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IRON-DEXTRAN CARCINOGENESIS IN RATS: INFLUENCE OF DOSE ON THE NUMBER AND TYPES OF NEOPLASM INDUCED

by

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Groups of 16, 32, 64 and 128 male CB Wistar rats were given, respectively, 16, 8, 4 and 2 subcutaneous injections of 0.75 ml iron-dextran (" Imferon ") at weekly intervals: 64 untreated rats served as controls. Injection site tumours arose in 8, 11, 7 and 8 rats, respectively, in the four groups treated with iron-dextran. The average time of tumour induction was similar in the four test groups but the grade of malignancy of tumours was directly related to the total dose of iron-dextran administered. The wider implications of this latter observation are briefly discussed. The incidence of tumours at locations distant from the injection site was higher in the 128 rats which received only two injections of iron-dextran than in the 64 untreated controls. However, the risk of dying with a distant tumour was not statistically higher in the combined *test groups than in the control group. Several of the distant neoplasms in the iron-dextran treated animals were of unusual types but their relationship to treatment with iron-dextran remains uncertain.*

The carcinogenicity of iron-dextran in laboratory animals, particularly mice and rats, is now undisputed. In dose schedules smaller than those used clinically (but much larger in relation to body weight) iron-dextran produces a high incidence of local sarcomas after repeated subcutaneous injection (Richmond, 1959, 1960; Haddow and Horning, 1960; Golberg *et al.*, 1960; Lundin, 1961; Fielding, 1962; Kunz *et al.*, 1963; Haddow and Roe, 1964; Roe *et al.*, 1964).

Many aspects of this process are still obscure, however, and two of these are the subject of the present investigation. *First*, although most workers have established a broad dose-response relationship, Golberg *et al.* (1960) claimed that low doses showed little or no carcinogenic activity and suggested that there might be a demonstrable threshold dose below which tumours were not induced. The same group of workers stressed that systemic iron-overloading tended to delay removal of iron from the injection site and that this was an essential feature predisposing to tumour formation (Baker *et al.*, 1961). *Secondly*, it is still uncertain whether the administration of iron-dextran increases the incidence of tumours at locations other than the sites of injection. Some studies have led to the conclusion that the incidence of such tumours is higher than in untreated controls (Langvad, 1965); others that the spectrum of tumours is wider and that neoplasms of distinctly unusual types are prone to occur in animals receiving iron-dextran (Haddow and Horning, 1960; Haddow, 1963). The former conclusion has not been confirmed in subsequent investigations whilst the latter needs further corroboration (Lundin, 1961; Roe *et al.*, 1964). Roe and Lancaster (1964) stressed the importance of

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including large numbers of untreated controls so that the incidence and distribution of both common and rare spontaneous neoplasms would be sufficiently well established for this purpose.

MATERIAL AND METHODS

Rats

Random-bred male rats of the Chester Beatty stock Wistar strain, 6-7 weeks old and weighing approximately 200 g, were used. The animals were housed in metal cages, 8 rats in each, and fed on cubed diet No. 86 (Dixon & Sons, Ware, Herts., England), details of which have been given in a previous paper (Roe *et al.*, 1964); water was supplied *ad libitum*.

Iron preparations

Iron-dextran (" Imferon "; Batch No. 246/5) was supplied by Bengers Ltd. (now Fisons Pharmaceuticals Ltd., Holmes Chapel, Cheshire, England); this preparation contains 50 mg iron ml.

Experimental details

Three hundred and four rats were divided at random into five groups. Four of these received weekly doses of iron-dextran, 0.75 ml being injected subcutaneously into the flank. The fifth group received no treatment. The numbers of animals in each group, the different courses of injections, and the total amounts of iron administered are shown in Table I. All rats were examined regularly and killed if they developed tumours at the injection site or elsewhere, or became obviously sick. They were killed with ether and a detailed post-mortem examination was carried out, following the scheme described by Roe (1965). Tissues were fixed in Bouin's solution and sections were cut at 5 μ and stained as follows: haematoxylin and eosin; haematoxylin and van Gieson; Heidenhain's haematoxylin; Perl's method for iron; Gordon and Sweet's silver impregnation method for reticulin; periodic acid-Schiff for glycogen and glycoproteins.

TABLE I

DETAILS OF TREATMENT

Group	No. of rats	No. of weekly injections of 0.75 ml iron-dextran	Total amount of iron administered (mg)
A	16	16	600
B	32	8	300
C	64	4	150
D	128	2	75
E	64	None	None

RESULTS

I. Development of neoplasms at injection sites

The number of tumours encountered in the four test groups (A-D) and details of their temporal distribution are given in Figure 1.

It is clear that the *incidence* of neoplasms at the injection site is directly related to the total dose of iron-dextran administered. The occurrence of tumours in animals from Group D (which received only two injections of iron-dextran) is particularly noteworthy; it provides no grounds for the view that there is a threshold dose below which there is no risk of inducing neoplasia at the injection site. But it should be stressed that the two injections of iron-dextran supplied 75 mg iron (= 375 mg/kg body weight) which, since the rats were healthy and not iron-deficient, must certainly have led to saturation of iron stores and to overloading (see Discussion).

Differences in the *time of onset* of injection site tumours in the four experimental groups were not clearly established. This is surprising but, as noted previously (Roe *et al.*, 1964), it may (in part) reflect the difficulties in palpating small tumours through thickened skin.

II. Histology of injection site tumours

The histological types of neoplasms seen at injection sites are listed in Table II.

Sarcomas

Pleomorphic sarcomas were confined to Group A, confirming earlier work in which the most malignant tumours were seen in animals receiving the highest doses of iron-dextran (Roe *et al.*, 1964). These were large, rapidly growing tumours, quickly ulcerating through the skin. Histological sections showed anaplastic lesions with considerable cellular pleomorphism (Fig. 2). Binucleate and multinucleate forms were com-

NEOPLASMS IN RATS GIVEN 16, 8, 4, 2 or 0 SUBCUTANEOUS INJECTIONS OF IRON-DEXTRAN

NO. OF RATS IN GROUP	No. of injections of Iron-Dextran (0·75mL each)	TIME OF DEATH (days)								TOTAL INJECTION SITE TUMOURS		TOTAL TUMOURS AT OTHER SITES
		<100	101 - 200	201 - 300	301 - 400	401 - 500	501 - 600	601 - 700	701 - 800	SARCOMAS	FIBROMAS	
A 16	16		○○Ⓐ	○	●○○	●●●●○ ○	●●●			8	0	1
B 32	8			●	○○	●○○○○ ○○○○○ ○○Ⓗ	●●●●● ◑◑◑◑○ ○○○○Ⓜ	○		8	3	2
C 64	4	○	○	○	○○○○○ ○○○Ⓓ	◑○○○○ ○○○○○ ○○○○○ ○○○○○ ○○Ⓔ	●●◑◑○ ○○○○○ ○○○○○ ○○Ⓕ	●●○○○ ○○Ⓖ	○○○	4	3	4
D 128	2	○○		○○Ⓘ	○○○○○ ○○○○○ ○○○○Ⓓ ⓁⓄ	●●●●◑ ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○○Ⓚ	◑○○○○ ○○○○○ ○○○○○ ○○○○○ ○○○○Ⓛ Ⓜ Ⓝ Ⓟ Ⓠ	*◑○○○○ ○○○○○ ○○○○○ ○○ⒼⒽⒾ ⒺⒺ	○○○○○ ○○○○○ ⒹⓈⓉ	5*	3*	20**
E 64	0	○○	○	○○	○○○○○ ○○○○○ ○○	○○○○○ ○○○○○ ○○○Ⓔ	○○○○○ ○○○○○ ○	○○○○○ ○○○○○ ○○○○○ ⒺⒽⒾⓊ	○○○	0	0	5

* = 1 LOCAL FIBROMA & 1 SARCOMA IN THE SAME ANIMAL; ** INCLUDES 2 DISTANT TUMOURS IN ONE ANIMAL.

○ = RATS WITHOUT TUMOURS ● = RATS WITH LOCAL SARCOMAS ◑ = RATS WITH LOCAL FIBROMAS

FIGURE 1

A = Carcinoma of the bladder
B = β-cell adenoma of pancreas
C = Parathyroid adenoma
D = Epidermoid carcinoma of skin
E = Localised lymphocytic neoplasm of lung
F = Exocrine adenoma of pancreas
G = Sarcoma of testis
H = Adenocarcinoma of colon
I = Anaplastic carcinoma of pancreas
J = Carcinoma of breast
K = Papillomatosis of bladder
L = Subcutaneous sarcoma
M = Retroperitoneal sarcoma
N = Subcutaneous fibroma
P = Neurofibroma
Q = Hepatoma
R = Thymoma
S = Localised lymphocytic neoplasm of salivary gland
T = Generalised reticulum-cell sarcoma
U = Neuroblastoma

mon and many cells were undergoing division, some of them with grossly abnormal mitotic figures. There was negligible collagen formation. A few iron-laden macrophages were scattered throughout the tumours and traces of iron pigment were occasionally seen within tumour cells.

Spindle cell sarcomas occurred in all four test groups. They were usually smaller than the pleomorphic sarcomas and ulceration of the skin was less frequently seen. Such lesions were reasonably well differentiated, many of them showing quite marked collagen formation (Fig. 3). Macrophages were present in and around the tumours and, as in the case of the pleomorphic tumours, a little iron pigment was sometimes demonstrable within tumour elements.

Early malignant changes were seen in several instances, either alone or in company with a well-developed pleomorphic or spindle cell lesion. These were small foci of abnormal fibroblasts, often associated with an area of degenerate ground substance (Fig. 4). In lesions showing more intense fibroblastic proliferation, columns of abnormal cells could be seen extending outwards and infiltrating the dense masses of macrophages which invariably accumulate at the injection site (Fig. 5).

No metastases were seen in any animals.

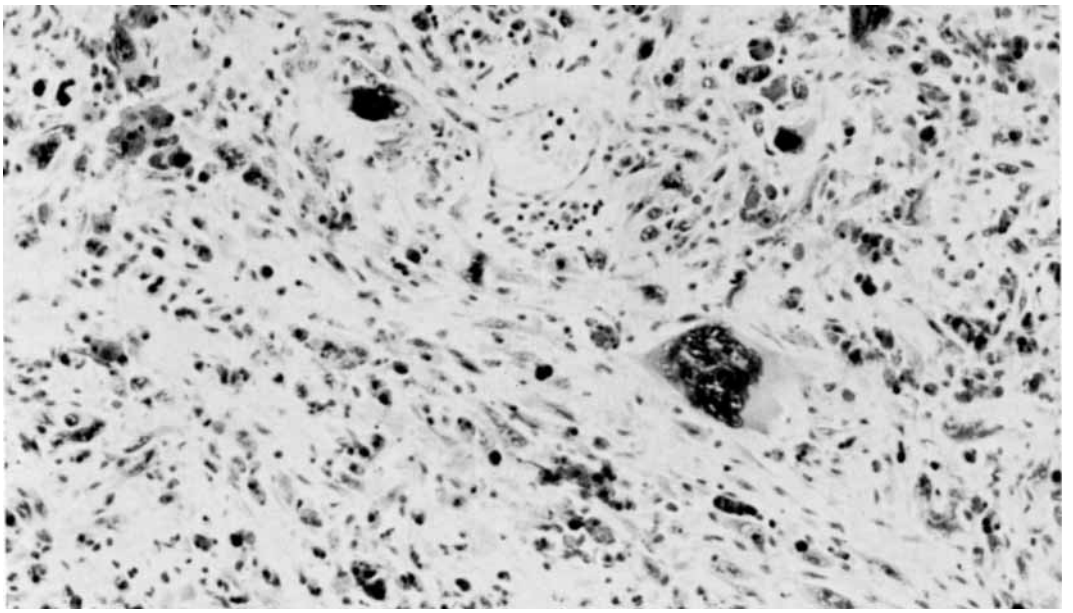


FIGURE 2

Poorly-differentiated pleomorphic sarcoma arising at the injection site in a rat after 16 weekly injections of iron-dextran (Group A). Haematoxylin and eosin. ×200.

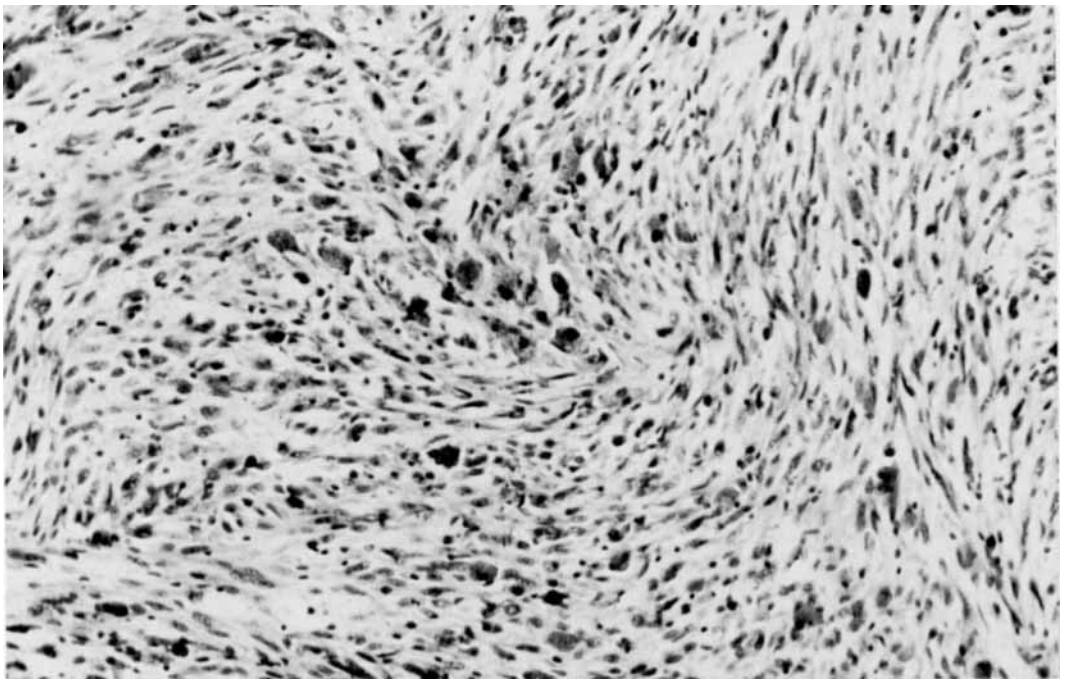


FIGURE 3

Well-differentiated spindle cell sarcoma arising at the injection site in a rat after 8 weekly injections of iron-dextran (Group B). Haematoxylin and eosin. ×200.

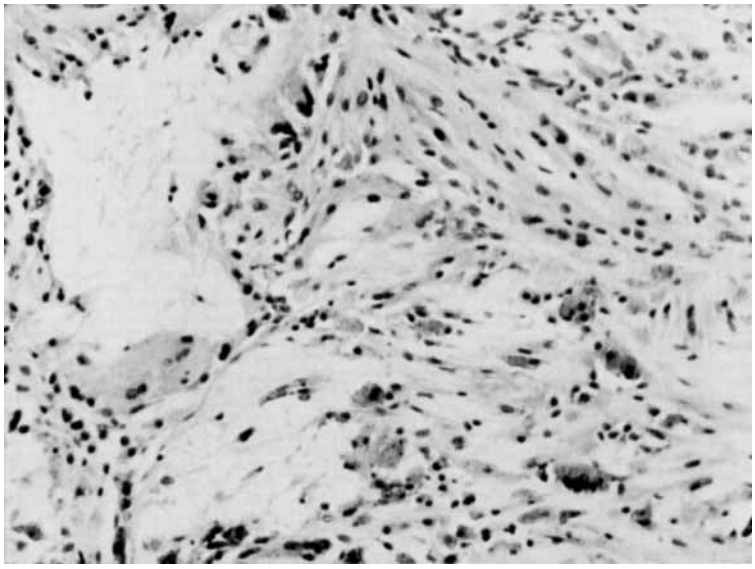


FIGURE 4

An early neoplastic focus at the injection site, showing abnormal fibroblasts surrounded by zones of ground substance. Haematoxylin and eosin. ×200.

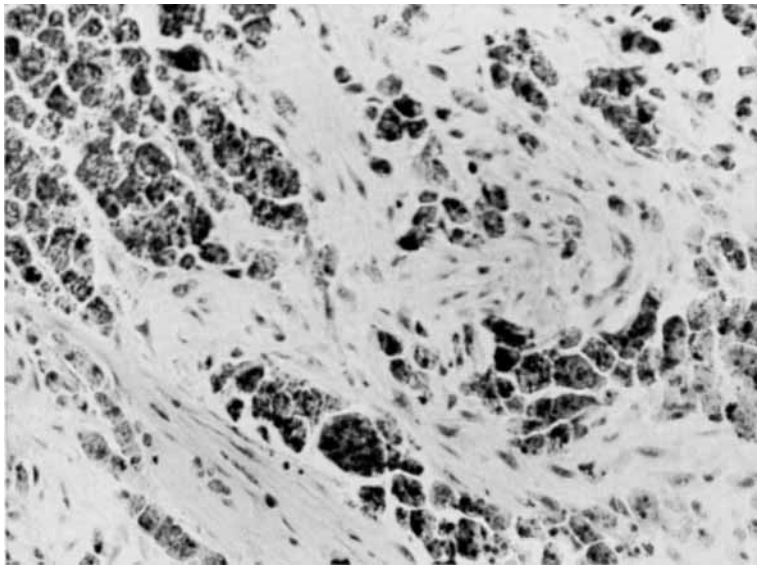


FIGURE 5

Another early lesion, demonstrating the extension of well-differentiated tumour elements amongst iron-laden macrophages. Haematoxylin and eosin. × 200.

Fibromas

These tumours were confined to rats in Groups B, C and D, which received the lower doses of iron-dextran. The lesions were well demarcated and unequivocally benign (Fig. 6). No suspicious areas, suggesting malignant transformation, were encountered and in the

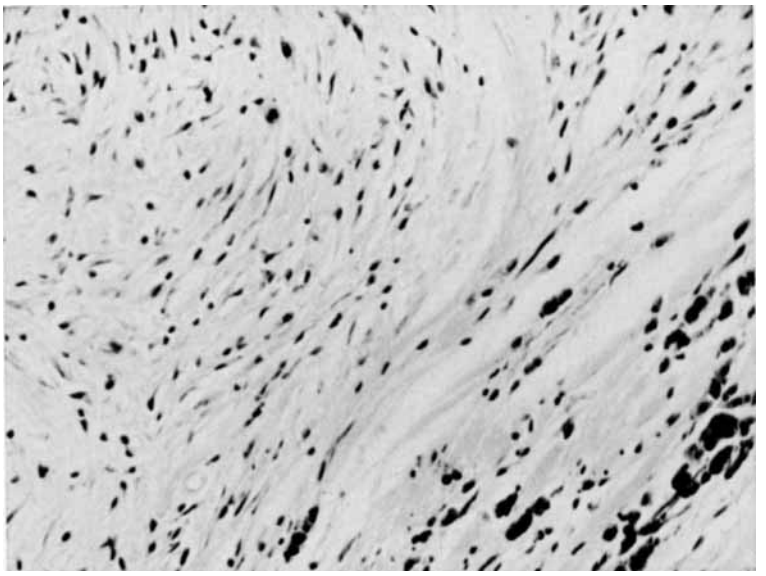


FIGURE 6

Fibroma arising at the injection site in a rat after four weekly injections of iron-dextran (Group C). Macrophages, loaded with iron, are present in one corner of this field. Haematoxylin and eosin. ×200.

one rat where a fibroma and a sarcoma arose together at the same injection site, the two tumours were entirely separate and could be distinguished with ease.

III. Distant tumours

A wide variety of distant neoplasms was encountered. These have already been included in Figure 1, but for ease of reference they are also listed in Table III; some of the lesions are illustrated in Figures 7 to 12.

Three points should be stressed from the start. A striking range of distant tumours was found, including lesions of epithelial as well as connective tissue origin. Many of these arose at sites in which "spontaneous" neoplasms are rarely encountered (see Roe, 1965). Thirdly, no distant lesions were seen in rats with injection site tumours. No significance can, however, be attached to the third of these features since the majority of animals with injection site neoplasms were killed before 600 days; most distant tumours were encountered after this time. If this difference is taken into account, only 2-3% of the animals with injection site tumours would have been expected to develop distant neoplasms; the fact that none did so is clearly without statistical significance.

At first sight, the high incidence of distant tumours in Group D, particularly when compared with that in the control animals (Group E), is impressive. However, closer analysis of the findings leads to the conclusion that they provide no real evidence that iron-dextran increased the risk of development of distant neoplasms.

TABLE II

HISTOLOGICAL TYPES OF NEOPLASMS FOUND AT INJECTION SITES

Tumours	Group A	Group B	Group C	Group D
Sarcomas: pleomorphic	3	—	—	—
spindle cell	5	8	4	5
Fibromas	—	3	3	3
Total	8	11	7	8

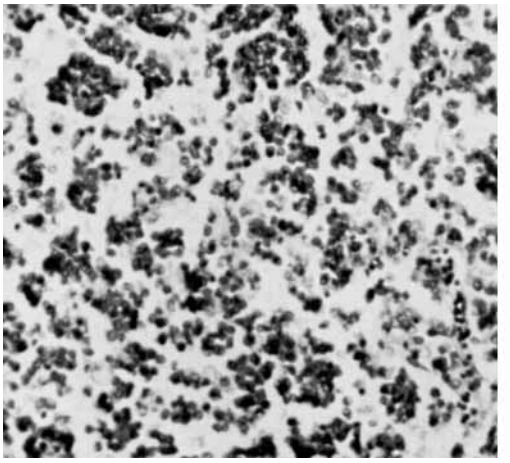
First and foremost, the results indicate no trend in risk in relation to the total dose of iron-dextran administered, although differences in survival between Groups A-D may have masked such a relationship. Secondly, it is doubtful whether it is valid to present "total numbers" of tumours when, as Table III demonstrates, they are so heterogeneous a group. Some of the tumours listed were rapidly growing and contributed to the cause of death; others were merely incidental findings.

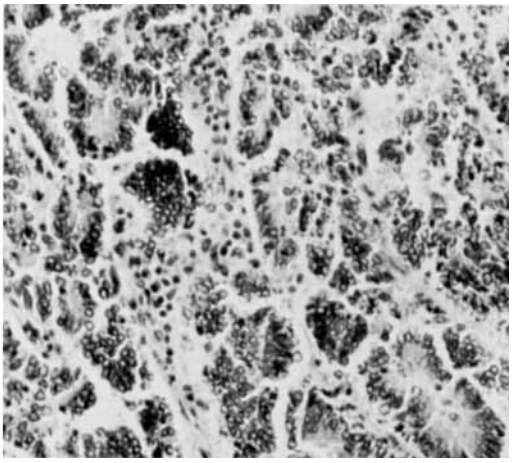
In view of the former difficulty—and despite the latter—an attempt was made to reassess the results statistically by comparing animals in the five groups with regard to their comparative risk of dying with a tumour at various times. Since a high proportion of rats in all groups succumbed between 400 and 600 days (Fig. 1), this period was taken for analysis by the X^2 test. During this time, 150 iron-dextran treated animals (Groups A-D) died; 11 rats had tumours of distant sites and 139 did not. During the same period, 25 untreated controls in Group E died and only one of these bore a tumour. The probability that rats treated with iron-dextran ran a greater risk of dying with a distant tumour failed to reach the customary 5% level of significance ($X^2 = 0.27$ on 1 d.f., $P > 0.5$). Similar calculations for the periods 0-400 days and 600-800 days also failed to reveal any significant

TABLE III

SUMMARY OF DISTANT TUMOURS

Group A	Group B	Group C	Group D	Group E
Transitional cell carcinoma of bladder	β-cell adenoma of pancreas Adenomata of parathyroid	Squamous carcinoma of skin with lymph-node metastases Exocrine adenoma of pancreas Sarcoma of testis Reticulum cell sarcoma of lung	Squamous carcinoma of skin Anaplastic carcinoma of pancreas Adenocarcinoma of colon Carcinoma of breast (2) Papillomatosis of bladder Spindle cell sarcoma of flank Retroperitoneal sarcoma (2) Fibroma of abdominal wall Neurofibroma of diaphragm Hepatoma (3) Thymoma Lymphocytic neoplasm of lung (3) Lymphocytic neoplasm of salivary gland Reticulum cell sarcoma	Spindle cell sarcoma of flank (2) Localized lymphocytic neoplasm of lung (2) Neuroblastoma
Total No. of distant tumours	Total No. of distant tumours	Total No. of distant tumours	Total No. of distant tumours	Total No. of distant tumours
1	2	4	20	5
% of rats in group with distant tumours	% of rats in group with distant tumours	% of rats in group with distant tumours	% of rats in group with distant tumours	% of rats in group with distant tumours
6.3	6.3	6.3	14.1	7.8





FIGURES 7-12

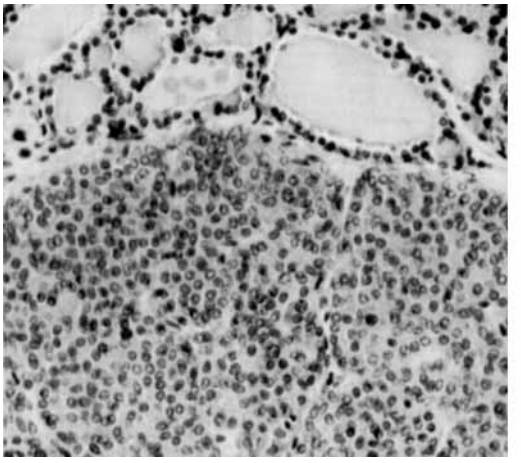
Distant tumours arising in rats treated with iron-dextran:

FIGURE 7

Anaplastic carcinoma of pancreas (Rat, Group D). Haematoxylin and eosin. ×200.

FIGURE 8

Adenocarcinoma of colon (Rat, Group D). Haematoxylin and eosin. ×200.



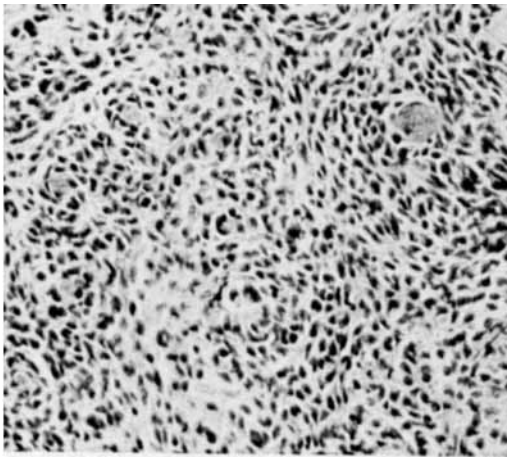


FIGURE 9

Adenoma of parathyroid (Rat, Group B). Haematoxylin and eosin. ×200.

FIGURE 10

Sarcoma of testis (Rat, Group C). Haematoxylin and eosin. ×200.

difference between treated and control animals ($X^2 = 2.57$ on 1 d.f., $P > 0.1$; and $X^2 = 0.018$ $P = 1.0$; respectively).

Despite the fact that a relatively large number of untreated rats were observed in the present experiment, insufficient information about the occurrence of unusual tumours emerged for any statistical evaluation of the results in respect of any individual type of tumour. We can, therefore, do no more than stress the wide array of distant tumours seen in Groups A-D and admit that the proportion of them which can be attrib-

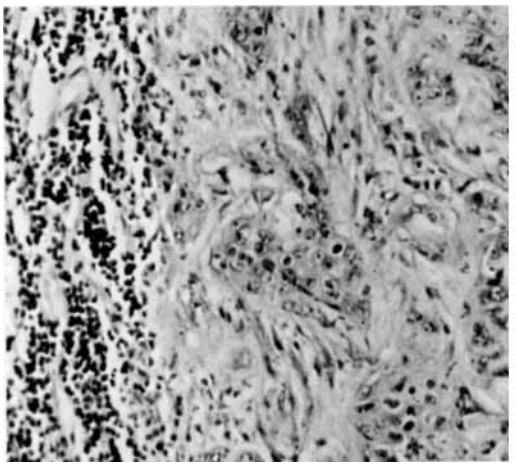


FIGURE 11

Lymph-node metastasis from epidermoid carcinoma of skin. (Rat, Group C). Haematoxylin and eosin. ×200.

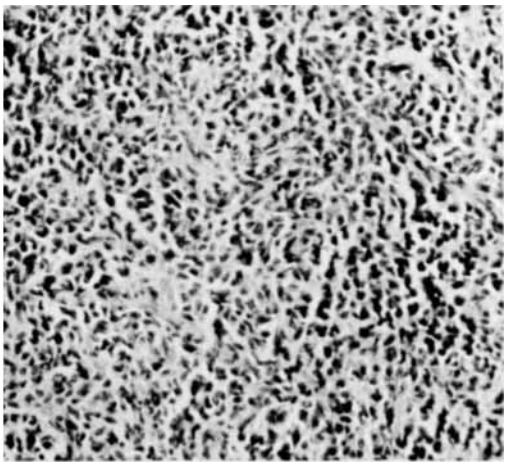


FIGURE 12

Neuroblastoma of adrenal medulla in untreated rat (Group E). Haematoxylin and eosin. ×200.

uted to treatment with iron-dextran cannot, as yet, be determined.

DISCUSSION

The present work confirms once again that iron-dextran is carcinogenic in the rat and indicates that as few as two subcutaneous injections of this material may lead to local tumour formation. This finding is certainly disturbing if carcinogenesis by iron-dextran is indeed a purely local phenomenon (Haddow, 1963; Roe, 1961, 1966). But, as mentioned earlier, two injections of 0.75 ml of iron-dextran are equivalent to a dose of 375 mg iron/kg body weight, so that iron-overloading must have been produced. Whether local tumours would have developed in Group D without this complicating factor remains an open question, and emphasizes the importance of studying animals whose iron stores have been depleted.

The local tumours induced by iron-dextran in the present series were similar to those described in previous reports and merit no special comment. The findings in the high dose group (Group A) are, however, of special interest: the most malignant pleomorphic lesions were confined to these animals (Roe *et al.*, 1964) and, conversely, no fibromas were seen in them. The distinction between fibromas and sarcomas was straightforward in material from all four test groups and no equivocal tumours were encountered. The early lesions have been less thoroughly documented (Baker *et al.*, 1961; Fielding, 1962) and their occurrence with other, well-developed neoplasms provides further support for the view that the injection site tumours are frequently multifocal (Roe *et al.*, 1964). These early lesions are somewhat reminiscent of the " premalignant fibroblastic nodules " produced in rats by subcutaneous implants of polycyclic hydrocarbons (Vasilief, 1959) although we regard the changes produced by iron-dextran as definitely malignant. Metastases from injection site sarcomas have been recorded in other accounts (Haddow and Horning, 1960; Lundin, 1961), but none were encountered in the present series.

Some of the problems which arose in the assessment of the present results with respect to the occurrence of distant tumours in rats treated with iron-dextran have already been stressed. In particular, the numbers and distribution of distant tumours in test animals showed no correlation with the amount of iron-dextran administered. Furthermore, comparison with normal rats was particularly difficult since the incidence of spontaneous tumours in the latter was unexpectedly low—smaller, for example, than in the normal rats used as controls in pre-

vious experiments reported from this laboratory (Roe *et al.*, 1964). The control animals were, as usual, carefully matched with respect to strain, age, and sex, and reasons for their low yield of tumours in the present work are obscure. Despite the lack of statistical evidence, the remarkable range of types of distant neoplasms encountered in the test groups reinforces earlier suspicions that iron-dextran may predispose to the development of a variety of unusual distant tumours (Haddow and Horning, 1960; Haddow, 1963). But until many more data are available from very large numbers of untreated rats of the same strain, it is virtually impossible to decide how many of the distant tumours seen in the test groups are really attributable to treatment with iron-dextran.

In conclusion, the most interesting features of the present study are the absence of any evidence of a threshold dose for the induction of local sarcomas, and the clear demonstration that the dose of iron-dextran influences not only the incidence but also the type of tumour which arises at the injection site. This observation, which confirms that of Roe *et al.* (1964), has wide implications both in relation to studies of the mechanisms of carcinogenesis and to the use of the subcutaneous route for screening chemical agents for carcinogenicity (Grasso and Golberg, 1966).

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CARCINOGÉNÈSE INDUITE PAR LE FER-DEXTRANE (IMFÉRON) CHEZ DES RATS: INFLUENCE DE LA DOSE SUR LE NOMBRE ET LA NATURE DES TUMEURS PROVOQUÉES

*Des groupes de 16, 32, 64 et 128 rats Wistar CB mâles ont reçu respectivement 16 8, 4 et 2 injections sous-cutanées de 0,75 ml de fer-dextrane (Imféron) une fois par semaine; le groupe témoin se composait de 64 rats non traités. Des tumeurs sont apparues au point d'injection chez 8, 11, 7 et 8 rats respectivement dans les quatre groupes traités. La durée moyenne d'induction de la tumeur était à peu près la même dans les quatre groupes traités mais le degré de malignité des tumeurs était directement lié à la dose totale administrée. Les auteurs examinent brièvement les conséquences générales qu'on peut tirer de cette dernière observation. L'incidence des tumeurs à des localisations distantes du point d'injection était plus grande chez les 128 rats qui n'avaient reçu que deux injections que chez les 64 témoins. Toutefois, le risque de mourir d'une tumeur à distance n'était pas statistiquement plus élevé dans l'*ensemble *des groupes traités que dans le groupe témoin. Chez les animaux traités, plusieurs des tumeurs à distance étaient d'un type inhabituel, mais on ne sait pas au juste quelle relation il y a entre ces tumeurs et le traitement.*

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