 0·5 | 0·3 | 0·6 | 0 | 0·6 | 0 | 0·8 | 0·5 | 0 | 0·25 | 1·5 | 0 | 0 | 0 | 10 |
| 8 | 15 | Ferric potassium oxalate | 0 | 0·5 | 1·7 | 1·5 | 0·4 | 1·4 | 0·2 | 1·4 | 1·2 | 0·8 | 0·1 | 2·0 | 0·4 | 0 | 0 | 53 |
| 9 | 10 | Iron peptonised | 0·9 | 0·6 | 0·8 | 0·9 | 0 | 0·1 | 0 | 1·4 | 0·4 | 0 | 0 | 0·6 | 0 | 0 | 0 | 0 |
| 10 | 10 | Ferric hydroxide saccharated "Fe–OS" | 0·3 | 0·2 | 0 | 0 | 0·5 | 0·9 | 0 | 0·6 | 0·2 | 0 | 0 | 0·7 | 0 | 0 | 0 | 0 |
| 11 | 10 | Ferric hydroxide-dextran complex "Fe–Dex" | 0·3 | 0·5 | 0 | 0 | 0·2 | 0·1 | 0 | 0·5 | 0·1 | 0 | 0 | 0·1 | 0 | 0 | 0·1 | 0 |
| 12 | 10 | Ferric hydroxide-dextrin complex "Fe–Din" | 0 | 0 | 0 | 0 | 0 | 0·7 | 0 | 0·5 | 0·1 | 0 | 0·3 | 0·8 | 0 | 0 | 1·2 | 0 |
| 13 | 10 | Ferric chloride | 0 | 0 | 0 | 0 | 0 | 0·3 | 0 | 1·3 | 0·5 | 0·3 | 0·3 | 0·5 | 0 | 0 | 0 | 0 |
| 14 | 10 | Ferrous chloride | 0 | 0 | 1·8 | 2·4 | 1·0 | 0·6 | 0 | 0·8 | 1·8 | 0 | 0 | 0·8 | 0·6 | 0 | 0 | 80 |
| 15 | 5 | Ferrous sulphate | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0 | 100 |

* In addition to the agents listed in this column, all animals received a single sensitising dose of dehydrotachysterol as described in the text.

amount (Groups 5, 6, 7, 8 and 13); still other compounds induced a high mortality rate even when thus injected (Groups 14 and 15). Many of the iron preparations in this series, appear to produce tachyphylactic responses in that a second injection given a few minutes after a first dose is better tolerated.

## Discussion

It has long been known that the absorbability, stability and the rate of diffusion of various iron preparations depends largely upon the carrier (anion or ligand) to which the metal is attached. Using the Prussian-blue reaction for the demonstration of iron in tissues, it could also be shown histochemically that iron thus administered in various forms, possesses an affinity for different organs depending upon the carrier; the largest amounts of the metal are usually found in the organs that respond with calcification in the sensitised rat. It is difficult to understand how mere differences in the solubility, stability or diffusibility of iron compounds could account for their vastly different specific organ affinities. *A priori*, it might be expected that a stable and highly diffusible compound could best traverse the capillary walls and spread through tissues throughout the organism without organ selectivity. Our observations show, however, that the distribution of the various iron preparations differs not only quantitatively but also qualitatively.

Since ferrification and subsequent calcification are predominantly limited to connective tissue elements, which are structurally quite similar, it may be reasonably assumed that the biochemical constitution of the stroma is largely dependent upon the adjacent parenchymal elements. For example, the connective tissue compounds of the pancreas, salivary glands and thyroid do not differ essentially in their histologic structure. This similarity is even more striking when we compare the stroma of the thyroid with that of the parathyroids, which are situated within the same regional blood vessel, lymph vessel and nervous supply systems. Yet, the calciphylactic sensitivity of these structures is not the same. These findings suggest that through calciphylaxis we may be able to detect differences in the biochemical constitution of the stroma in organs whose connective-tissue framework shows no morphologic evidence of specificity.

Only in one instance was it possible to identify a structural difference in the connective tissue that could account for its selective calciphylactic sensitivity. The "anaphylactoid shock organs" of the rat and particularly the lips are rich in mast cells and these discharge their granules during the anaphylactoid reaction that occurs for example after treatment with a compound such as Fe–Din. Even Fe–Dex can produce such a discharge although only at higher dose levels than those used in the present experimental series. In these cases, the discharge of the mast cell granules appears to be causally related to the development of the anaphylactoid oedema. In the calciphylactically sensitised rat the induction of an anaphylactoid reaction by such an iron-containing compound results in

the excess localisation of iron in the target organ. Histologic examination of the lips in Fe–Din treated rats shows that in fact Prussian-blue positive iron granules tend to localise in and around the walls of small vessels, wherever mast cell discharge is intense and presumably these iron deposits are responsible for the subsequent attraction of calcium.

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## References

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