Int. J. Cancer: 3, 415-423 (1968)

IRON-DEXTRAN INDUCTION OF DISTANT TUMOURS IN MICE

by

E. LANGVAD
The Fibiger Laboratory [1], *Kgs. Lyngby, Denmark*

The systemic carcinogenicity of iron-dextran has been investigated after single and repeated injections of Imferon in mice and rats. Systemic iron deposits induced by iron-dextran resulted in a stepwise development of reticulosarcomas. Such a histologic reaction, however, was not seen in relation to iron deposits following iron-carbohydrate complex (Intrafer) administration. Distant tumours arising in the lymphoreticular structures showed a uniform latency in test and control mice, while tumours of other origin showed a prolonged latency as compared to such tumours in control animals. It is suggested, therefore, that iron-dextran may act as a co-carcinogenic factor in the case of lymphoreticular neoplasia, increasing host susceptibility to the oncogenic virus presumably present in both control and test animals. The non-lymphoreticular distant and local tumours may stem from a different and direct carcinogenic action of iron since these tumours showed a prolonged latency and were seen after the administration of either iron-dextran or iron-carbohydrate complex.

The results indicate an inconclusive dose-response relationship, though no significant difference was found between tumour yield in the larger groups receiving 500 and 25 mg Fe^{+++}/kg body weight, the latter dose being within the therapeutic level on a comparative weight basis.

Iron-dextran has been found to be carcinogenic in laboratory animals, particularly mice and rats. Repeated subcutaneous injections were followed by a high incidence of sarcomas at the site of injection (Richmond, 1959, 1960; Haddow and Horning, 1960; Golberg *et al.*, 1960; Lundin, 1961; Haddow and Roe, 1964; Haddow *et al.*, 1964; Roe *et al.*, 1964). In studies by Haddow and Horning the total dose represented about 150 times the clinical dosage as calculated on a comparative weight basis. These authors held that the tumours were directly due to the local action of iron-dextran and that the absolute amount administered—i.e. the local dose—was decisive for the neoplastic response, while the dosage in relation to body weight was of minor or no significance. However, others have pointed out that the relation of the total dose to body weight could not be disregarded. Thus Baker *et al.* (1961) submitted that the high dosages used in previous animal experiments caused a systemic iron-overloading. This, they suggested, might lead to a delayed removal of iron from the injection site, thereby favouring local tumour formation.

In most studies a broad dose-response relationship has been established. Roe and Carter (1967) found that the grade of malignancy of the local tumours produced in rats was directly related to the total dose of iron-dextran. However, Golberg and colleagues (1960) reported that low total doses produced little or no carcinogenic effect. They theorized that there might be a threshold dose below which no carcinogenic effect could be demonstrated.

Previous investigations have also shown that the administration of iron-dextran may be followed by the development of tumours at locations

[1] Under the auspices of the Danish Cancer Society.

Received: 7 December, 1967.
Approved: 22 April, 1968.

remote to the site of injection (Haddow and Horning, 1960; Langvad, 1965, 1966). These neoplasms represented a wide spectrum of types which do not usually occur in the type of experimental animals used (Haddow and Horning, 1960; Haddow, 1963). Similar observations were made by Roe and Carter (1967), but the relationship to the administration of iron-dextran remains uncertain.

To clarify this point, we have investigated in mice and rats the systemic carcinogenic effect of high-dosage iron-dextran and of amounts comparable per kg body weight to clinical dosage.

MATERIAL AND METHODS

Iron preparations

A: Iron-dextran (Imferon); Batch No. B 10216 was supplied by Pharmacia, Copenhagen. The preparation contains 50 mg Fe^{+++}/ml.

B: Iron-carbohydrate complex (Intrafer) was also supplied by Pharmacia, Copenhagen. This preparation contains 20 mg Fe^{+++}/ml.

Animals

Inbred mice of the ST/a, C3H, AKR/a, and DBA/2 strains as well as inbred rats of the Wistar strain were used. The animals were approximately 6 weeks old. The mice weighed 20 g and the rats 100 g at the beginning of the experiments. All animals were housed in polypropylene plastic cages, and fed on a cube diet (Hvidesten, C. Chr. Andreasen & Co., Sluseholmen, Copenhagen S.V.); water was supplied *ad libitum*.

Experimental details

The effect of Intrafer was studied in ST/a mice given a single intravenous dose of 50, 250 or 500 mg Fe^{+++} per kg body weight in the ventral tail vein. The animals were observed until spontaneous death.

The effect of several subcutaneous injections of Imferon was studied in C3H, AKR/a, ST/a, and DBA/2 mice as well as in Wistar rats. These animals received weekly doses of 5-10 mg Fe^{+++} per mouse (i.e. 250-500 mg per kg body weight), and 12.5-25 mg Fe^{+++} per rat (i.e. 125-250 mg per kg body weight). Subcutaneous injections were made at the same site in the left flank. In one experiment an equally divided (5+5 mg) dose was given simultaneously in the left and right flanks. Controls received Tyrode's solution. The animals were observed until spontaneous death except in one experiment in which the surviving animals were killed after 52 weeks.

The effect of a single subcutaneous injection of Imferon was studied at doses of 25-2500 mg Fe^{+++} per kg body weight in ST/a mice, which were observed until spontaneous death. Controls received Tyrode's solution.

RESULTS

Among 60 ST/a mice given a single intravenous injection of the iron-carbohydrate complex Intrafer in doses of 50, 250, or 500 mg Fe^{+++}/kg body weight, 43.3% died within 20 weeks after the injection without tumours. Among the remaining animals no tumours were found in the groups given 50 and 500 mg iron. In the second group, treated with 250 mg Fe^{+++}/kg body weight, 15 mice survived for more than 20 weeks. Five of these (30%) died with tumours. In two of them sarcomas were found arising from the connective tissue of the pelvic floor. These might be considered local tumours due to the accidental paravenous injection of Intrafer. In the untreated control group 6 out of 38 animals (15.8%) died with tumours. This difference between the tumour incidence in the second group and the control group failed to reach the customary 5% level of significance ($x^2=2.04$ on 1 d.f.—$P>0.10$).

The results of Imferon treatment are summarized in Tables I and II. From Table I it appears that repeated Imferon injections caused a significant number of local tumours in Wistar rats. However, in four strains of inbred mice no significant increase in the incidence of local tumours was observed after similar treatment. The influence on the development of spontaneous neoplasms was studied in AKR/a and C3H mice. It appears from the Table that the incidence of leukaemia in AKR/a mice remained uninfluenced by Imferon treatment, while the incidence of mammary carcinoma in C3H mice seemed even lower in the treated mice than in the controls.

Table II shows the effect of single subcutaneous injections of Imferon on tumour incidence and survival time of male and female ST/a mice. It may be seen that the female mice—treated as well as controls—survived for periods nearly twice as long as the male mice.

Tumours at the site of injection were seen in 3 treated female mice. The total incidence of other

TABLE I

TUMOUR INCIDENCE AND SURVIVAL TIME OF RATS AND MICE RECEIVING REPEATED DOSES OF IMFERON

Strain	Treatment		No. of animals	No. of tumour-positive animals	Mean survival time (weeks)
	Single dose	No. of doses			
Wistar rats	125 mg Fe^{+++}/kg	46	10	6 local sarcomas	79
"	250 mg Fe^{+++}/kg	46	10	5 local sarcomas	84
"	Tyrode 0.5 ml	46	8	0	129
ST/a mice	250 mg Fe^{+++}/kg	17	39	1 local sarcoma	52 [1]
"	Tyrode 0.1 ml	17	39	0	52
DBA/2 mice	250 mg Fe^{+++}/kg	14	20	0	22
"	250+250 mg Fe^{+++}/kg	14	20	0	14
"	500 mg Fe^{+++}/kg	14	20	0	14
"	Tyrode 0.2 ml	14	20	1 local sarcoma	37
C3H mice	250 mg Fe^{+++}/kg	$\leqq$ 52	28	1 mammary carcinoma	54
"	Tyrode 0.1 ml	$\leqq$ 52	21	4 mammary carcinoma	121
AKR/a mice	250 mg Fe^{+++}/kg	$\leqq$ 52	20	14 leukaemias	40
"	Tyrode 0.1 ml	$\leqq$ 52	20	16 leukaemias	30

[1] The ST/a mice were killed after 52 weeks. All the other animals were observed until spontaneous death.

TABLE II

TUMOUR INCIDENCE AND SURVIVAL TIME OF ST/A MICE AFTER A SINGLE DOSE OF IMFERON

Treatment mg Fe^{+++}/kg	Sex	No. of animals	No. of animals with distant tumours [1]	Percentage tumour incidence	Mean survival time (weeks)
2500 mg Fe^{+++}/kg	male	9	1	11	52
1250 "	,,	10	0	0	50
1000 "	,,	6	0	0	38
750 "	,,	10	1	10	48
500 "	,,	44	4	9	40
25 "	,,	43	3	7	39
Tyrode 0.2 ml	,,	39	2	5	46
2500 mg Fe^{+++}/kg	female	8	6 (1)	88	77
1250 "	,,	10	7	70	72
1000 "	,,	10	2 (1)	30	77
750 "	,,	10	3	30	74
500 "	,,	53	24 (1)	47	89
25 "	,,	46	19	41	89
Tyrode 0.2 ml	,,	60	13	22	87

[1] Number of animals with local tumours given in parentheses.

tumours was 7.4% in all the treated males, as compared to 5.1% in the corresponding control group. This difference was not statistically significant. In the females the total tumour incidence was 44.5% (46.7% including the three local tumours), as compared to 21.7% in the corresponding control group. This difference was highly significant ($x^2=9.22$ on 1 d.f. $P<0.005$).

The figures presented in Table II suggest a dose-response relationship at a high dosage level. However, no significant difference was found between the tumour incidences in the two larger groups receiving 25 and 500 mg Fe^{+++} per kg body weight respectively ($x^2=0.34$ on 1 d.f. $P>0.50$). They may, therefore, be treated as one group.

TABLE III

TUMOURS FOUND IN ST/A MICE AFTER A SINGLE INJECTION OF IMFERON AT DOSES OF 25 AND 500 MG Fe^{+++}/KG

Total No. of animals: 285 [1]	Female		Male	
	Treated [2]	Controls	Treated [2]	Controls
Lymphatic leukaemia	9	4	0	1
Lymphocytic neoplasm of thymus	1	0	0	0
Lymphosarcoma	1	1	0	0
Reticulosarcoma	18	4	0	0
Adenoma or carcinoma of the lung	2	1	2	0
Adenocarcinoma of the colon	1	0	0	0
Gastric adenocarcinoma	2	0	0	0
Anaplastic carcinoma of pancreas	0	0	1	0
Hepatoma	1	0	2	1
Haemangioendothelioma of the liver	1	0	0	0
Haemangioendothelioma in the interscapular region	0	0	1	0
Cavernous haemangioma of the liver	0	0	1	0
Spindle cell sarcoma at site of injection	1	0	0	0
Spindle cell sarcoma, subcutaneous, non-local	1	1	0	0
Spindle cell sarcoma retroperitoneally	1	0	0	0
Pleomorphic sarcoma retroperitoneally	2	0	0	0
Pleomorphic sarcomas elsewhere	3	1	0	0
Osteoclastoma of the thoracic wall	0	1	0	0

[1] A total of 45 animals, which died before the 20th week, has been excluded. No tumours were found in these animals.
[2] Dosage 25 and 500 mg.

TABLE IV

INCIDENCE AND LATENCY OF LYMPHORETICULAR AND NON-LYMPHORETICULAR NEOPLASMS IN ST/A MICE AFTER A SINGLE INJECTION OF IMFERON AT A DOSE OF 25 AND 500 MG Fe^{+++}/Kg.

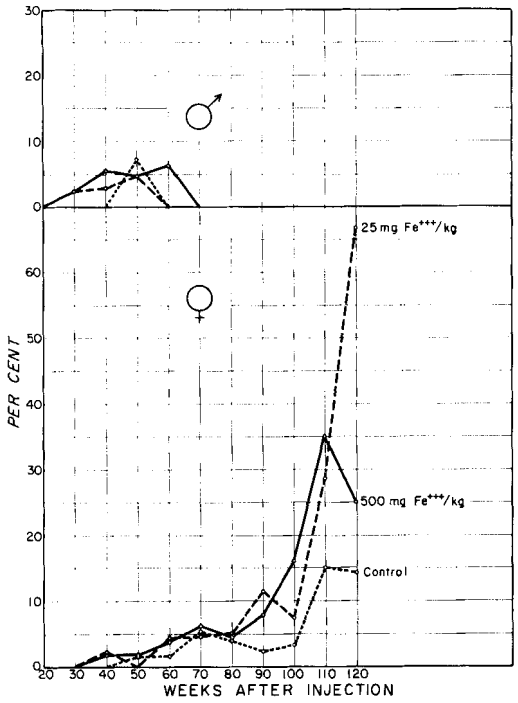
	Sex	Total no. of animals	Lymphoreticular neoplasms				Non-lymphoreticular neoplasms			
			No. of animals with neoplasia	Tumour incidence (%)	Mean latency (weeks)	Latency as percentage of control life-span [1]	No. of animals with neoplasia	Tumour incidence (%)	Mean latency (weeks)	Latency as percentage of control life-span [1]
Imferon	Female	99	29	29.3	75.3	82	15	15.1	95.4	104
,,	Male	87	0	0			7	8.0	37.6	79
Control	Female	60	9	15.0	75.8	82	4	6.7	76.5	83
,,	Male	39	1	2.5	40.0	96	1	2.5	43.5	91

[1] Control life-span calculated as mean survival time of tumour-negative control animals from start of experiment.

While the tumour incidence of the treated males of this combined group of animals receiving 25 or 500 mg Fe^{+++} did not show any significant increase, the tumour incidence of the treated females was about twice as high as that of the controls. This increase was statistically significant ($x^2=8.78$ on 2 d.f., $P<0.005$).

Table III shows the different types of tumours which were observed in ST/a mice receiving 25 or 500 mg Fe^{+++}/kg body weight and in the controls. In the females, treated as well as controls, more than half of the tumours were of a lymphoreticular type while only one such tumour was found among the males in an untreated animal. Only one local tumour, a spindle-cell sarcoma, was found.

In Table IV the tumours have been grouped as lymphoreticular neoplasms (leukaemias, reticulosarcomas, lymphocytic neoplasms) and non-lymphoreticular tumours, the latter group comprising all other neoplasms listed in Table III. The table shows that the incidence, but not the



latency period, of the lymphoreticular neoplasia in female mice was influenced by Imferon treatment. The increase of these tumours was statistically significant ($x^2=4.14$ on 1 d.f., $P<0.05$). No tumours of this group were seen in treated male animals.

Table IV also shows an increased incidence of non-lymphoreticular tumours in both female and male mice after a single dose of Imferon, but this increase was not statistically significant.

The figures in Table IV indicate that the non-lymphoreticular neoplasms in treated female mice developed after a longer latency period than in the control mice. Figure 1 shows the incidence of tumours within successive 10-week periods after injection expressed as a percentage of surviving animals. It may be seen that, up to the 80th week after the administration of Imferon, the tumour

◄ FIGURE 1

Tumour incidence within successive 10-week periods calculated as percentage of surviving ST/a mice after a single subcutaneous injection of Imferon at a dose of 25 and 500 mg Fe+++ per kg body weight.

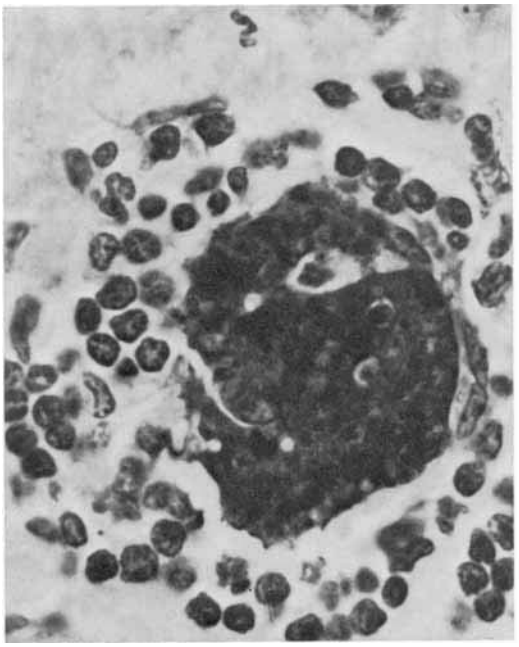


FIGURE 2

Iron accumulation in the liver with associated lymphocyte infiltrate in an ST/a mouse 86 weeks after a single subcutaneous injection of 1,000 mg Fe+++ per kg body weight. Perl's method for iron and haematoxylin and eosin. ×1,000.

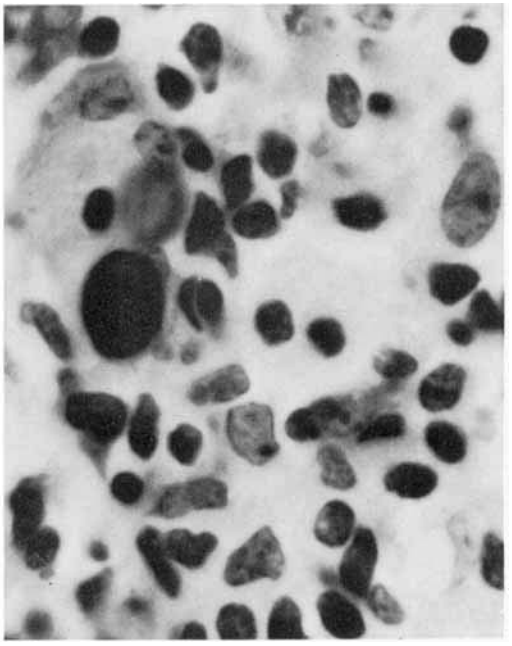


FIGURE 3

Mixed lymphocyte-reticulum cell infiltrate in the liver of an ST/a mouse 82 weeks after a single subcutaneous injection of 1,000 mg Fe+++ per kg body weight. Note globular iron deposit. Perl's method for iron and haematoxylin and eosin. ×1,000.

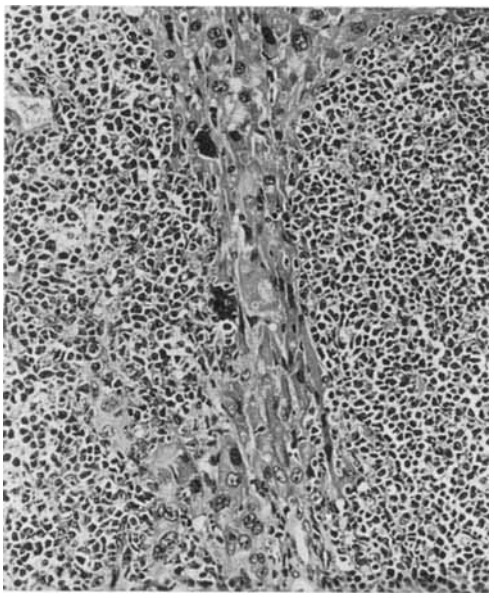


FIGURE 4

Reticulosarcoma in an ST/a mouse 72 weeks after a single subcutaneous injection of 750 mg Fe^{+++} per kg body weight. Iron accumulations are seen adjacent to a strand of remaining liver parenchyma. Perl's method for iron and haematoxylin and eosin. ×160.

incidence in the treated animals did not differ much from that of the controls. However, after the 80th week the risk of tumour development appeared considerably higher in the treated females. This increase is explained by the late appearance of non-lymphoreticular tumours, which after the 80th week occurred at a significantly higher rate in the treated females than in the controls ($x^2=4.23$ on 1 d.f., $P<0.05$).

Histological examination of animals succumbing shortly after the administration of iron-dextran at the dose-level 500 mg Fe^{+++}/kg body weight revealed iron deposits in the perivascular reticuloendothelial structures, especially in liver, lungs, and spleen. However, in animals examined more than 20 weeks after the injection of Imferon such iron deposits were rarely seen. At dose level 25 mg Fe^{+++}/kg no iron deposits could be found on light microscopy. At later stages of the experimental period foci of lymphocyte infiltrates were seen in the perivascular structures, especially in the lungs and in the periportal spaces of the liver in both experimental groups. As the animals grew older, mixed infiltrates dominated by reticulum cells were the common findings. These infiltrates attained considerable size, finally invading and

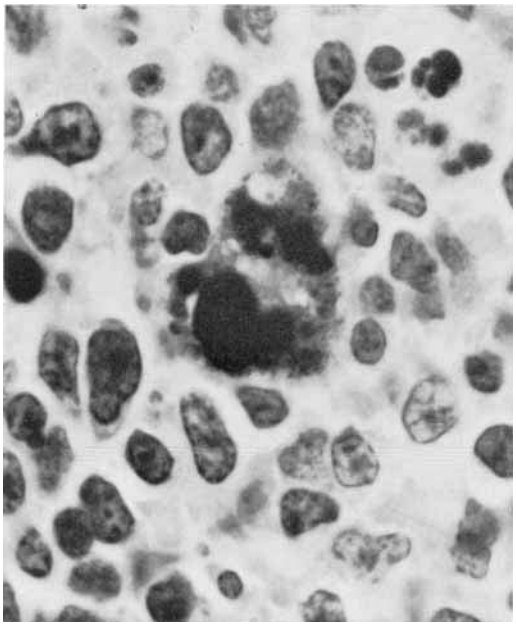


FIGURE 5

Reticulosarcoma shown in Figure 4. Perl's method for iron and haematoxylin and eosin. ×1,000.

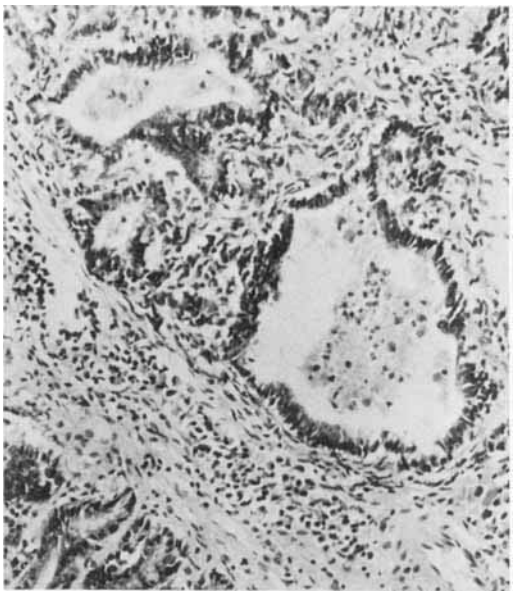


FIGURE 6

Adenocarcinoma of the colon in an ST/a mouse 31 weeks after a single subcutaneous injection of 500 mg Fe^{+++} per kg body weight. Perl's method for iron and haematoxylin and eosin. ×160.

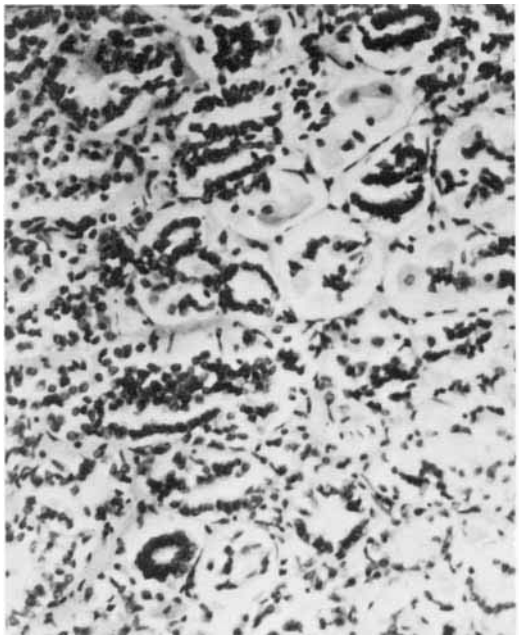


FIGURE 7

Gastric adenocarcinoma in an ST/a mouse 100 weeks after a single subcutaneous injection of 500 mg Fe^{+++} per kg body weight. Haematoxylin and eosin. ×160.

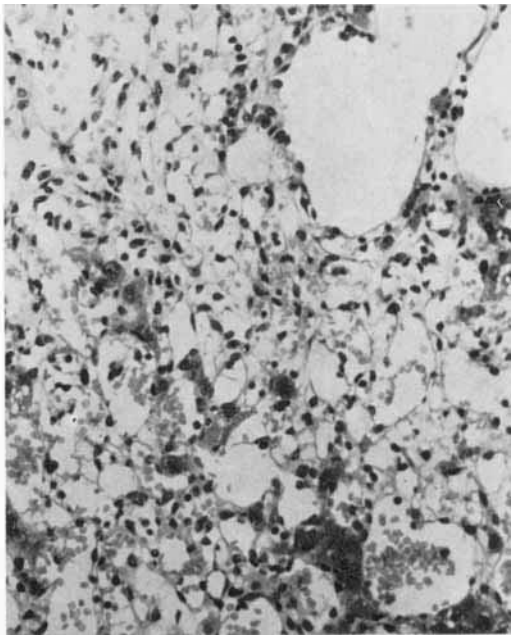


FIGURE 8

Haemangioendothelioma of the liver in an ST/a mouse 90 weeks after a single subcutaneous injection of 2,500 mg Fe^{+++} per kg body weight. Perl's method for iron and haematoxylin and eosin. ×160.

replacing the surrounding tissue. The gradual transition of the infiltrates from lymphocytic into reticulum cell infiltrates and further into frank reticulosarcomas was similar to that previously described (Langvad, 1966) in relation to persistent iron deposits following much higher doses of iron-dextran (Fig. 2-5). Some of the non-lymphoreticular lesions are illustrated in Figures 6-8.

After intravenous administration of colloidal iron-carbohydrate complex (Intrafer), iron deposits were found at locations similar to those seen in the iron-dextran treated animals. However, the characteristic cellular reactions were not observed in Intrafer treated animals.

DISCUSSION

The present work confirms earlier observations that iron-dextran may exert a systemic as well as a local carcinogenic action. Against this background it seems reasonable to reconsider the question of whether the total dose rather than the local dose plays a decisive role. The reactions observed in animals given high doses of Imferon suggest a relationship between total dose and response. However, no statistically significant differences were found between the groups receiving 25 and 500 mg Fe^{+++}/kg body weight, and it seems doubtful to begin with whether there is such a thing as a " safe " iron-dextran threshold dose. The iron-dextran complex seems to be treated as particulate matter by the organism and consequently is deposited and concentrated in the reticuloendothelial system (RES). The high molecular weight carrier leads to a non-physiological differential uptake of iron-dextran by cells which have no specific function in iron metabolism. Thus even relatively small doses may lead to a transient overloading of individual RES cells.

High iron-dextran doses result in systemic accumulations of histologically demonstrable iron. This may be followed by the appearance of multifocal cellular reactions frequently developing stepwise into reticulo-sarcomas or by other neoplasia arising close to the iron deposits. At small doses, where no iron deposits are found by light microscopy, the histological events are similar and the final outcome identical. This might indicate that

even the transient presence within the cells of small amounts of the high molecular compound may introduce a stage of latent neoplasia. Some dextrans have been shown to be carcinogenic, inducing leukaemias and reticulosarcomas in rats and mice (Hueper, 1959). The tumours arose in organs where the macromolecular substance was retained for prolonged periods of time. Reactive cellular proliferations were seen and interpreted as a preparatory state for the development of morphological malignancy.

Several experiments aimed at elucidating the local tumorigenic effect of iron-dextran have failed to establish the systemic carcinogenicity. The fact that many animals died as a result of treatment or were killed when local tumours developed may have prevented most of the experimental animals from surviving the induction period of distant tumours. This feature is clearly seen in the results obtained with C3H mice (Table I). The mean survival time of the treated animals was 54 weeks, while the controls had a mean survival of 121 weeks. The mean latency of the mammary tumours found in the control group was 93 weeks. Similarly, the absence of lymphoreticular tumours in male animals may to a large extent be explained by the short mean life expectancy of male animals (48 weeks) as compared to the 75-week mean induction period of this group of malignancies.

The non-lymphoreticular tumours appeared at a relatively early stage in the males, but no significant differences could be demonstrated between treated and untreated animals. In the females the first non-lymphoreticular tumour was found 31 weeks after the injection of iron-dextran. However, a significant increase of tumours of this type was not observed until after the 80th week.

The concentration of iron in the RES might be essential for the systemic carcinogenic effect of Imferon. The morphological events following iron-dextran accumulation in the reticuloendothelial cells in various organs support this view. Similar changes were not observed in relation to Intrafer accumulation in the RES cells. While the known local tumorigenic properties of heavy metals probably depend on a direct action of the metal ions, differences observed in the present work between the biological effect of Imferon and Intrafer indicate that the presence of the metal as a complex is of major importance for the systemic carcinogenic effect.

Imferon treatment increased the incidence of lymphoreticular tumours without influencing the induction period. This may indicate that the aetiology of these tumours is the same in treated and untreated animals and that Imferon is functioning as a co-carcinogenic factor. Since the murine lymphoreticular neoplasms may very well be of viral origin the effect of Imferon might be explained by a decrease of immunological resistance to an oncogenic virus. Previous studies (Langvad, 1966) of the systemic iron carcinogenesis mechanism revealed that Intrafer accumulation in the RES system does not interfere with the treated animals' ability to produce antibodies against polyoma virus. Similar studies of Imferon have yet to be made.

So far as the non-lymphoreticular neoplasms are concerned, the prolonged induction period may indicate a direct carcinogenic effect of iron-dextran rather than an increased susceptibility to carcinogenic factors also present in the untreated control animals.

The induction mechanism of the local and distant non-lymphoreticular neoplasia may be assumed to be the same with both iron-dextran and iron-carbohydrate complex. Thus, in the Intrafer experiment, a number of non-lymphoreticular neoplasia were to be expected. Two local and two distant tumours of this type were seen in the test animals. Although the total number of tumours in the treated animals was not significantly increased, the incidence of non-lymphoreticular tumours was significantly higher in the treated group than in the control group ($x^2=7.30$ on 1 d.f., $P<0.005$).

Although the present observations seem to support the assumption that Imferon exerts both a direct and an indirect carcinogenic action, further evidence is obviously needed before this hypothesis can be fully evaluated. However, it seems beyond doubt that, in addition to its previously described local tumorigenic effect, Imferon may also cause distant tumours in mice and rats at total doses well below those accepted in human therapy.

ACKNOWLEDGEMENTS

This work has been supported by grants from the Danish National Research Foundation and the Daell Foundation.

INDUCTION DE TUMEURS A DISTANCE PAR LE FER-DEXTRANE CHEZ LA SOURIS

On a étudié la carcinogénicité d'action générale du fer-dextrane après des injections uniques ou répétées d'Imferon à des souris et des rats. Les dépôts de fer dans l'organisme, provoqués par le fer-dextrane, ont entraîné par degrés la formation de réticulosarcomes. En revanche, cette réaction tissulaire n'a pas été observée avec des dépôts de fer consécutifs à l'administration d'un complexe fer-glucide (Intrafer). Les tumeurs apparaissant à distance dans les structures lymphoréticulaires ont présenté une latence uniforme chez les souris d'épreuve et les témoins, tandis que les tumeurs d'une autre origine présentaient une latence prolongée par rapport aux mêmes tumeurs chez les animaux témoins. Il y a donc lieu de penser que le fer-dextrane pourrait avoir un rôle co-carcinogène dans les cas des tumeurs lymphoréticulaires, augmentant la réceptivité de l'hôte au virus oncogène que l'on suppose présent tant chez les animaux témoins que chez les animaux d'épreuve. Il est possible que les tumeurs à distance ou locales non lymphoréticulaires proviennent d'une action carcinogène différente et directe du fer, puisque ces tumeurs ont présenté une latence prolongée et ont été observées après administration soit de fer-dextrane soit du complexe fer-glucide.

Les résultats indiquent une relation dose-réponse non concluante, mais on n'a pas trouvé de différence significative de rendement tumoral entre les groupes nombreux qui ont reçu respectivement 500 et 25 mg de Fe^{+++} par kg de poids corporel, cette dernière dose se situant à l'intérieur des limites thérapeutiques, compte tenu des poids relatifs.

REFERENCES

BAKER, S. P. DE C., GOLBERG, L., MARTIN, L. E., and SMITH, J. P., Tissue changes following injection of iron-dextran complex. *J. Path. Bact.*, **82**, 453-470 (1961).

GOLBERG, L. E., and SMITH, J. P., Iron overloading phenomena in animals. *Toxicol. appl. Pharmacol.*, **2**, 683-707 (1960).

HADDOW, A., and HORNING, E. S., On the carcinogenicity of an iron-dextran complex. *J. nat. Cancer Inst.*, **24**, 109-147 (1960).

HADDOW, A., Advances in knowledge of the carcinogenic process, 1958-1962. *Acta Un. int. Cancr.*, **19**, 453-457 (1963).

HADDOW, A., and ROE, F. J. C., Iron dextran and sarcomata. *Brit. med. J.*, ii, 119-121 (1964).

HADDOW, A., ROE, F. J. C., and MITCHLEY, B. V. C., Induction of sarcomata in rabbits by intramuscular injection of iron-dextran (" Imferon "). *Brit. med J.*, i, 1593-1594 (1964).

HUEPER, W. C., Carcinogenic studies on water-soluble and insoluble macro-molecules. *Arch Path.*, **67**, 889-617 (1959).

LANGVAD, E., " Imferon ", carcinogen or co-carcinogen? Internal factors determining the course of oncogenic virus infection? *In* H. Bergstrand and K. E. Hellström (*ed.*), *Symposium on virus and cancer*. Unio Nordica Contra Cancrum, p. 109, Tryckeri Balder AB, Stockholm (1965).

LANGVAD, E., Imferon induction of lung tumours in mice, *In* L. Severi (*ed.*), *Lung tumours in animals, Proceedings of the Third Quadrennial Conference on Cancer*, University of Perugia, 897-904 (1966).

LUNDIN, P. M., The carcinogenic action of complex iron preparations. *Brit. J. Cancer*, **15**, 838-847 (1961).

RICHMOND, H. C., Induction of sarcoma in the rat by iron-dextran complex. *Brit. med. J.*, i, 947-949 (1959).

RICHMOND, H. G., The carcinogenicity of an iron-dextran complex. *In* R. W. Raven (*ed.*), *Cancer progress*, p. 24-33. Butterworth Medical Publications, London (1960).

ROE, F. J. C., HADDOW, A., DUKES, C. E., and MITCHLEY, B. C. V., Iron-dextran carcinogenesis in rats: Effects of distributing injected material between one, two, four or six sites. *Brit. J. Cancer*, **18**, 801-808 (1964).

ROE, F. J. C., and CARTER, R. L., Iron-dextran carcinogenesis in rats: Influence of dose on the number and types of neoplasms induced. *Int. J. Cancer*, **2**, 370-380 (1967).