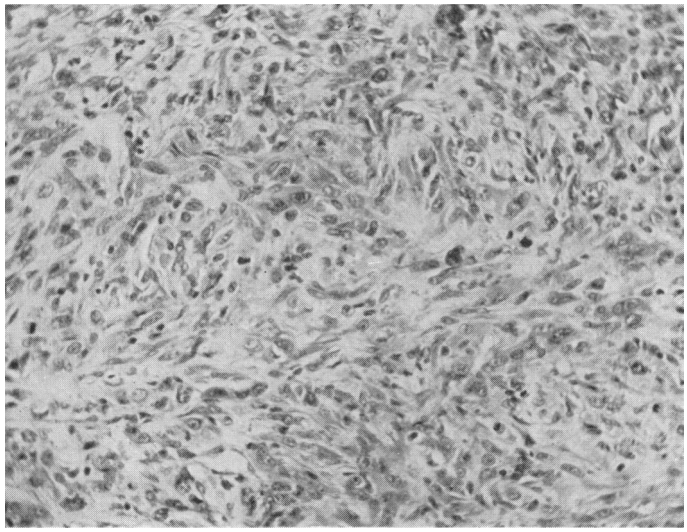

FIG. 1

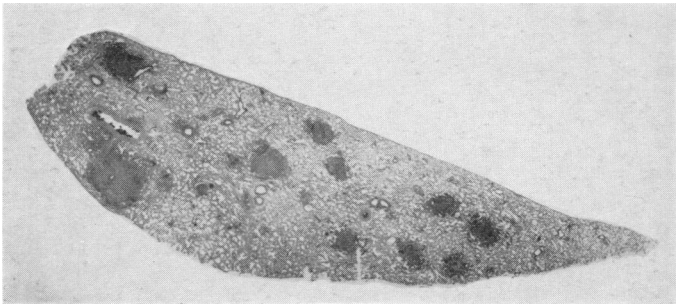

FIG. 2

FIG. 1.—Pleomorphic sarcoma at site of intramuscular injection of imferon in Rabbit No. 4. Mitotic figures are fairly numerous. (H. and E. ×180.)

FIG. 2.—Multiple metastatic deposits in lung from imferon-induced tumour in Rabbit No. 6. (H. and E. ×2.6.)

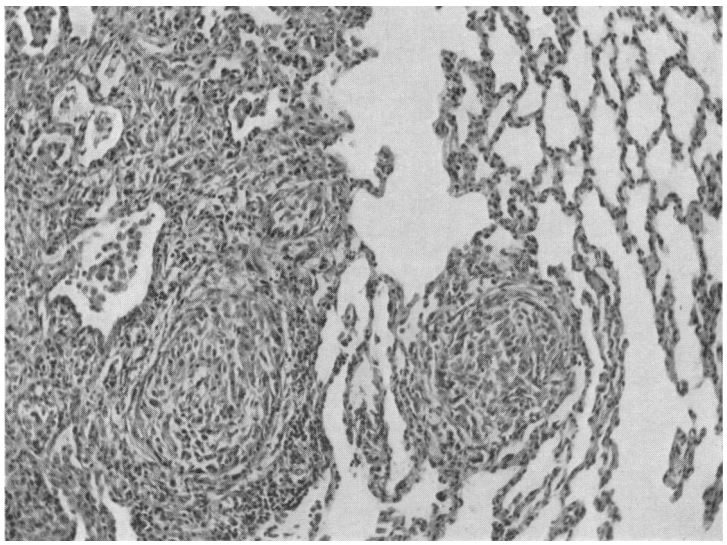

FIG. 3

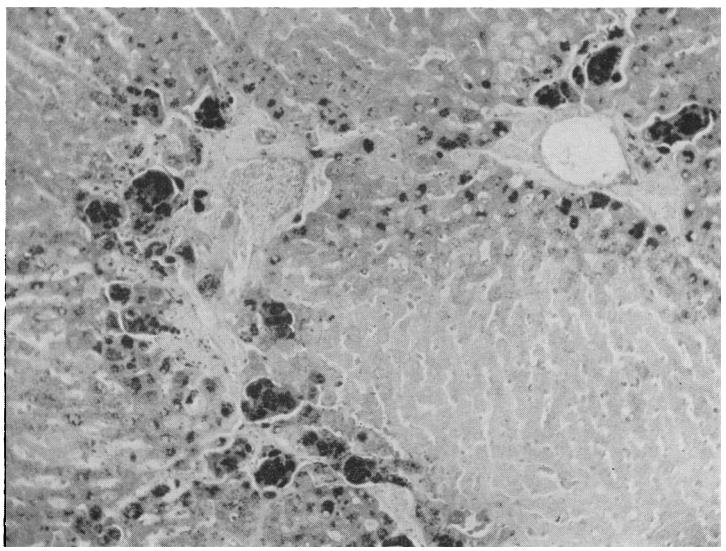

FIG. 4

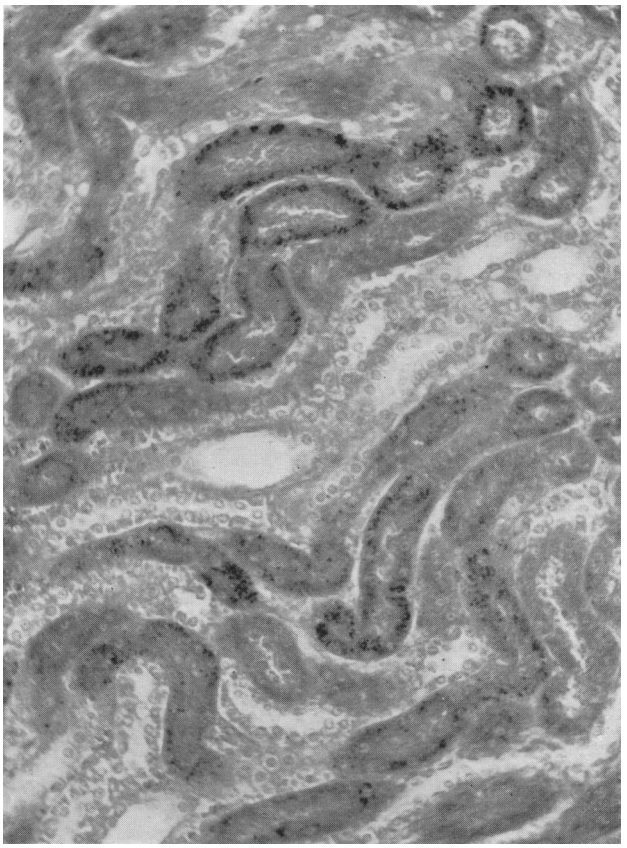

FIG. 5

FIG. 3.—Edge of metastatic deposit in lung from Rabbit No. 6. Note the occlusion of two small blood-vessels by tumour tissue. (H. and E. ×105.)

FIG. 4.—Periportal distribution of haemosiderin in liver of Rabbit No. 4. (Perl's reaction. ×105.)

FIG. 5.—Haemosiderin in tubular epithelium from juxtamedullary region of kidney in Rabbit No. 4. (Perl's reaction. ×220.)

Induction of Sarcomata in Rabbits by Intramuscular Injection of Iron-dextran ("Imferon")

A. HADDOW,* M.D., D.SC., PH.D., F.R.S.; F. J. C. ROE,* D.M., M.C.PATH.; B. C. V. MITCHLEY*

[WITH SPECIAL PLATE]

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Sarcomata have been induced at the site of repeated injection of the iron-dextran complex, "imferon," in rats, mice, and hamsters of various strains (Richmond, 1957, 1959, 1960; Haddow and Horning, 1960; Lundin, 1961). Haddow and Horning (1960) failed to induce tumours in 18 guinea-pigs injected subcutaneously with imferon. However, the injections were poorly tolerated in this experiment and only eight of the animals survived for more than one year. In the same paper they reported that six rabbits had been given 28 intramuscular injections of imferon at weekly intervals, and that there was no evident pathological condition 16 months after the start of treatment. The present communication records subsequent events in this experiment.

Materials and Methods

Rabbits.—Six random-bred rabbits (3 males and 3 females), born and reared within the Institute, were used for the experiment. They were housed in metal cages and fed the mixed diet given routinely to all rabbits in the colony. This diet includes: oats; bran; a pelleted diet containing various cereals, fish-meal, meat and bone-meal, skimmed milk, yeast, salt, and cod-liver oil; carrots; and cabbage. Water was provided *ad libitum* at all times. The rabbits were approximately 6 months old at the start of the experiment.

Imferon.—The imferon used was of the manufacturer's Batch No. 3975/5.

Experimental and Results

Each of the six rabbits was injected once a week for 28 weeks with 2 ml. of imferon into the right thigh muscle. Three of the rabbits which survived for 37 months or more from the time of the first imferon injection received also up to eight injections of 100 mg. of tetracycline into the muscle of the left loin. These latter injections were given as a prophylactic against enteritis which was occurring sporadically within the rabbit colony at the time. Apart from this, none of the six rabbits received any other treatment.

The results of the experiments are summarized in the Table and illustrated in Figs. 1–5 (Special Plate). Apart from the changes summarized, evidence of slight coccidiosis was present in the livers of all six rabbits. The iron in the livers was distributed periportally (see Fig. 4). It was in the form of haemosiderin, and contained in Kupffer cells or possibly in macrophages that had migrated there from the injection site or elsewhere. In Rabbit No. 4 one lobe of the liver was completely replaced by large groups of siderocytes and wide bands of fibrous tissue.

Attempts were made to transplant both the injection-site tumours. Fragments of the tumour which arose in Rabbit No. 4 were implanted intramuscularly into six rabbits and subcutaneously into a similar number. None of the implants grew. The tumour which arose in Rabbit No. 6 was transplanted subcutaneously into four cortisone-treated (details of cortisone: 10 mg./kg. at time of transplant, then 5 mg./kg. twice weekly for 12 weeks) rabbits. Again none of the implants grew.

Portions of the tumour from Rabbit No. 6 were grown in tissue-culture by Dr. D. T. Hughes. Cells with a characteristically malignant type of growth pattern—that is, no contact inhibition—were observed growing out from the primary explants and as distinct colonies in subcultures. The wide diversity of cell size and nuclear size observed in sections of the tumour persisted in tissue culture. So far as the nuclei were concerned a range from micronuclei (probably less than 1/10 diploid) to giant nuclei (possibly octaploid) was seen. The occurrence of both micronuclei and large nuclei in the same cells suggested chromosome non-disjunction, since no stages of amitotic division were seen.

* Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London.

Tumour Induction in Rabbits at Site of Injection of Imferon

Rabbit No.	Sex	Treatment Data	Age at Death (Months)	Time Elapsed Since First Injection (Months)	Appearance of Injection Site at Necropsy	Evidence of Metastatic Spread	Liver Changes	Other Organs
1	M	28 once-weekly injections of imferon intramuscularly into right thigh, beginning when animals 6 months old	22	16	Iron-laden macrophages only	—	Multifocal necrosis. Iron + +	
2	M		40	34	Slight fibrosis and iron-laden macrophages only	—	Multifocal necrosis. Nodular regeneration. Iron + +	*Spleen*—numerous siderocytes. *Kidney*—haemosiderin in tubular epithelium
3	F		40	34	Iron-laden macrophages only	—	Early cirrhosis. Iron + +	
4	M		45	39	40 × 35 × 30 mm. pleomorphic sarcoma. Iron-laden macrophages present in tumour, but little iron in sarcoma cells	—	Cirrhosis. Nodular regeneration. Iron + +. Atrophy and fibrosis of one lobe	*Spleen*—numerous siderocytes. *Kidney*—haemorrhages in cortex and haemosiderin in tubular epithelium. *Adrenals*—haemosiderin in medulla
5	F		46	40	Iron-laden macrophages only	—	Cirrhosis. Nodular regeneration. Iron + +	*Kidney*—haemosiderin in tubular epithelium
6	F		54	48	35 × 30 × 30 mm. partly necrotic pleomorphic sarcoma invading surrounding muscle. Iron-laden macrophages between tumour cells	Numerous metastases in lungs	Slight fibrosis. Iron + +	*Mammary adenocarcinoma*. *Kidney*—haemorrhagic cyst

Discussion

There is no precise information regarding the incidence of spontaneous sarcomata in the thigh muscles of random-bred rabbits such as those used in the present experiment. It can be said, however, that no such tumours have been seen within our colony, which at any one time consists of between 20 and 40 adult rabbits and their young, during a period of over 10 years. In any case, it would seem to be highly likely that the two sarcomata seen in the experiment described arose as a direct result of the injections of iron-dextran which they received. In particular, the proximity of the primary tumours to the sites of deposition of iron provides a compelling reason for believing that the latter were causally related to the former. Histologically, both tumours were undoubtedly malignant and one had metastasized to the lung. One of the tumour-bearing rabbits was male and one female.

Both tumours arose during the latter half of the life-span of the rabbits ; the first, three and a quarter years after the first injection of imferon when the rabbit was nearly 4 years old ; the second, four years after the first injection when the rabbit was 4½ years old. These long, latent intervals fit in with the view that induction-time is related directly to life-span. In the experiments reported by Haddow and Horning (1960) the shortest tumour-induction time seen in imferon-injected mice was six months, and in imferon-treated rats 10 months. These minimum latent intervals are of the order of one-quarter to one-third of the life-span of the respective species. These considerations add weight to the argument that the lack, so far, of evidence for the carcinogenicity of iron-dextran complexes in man provides no sure grounds for believing that they are carcinogenically inactive in the human species. It remains entirely possible that the minimum induction-time exceeds the period since the introduction of iron-dextran as a form of therapy.

Liver changes were seen in all six rabbits. They included multifocal necrosis, cirrhosis, nodular regeneration, and deposition of iron pigment, mainly in the cells of the reticulo-endothelial system (Kupffer cells). It is highly probable that these liver changes were due to iron-overloading. Golberg and Smith (1960a, 1960b) found that vitamin-E deficiency precipitated the appearance of cirrhosis in rats overloaded with iron. We have no information regarding the vitamin-E status of the diet fed to the rabbits in the present experiment. The presence of large numbers of iron-laden macrophages—that is, siderophages—in the spleen and the presence of iron-pigment in the tubules of the renal cortex are also signs of iron-overloading (see Golberg and Smith, 1958).

Mammary adenocarcinomata are of infrequent occurrence in rabbits of the Chester Beatty colony. Nevertheless, it would not be justifiable to regard the tumour of this type seen in the rabbit which survived for four and a half years as due to iron-dextran treatment. A larger experiment which included matched untreated control rabbits would be needed to study this point.

Baker, Golberg, Martin, and Smith (1961) studied the changes at the site of injection of iron-dextran in mouse, rabbit, dog, and man, using ^{59}Fe-labelled iron. Local retention was found to be dependent upon species, sex, route of injection, dosage, and changes at the site due to previous injections. The highest proportion of iron was found to remain at the site of injection in the male rat given repeated massive injections into the same subcutaneous site. Rabbits, and especially dogs, had much less residual iron with a correspondingly high hepatic uptake. On the other hand, the changes following repeated intramuscular injections—namely, muscle necrosis and the accumulation of siderophages—were reported to be similar in rabbits, rats, and mice. Elsewhere in the same paper, on the basis of studies on anaemic piglets, Baker *et al.* (1961) suggest that iron present in siderophages at the injection site may be mobilized easily, and that this is likely to happen in the anaemic human to whom iron-dextran injections are given. The present experimental findings suggest that, irrespective of the extent to which the local response in rabbits differs from that in rats and mice, and irrespective of the distribution of iron following injection, the end-result—namely, sarcoma at the injection-site—may be the same. In studies of the sites of injection of iron-dextran in 15 humans, Baker *et al.* (1961) reported no pathological changes in 13, but fibrosis and heavy accumulation of siderophages in the remaining two, both of whom had received massive doses. Taking all these findings together it is not possible, on the basis of early tissue changes to iron-dextran, to dismiss the possibility that some humans injected with the material will eventually develop tumours at the site of administration.

The conflicting views (*a*) that iron-dextran is potentially hazardous for man, and (*b*) that it presents little or no hazard, have been discussed at length elsewhere (Haddow and Horning, 1960 ; Baker *et al.*, 1961 ; Roe, 1961 ; Roe and Lancaster, 1964). Similarly, the possible mechanisms by which iron-dextran induces cancer have been discussed and viewed against a background of other examples of carcinogenesis by metallic compounds (Haddow and Horning, 1960 ; Roe and Lancaster, 1964). These aspects of the findings will not therefore be further discussed here. The main conclusion to be drawn from the results presented in this communication is that the induction of tumours at the site of injection of iron-dextran in yet another species of animal, the rabbit, sounds again the note of caution in connexion with clinical use of this material. Moreover, the warning note is louder than before, in so far as the rabbit, unlike the rat and the mouse, but perhaps like man, shows less tendency for iron to be retained where it is injected.

Summary

One out of three male and one out of three female random-bred rabbits, injected once weekly for 28 weeks with 2 ml. of iron-dextran (imferon) into the muscle of the right thigh, developed local sarcomata. The tumours arose 39 months and 48 months, respectively, after the first injection. The second of the two tumours had metastasized to the lungs.

An adenocarcinoma of mammary-gland origin in the second of the sarcoma-bearing rabbits is regarded as probably spontaneous in origin.

Liver changes, including accumulation of iron, necrosis, nodular regeneration, and cirrhosis, were seen in all six rabbits.

The findings are discussed.

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