On the Carcinogenicity of an Iron-Dextran Complex [1, 2]

ALEXANDER HADDOW *and* ERIC S. HORNING,[3, 4]
Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, England

SUMMARY

A recent observation, by H. G. Richmond, on the carcinogenicity of an iron-dextran complex, is confirmed and extended, the complex having proved markedly carcinogenic in the rat and mouse after subcutaneous injection of massive doses. The bulk of tumors appear at the site of injection as sarcomas arising from the iron-laden connective and reticuloendothelial tissues. The component dextran is inactive. It is still not known whether the potency of the complex is a function of the metal alone or of the entire complex. The various possibilities are considered, with particular reference to alternative mechanisms of action involving differing aspects of iron metabolism, and including the inactivation of vitamin E.—J. Nat. Cancer Inst. 24: 109–147, 1960.

THE GREAT range of chemicals capable of exerting carcinogenic effects has long been known to embrace specific metals and metalloids, *e.g.*, arsenic, beryllium, chromium, cobalt, nickel, and zinc—including some of the relatively common members of the transition elements. In most cases such action has been demonstrated only in somewhat limited or special conditions. Particularly noteworthy is the involvement of various metals and minerals—chromium, nickel, iron oxide, radioactive metals, arsenic, asbestos, and, possibly, beryllium—in the production of cancer of the respiratory tract, including the lung of man (*1*). In many such instances, certainly in the field of industrial toxicology, a difficulty is

[1] Received for publication October 9, 1959.

[2] The investigation in general has been supported by grants from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute, National Institutes of Health, Public Health Service.

[3] With the technical assistance of B. C. V. Mitchley and K. G. Moreman.

[4] The authors desire to acknowledge many helpful discussions with Professors F. Bergel and E. Boyland; Drs. L. A. Elson, L. Golberg, E. F. Hartree, and H. G. Richmond, and Mr. G. M. Timmis; and the valuable assistance of Mr. J. L. Everett. Many of the preparations were provided by Benger Laboratories, Ltd., through the kindness of Mr. B. D. Thornley, Dr. L. Golberg, and Mr. F. Fowler; the company also made a special grant toward the costs of this investigation. Various phthalocyanines were given by Dr. J. A. Elvidge, and other compounds by Imperial Chemical Industries, Ltd., and by Dr. M. W. Partridge. We are also grateful to Dr. E. H. Mercer and Mr. M. S. C. Birbeck for their assistance in electron microscopy, and to Mr. B. C. V. Mitchley, Mr. A. Green, and Mr. N. Casperd for technical assistance. The photography is the work of Mr. K. G. Moreman and his department.

encountered in that the suspected product may represent a complex mixture: for example, nickel ore may contain amounts of almost every other metal, including copper, cobalt, and the precious elements. In the list of metals, iron has not hitherto drawn special attention, though Argyll Campbell (*2*) studied the influence of iron oxide, in conjunction with precipitated silica, on the incidence of primary lung tumors in mice. Faulds and Stewart (*3*) reported that in 1948–53, 15 percent of 89 consecutive autopsies of hematite miners showed cancer of the lung, and that the tumors were usually located in areas of fibrosis due to siderosilicosis. Further, Bonser, Faulds, and Stewart (*4*) believed that silica might be the common factor in the environmental exposure of the asbestos worker and the hematite miner, that fibrosis precedes malignant change in the lung, and that both the asbestos and the hematite (iron oxide) might somehow convert the fibrogenic influence of silica into a carcinogenic action. It is, however, possible that iron oxide itself may possess greater relevance, than has been thought hitherto, as a factor common to the hazard in the chromate, hematite, nickel, and asbestos industries.

However, the whole subject has received a new stimulus through an unexpected observation recently made by Richmond (*5–7*) [*see also* Haddow (*8*)] on the carcinogenicity of an iron-dextran complex, which it is the purpose of the present paper to confirm and extend. The observation not merely has great interest in its own right, but it also carries, as it is hoped to show, many implications of potential importance, and has already prompted many new ideas and experiments. On both these grounds it appeared appropriate as the subject of a paper devoted to the memory of Jesse Greenstein, bearing in mind not only his contributions to the biochemistry of carcinogenesis in general, but also his special interests in the metal enzymes, in the role of iron, and especially in catalase.

In studies of the effects of repeated injection of trypan blue in the rat, in inducing pleomorphic histiocytic tumors, as had earlier been shown by Gillman and Gillman (*9*), Richmond wished to employ a "negative control," that is, a substance which, equally with trypan blue, would be taken up by the reticuloendothelium, but without producing tumors. Since the possibility that iron had any part in carcinogenesis had scarcely been entertained, and since large amounts of iron may accumulate in the body in a variety of conditions, such as hemochromatosis and transfusional, malnutritional, or occupational siderosis, Richmond chose as his control, in the expectation that iron must be noncarcinogenic if not altogether bland, the iron-dextran complex Imferon (Benger Laboratories, Ltd.). [This colloidal preparation was the subject of British Patent Specification 748,024 (London and Twigg), April 18, 1956.] Imferon is a sterile solution in water of a complex of ferric hydroxide and a low-molecular-weight dextran fraction. Each 2 ml. of the solution contains 100 mg. of iron, calculated as Fe, present as ferric hydroxide in complex—the amount of dextran being about 20 percent weight per volume. The solution also contains 0.9 percent sodium chloride. Estimation of the Number Aver-

age Molecular Weight [5] of dextran leads to a figure of approximately 2,500, corresponding to a chain of 15 anhydroglucose units. However, in electron micrographs (fig. 1) the particles may appear in large clusters; the true micellar and chain structure is not completely known, though X-ray diffraction and other studies (by Dr. J. Iball) are proceeding. Meantime, on account of its ready absorption from the intramuscular site, its low toxicity (*10*) (with an LD50 of 600–1200 mg.Fe/kg. by the intravenous route in mice, about one third that of saccharated oxide of iron), and its stability in the presence of proteins and electrolytes, it had found a wide application in the treatment of human iron-deficiency anemias, including those of pregnancy and rheumatoid arthritis (*11*), and of piglet anemia in veterinary practice (*12–15*). The British and American clinical literature is already extensive. A proportion of papers allude to undesirable side-effects, and the *British Medical Journal*, in 1954 (*16*), especially referred to the hazard of systemic allergic reactions when metals are administered either intramuscularly or intravenously, and believed that "It is therefore too early yet to advocate that the uncomplicated iron-deficiency anaemia so often seen in pregnancy should be treated by iron intramuscularly." In experimental siderosis induced by administration of the complex, Nissim (*17*; *see also 18, 19*) observed deposition of iron in the testes of mice, with resultant degeneration and atrophy of the seminiferous tubules. Extensive pharmacological studies of the factors in the local absorption and general distribution of such iron-polysaccharide complexes have been carried out by Golberg and his colleagues, who have also utilized the relatively low immediate toxicity of iron-dextran to investigate the effects of excessive administration and massive overload (*20–23; see also 24, 25*). The substance has further been used in intravital techniques for the demonstration of lymphatics (*26*) and in the determination of plasma volumes (*27*).

Surprisingly, Richmond found the iron-dextran complex itself to be carcinogenic, observing 37 local sarcomas in a total of 64 rats given the complex by repeated intramuscular injection, with a latent period of 10 to 14 months in adult rats and about 9 months in young rats. The early results were reported in a communication to the Pathological Society in January 1957. For several reasons it appeared desirable to test the complex in other species, and it is the purpose of this paper to confirm Richmond's discovery, to show that Imferon is a potent carcinogen in both the rat and mouse at least, certainly under the conditions employed, and lastly to consider the bearing which these facts may possess, upon the problems of carcinogenesis as a whole.

EXPERIMENTAL

The experiments so far complete and to be described were designed to test the carcinogenicity of iron-dextran in the mouse, rat, guinea pig, golden and cream hamsters, Chinese hamster, and rabbit. In parallel,

[5] Kindly provided by Dr. B. Ingelman, Pharmacia, Uppsala.

tests were also carried out in mice—in attempts to probe the mechanism of action—of the following agents: (1) the dextran component alone; (2) metallic sponge iron, dialyzed iron (Fe_2O_3 5%), various iron salts, *viz.*, ferric citrate, ferric salicylate and the ascorbate, ferrous sulfate, ferrous lactate, and ferrous gluconate, and other iron-containing substances or preparations such as ferrocene, ferritin, and ferrivenin (saccharated oxide of iron); (3) other metal complexes, *viz.*, bismuth-dextran, chromium-dextran, aluminium-dextran, and copper-dextran; (4) a range of chelating and related agents, *viz.*, 8-hydroxyquinoline, an 8-hydroxyquinoline copper complex, quinisatin oxine, catechol-3,5-disulfonic acid (Tiron), Versene, Versonal (Fe^3-specific), $\alpha\alpha$-dipyridyl, phenolphthalein phosphate, and $\beta\beta$-dimethylcysteine (DL-penicillamine); (5) in the same general connection, adenine, adenosine-5-phosphate, guanylic acid, and xanthine; (6) cobalt, zinc, and nickel octamethyltetrazaporphyrins, zinc tetrabenzporphyrin, and a cross-conjugated macrocycle copper porphyrin; (7) and finally, aluminium, tin, copper, chromium and hydroxymanganese phthalocyanines, and copper and chromium phthalocyanine tetrasulfonic acids.

All preparations were administered by repeated subcutaneous injection in the flank at weekly intervals, except in the case of an Imferon preparation given by forced-feeding to the rat, and of the administration of Imferon by intramuscular injection in the hind limb of the rabbit.

Iron-Dextran Complex (Imferon)

In a pilot or orienting experiment, 25 mice received 0.2 cc. Imferon (that is, the equivalent of 10 mg. Fe) subcutaneously in the right flank at approximately weekly intervals on 13 occasions. Before proceeding, it should be emphasized that such amounts represent, in relation to body iron, a swamping dose or overload both locally and systemically, as was evident from the histochemical demonstration of iron in the liver, spleen, kidney, testis, pancreas, intestine, pituitary, lymph nodes, and other sites. The relevance of this systemic effect, bearing in mind that the "clinical dose" for mice would be only one hundredth of that used in this experiment, will be further discussed.

The first observation made in this experiment was of the appearance in the treated mice, after some months, of a considerable area of epilation at the injection site (fig. 2). At 11 months from the beginning of the experiment, 1 mouse was found to have a tumor arising locally, which proved to be a spindle-cell sarcoma with frequent mitoses (fig. 3). Both the phagocytic cells of the tumor, and many of the malignant cells themselves, contained a pigment resembling hemosiderin, and evidently consisting of loosely bound iron, from the positive Prussian-blue reaction (fig. 6). In the following 6 months, that is, 18 months from the commencement, a total of 9 similar sarcomas developed in the 14 mice surviving at 1 year (*e.g.*, figs. 4, 5, and 7). This small-scale experiment appeared, therefore, to establish the carcinogenicity of Imferon for mice, under the conditions described. It also provided three other impres-

sions: namely, the rapidity of growth and progression of these tumors; their tendency to metastasize; and the propensity to the development not only of sarcomas at the injection site but also, in some cases, of proliferative lesions and malignant tumors in distant organs. In one mouse bearing a local sarcoma (fig. 10) one lobe of the liver contained hypertrophic areas (fig. 8), probably compensatory to liver damage rather than a true hepatoma. Another developed not only a sarcoma at the site of injection, and a tumor mass (in a hind limb) which proved to be secondary, but also a primary bronchogenic carcinoma—a tumor only rarely seen in mice (figs. 11, 12, and 13). Advantage has been taken of the favorable properties of iron in electron microscopic studies. In electron micrographs of several of the tumors described here, Dr. E. H. Mercer and Mr. M. S. C. Birbeck show islands of ferritin granules in the cytoplasm (fig. 14): the appearances are in many ways reminiscent of those seen under physiological conditions in certain organs in the process of aging, for example, in the macrophages of the spleen (fig. 15). According to Richter (*28*), who has studied the cellular transformation of injected colloidal iron complexes into ferritin and hemosiderin, the physical state of the ferric hydroxide micelles contained in iron-dextran, as judged by electron microscopy and electron diffraction, differs notably from the state of the ferric hydroxide in ferritin or hemosiderin. His findings show that the iron preparations used in therapy can be identified within cells, and that their intracellular disposition and fate can be followed at the molecular level.

In a second experiment, 30 stock mice received 0.3 cc. of iron-dextran by subcutaneous injection at weekly intervals for 7 months, *i.e.*, received a much higher individual and total dose than in the first experiment. Twenty-three of the mice survived 6 months, and of these, 18 developed sarcomas at the injection site, 8 of them with latent periods under 1 year (1 at 6 months, 3 at 8 months, 3 at 10 months, and 1 at 11 months). Hence, it would appear that larger and more protracted dosage materially accelerated tumor emergence. Fifteen of the primary tumors were transplanted, with successful transmission in 9. Figure 9 shows an example of one such primary sarcoma. In others, a tendency was observed, in sections including the skin, to the formation of masses or processes of enlarged and somewhat irregular basal epithelial cells, taking on a downward growth (figs. 16 and 17) and suggesting an influence, whether direct or indirect, on epithelium as well as the connective tissues. Throughout many of the sarcomas was noted a dense concentration of pigment-laden histiocytes, many of them multinucleate, but whether the iron-dextran had induced these histiocytes to divide, or merely to mobilize, proved difficult to ascertain. However, 3 tumors were recorded (additional to the sarcomas) which could be regarded as frank histiocytomas (fig. 20). One mouse showed liver damage, hepatoma, and hemangioma; in this, as in many of the livers examined, a prominent feature was the occurrence of spherical iron-containing inclusion bodies in the nuclei of the parenchymal cells (fig. 21).

Thus far, the iron-dextran used was the clinical preparation, with the property of efficient absorption—although the conditions of these experiments, as has been made clear, of necessity produced an iron overload both locally and systemically. However, two further experiments were conducted using laboratory preparations of Imferon, known to be poorly absorbed, which were provided by Benger Laboratories, Ltd. (batches 145Z and 107/59). In the first, on 11 weekly occasions, 20 stock mice received 0.2 cc. by subcutaneous injection. Of these, 19 survived to 6 months and 7 to 1 year. Tumors were recorded in 10, including an epithelioma of the skin at the injection site after a latent period of 6½ months (figs. 18 and 19), 7 sarcomas or fibrosarcomas with latent periods of 9 to 11 months (fig. 24), a fibroma at 11 months, and an intra-abdominal lymphosarcoma at 10 months (fig. 25). Of the sarcomas, 4 grew successfully on transplantation. In the second experiment with poorly absorbed material, 20 stock mice or their survivors (13 at 8 months) received 0.2 cc. of batch 107/59 by weekly subcutaneous injection over 7½ months. Of these, 7 developed tumors locally: 4 sarcomas, and 3 histiocytomas, the latter with latent periods of 5 months and the former of 7 months and upwards.

Imferon was also tested in a series of 30 male rats of the Chester Beatty inbred albino strain, which received 1 cc., weekly for 6 months, by subcutaneous injection in the flank. For convenience, injection was made into an area previously epilated. It was soon observed that regrowth of hair was greatly retarded or almost entirely inhibited, although a tuft of new hair frequently appeared at the site of subsequent injection, no doubt stimulated by trauma (fig. 26). All 30 of the treated rats were alive at 6 months, and, thereafter, yielded a total of 24 primary tumors, 19 sarcomas or fibrosarcomas, and 5 histiocytomas, several doubtless benign (figs. 22 and 29). Transplantation was attempted in 10 sarcomas, with success in 3. Generally speaking, the results of this experiment are similar to, and fully confirmatory of, those obtained by Richmond. In addition, 20 male rats received an oral Imferon solution (Benger's, batch 10163/1, 5% w/v Fe), 1 cc. being administered by forced-feeding weekly for 8 months, while 20 mice received 0.3 cc. of this preparation in the same way. These experiments have remained negative, except for a tumor in the right groin of 1 animal, which proved to be an adenocarcinoma of the mammary gland (fig. 27) and which showed a positive Prussian-blue reaction for iron. If this isolated tumor cannot with any assurance be attributed to treatment, nevertheless the finding is perhaps suggestive, since no tumor of this type has hitherto been observed to occur spontaneously in a male rat of this strain and must therefore be excessively rare. The distribution of iron in the intestinal mucosa of the same animal is shown in figure 23.

Among other species, 20 male cream hamsters received 0.5 cc. of iron-dextran subcutaneously each week for 10 weeks; only 1 developed a sarcoma, highly pleomorphic, after a latent period of 9 months. No tumors were recorded in a second series of 30 hamsters similarly treated. Two series of 12 and 20 Chinese hamsters received doses of 0.1 and 0.3 cc.

Imferon for 7 and 4 months, respectively, with relatively high mortality, a factor in which was an extraordinary frequency of damage to and hypertrophy of the liver (fig. 28), with hyperplastic foci and, in some cases, cholangioma and hepatoma. No tumors were observed at the site of injection. Two groups of 8 and 10 guinea pigs received 1 cc. of Imferon subcutaneously each week for 7 months. Treatment was poorly tolerated; only 5 and 3 animals survived 1 year, and no tumors were recorded. Six rabbits also received 28 weekly injections of 2 cc. of Imferon, in this experiment intramuscularly, but without evident pathology after 16 months.

The Component Dextran

Two experiments were conducted in groups of 30 and 20 stock mice to test the component dextran in 20 percent solution weight per volume (batches 10150/1 and 10164/1, Benger Laboratories, Ltd.). In both, weekly doses of 0.2 cc. were administered subcutaneously for 16 and 11 months; 12 and 16 animals survived in the respective groups. No tumors were recorded in either series.

Metals, Chelating Agents, and Metal Complexes

A range of metals, chelating agents of different kinds, and metal-containing complexes (listed above) was examined in order first to test the specificity of the carcinogenic effects of iron-dextran, and secondly in the hope of clarifying the mechanism of its action. The outcome of these experiments to date is summarized in table 1. While many are still in progress, a great contrast is already clearly apparent, between the carcinogenic potency of iron-dextran, and the inactivity or relatively low activity of most of these agents. Only one sarcoma and one benign tumor were obtained with aluminium-dextran, and none with the bismuth, chromium, or copper complexes, though it appears (private communication from Dr. L. Golberg) that these metal complexes are not at all comparable with Imferon, since they are not stably chelated, and hence liberate ionic metal on injection. The occurrence of an exceedingly low (apparent) yield of tumors with ferrous sulfate, lactate, and gluconate may be of interest, though probably more significant are the few tumors attributable to ferrivenin, which substance, however, Richmond found inactive. In short, these experiments have served to set out in sharp relief the altogether higher order of carcinogenic potency of the iron-dextran complex in the mouse and rat, under the conditions described.

DISCUSSION

It is still not certain whether this outstanding carcinogenic potency of the iron-dextran complex is a function of the metal alone or of the entire complex (which undoubtedly affords an exceptionally efficient mode of iron administration), or whether a special role is played by the

TABLE 1.—Activity of metals, chelating agents, and metal-containing complexes

Agent under test	Number and strain of mice	Number of weekly subcutaneous injections, and dose	Months of experiment to date and survivors	Tumors recorded
Metallic sponge iron..	20 CBA	16 20 mg.	15 (16)	Nil
Dialyzed iron (Fe_2O_3 5%).	20 CBA	16 0.2 cc.	15 (19)	Nil
Ferric citrate.....	20 Stock	33 5 mg.	9 (13)	Nil
Ferric salicylate.....	20 Stock	36 5 mg.	12 (14)	Nil
Iron ascorbate.....	20 Stock	43 0.2 cc. ≡0.3 mg. Fe	12 (11)	Nil
Ferrous sulfate.....	20 Stock	16 2.5 mg.	14 (13)	1 Fibroma
Ferrous lactate.....	20 Stock	21 5 mg.	12 (11)	1 Sarcoma
Ferrous gluconate....	20 Stock	13 5 mg.	12 (12)	1 Fibroma 1 Thymoma 1 Thecoma
Ferrocene.....	20 Stock	28 5 mg.	9 (17)	Nil
Ferritin (rat).....	20 (Albino rats)	10 20 mg.	7 (20)	Nil
	20 Stock (mice)	15 5 mg.	7 (12)	
Ferrivenin (saccharated oxide of iron).	20 Stock	13 0.2 cc.	14 (11)	2 Spindle-cell sarcomas 3 Histiocytomas
Bismuth-dextran (batch 10186/1, 0.5% Bi).	20 Stock	23 0.2 cc.	10 (14)	Nil
Chromium-dextran (batch 6459/1).	20 Stock	24 0.2 cc.	9 (16)	Nil
Aluminium-dextran (batch 6438/7).	20 Stock	14 0.2 cc.	14 (13)	1 Sarcoma 1 Benign histiocytoma
Copper-dextran (batch 11931/4).	20 Stock	6 0.1 cc. of 1 in 4 dilution	10 (13)	Nil
8-Hydroxyquinoline...	20 Stock	31 1 mg.	15 (17)	1 Hepatoma?
8-Hydroxyquinoline copper complex.	20 Stock	39 0.1 mg.	10 (14)	1 Pleomorphic sarcoma (*see* fig. 30)
Quinisatin oxine.....	20 Stock	41 1 mg.	9½ (19)	Nil
Catechol-3, 5-disulphonic acid (Tiron).	20 Stock	41 5 mg.	12 (13)	1 Thymoma 1 Lymphoma
Versene.....	20 CBA	21 0.2 cc. of 5% solution	14 (18)	Nil
Versonal Fe^3-specific	20 Stock	46 0.2 cc.	12 (17)	Nil
$\alpha\alpha$-Dipyridyl.....	20 Stock	40 5 mg.	10 (12)	1 Parotid tumor
Phenolphthalein phosphate.	20 Stock	13 0.2 cc.	11 (10)	Nil
$\beta\beta$-Dimethylcysteine (DL-penicillamine).	20 CBA	19 2 mg.	13 (18)	Nil
Adenine.....	20 CBA	28 5 mg.	14 (15)	Nil
Adenosine-5-phosphate	20 Stock	17 5 mg.	14 (19)	Nil
Guanylic acid (sodium salt).	20 Stock	25 5 mg.	14 (15)	Nil
	20 CBA	28 5 mg.	15 (18)	Nil
Xanthine.....	12 (Albino rats)	18 50 mg.	14 (12)	1 Sarcoma

TABLE 1.—Activity of metals, chelating agents, and metal-containing complexes —Continued

Agent under test	Number and strain of mice	Number of weekly subcutaneous injections, and dose	Months of experiment to date and survivors	Tumors recorded
Cobalt octamethyltetrazaporphyrin.	20 Stock	8 0.5 mg.	8 (20)	Nil
Zinc octamethyltetrazaporphyrin.	20 Stock	7 0.5 mg.	8 (17)	Nil
Nickel octamethyltetrazaporphyrin.	20 Stock	5 0.5 mg.	8 (12)	Nil
Zinc tetrabenzporphyrin.	20 Stock	8 0.5 mg.	9 (18)	Nil
Cross-conjugated macrocycle copper porphyrin.	20 Stock	4 0.5 mg.	10 (14)	Nil
Aluminium phthalocyanine.	20 Stock	11 0.5 mg.	8 (17)	Nil
Tin phthalocyanine...	20 Stock	34 0.5 mg.	8 (14)	Nil
Copper phthalocyanine	20 Stock	34 0.5 mg.	8 (17)	Nil
Chromium phthalocyanine.	20 Stock	11 0.5 mg.	8 (18)	Nil
Hydroxymanganese phthalocyanine.	20 Stock	8 0.5 mg.	9 (17)	Nil
Copper phthalocyanine tetra-3-sulfonic acid.	20 Stock	36 0.5 mg.	8 (20)	Nil
Copper phthalocyanine tetra-4-sulfonic acid.	20 Stock	25 0.5 mg.	8 (11)	Nil
Chromium phthalocyanine tetra-4-sulfonic acid.	20 Stock	23 0.5 mg.	8 (16)	Nil

component dextran. The possibility of some unsuspected contaminant in the fermentative production of the dextran appears very remote (private communication, Mr. B. D. Thornley) and can be excluded with confidence. As to the dextran itself, it must be recalled that other macromolecules, such as carboxymethylcellulose and polyvinylpyrrolidone, have also been incriminated as carcinogens (*29*). More recently, Hueper (*30*) has described the carcinogenicity of some but not all dextrans, also of polyvinylpyrrolidone and polyvinylalcohol, on parenteral inoculation in rats and mice. In these experiments, however, sarcomas were observed in those organs and tissues (such as the reticuloendothelium) in which the agents were stored, and not at the site of injection. The phenomenon would therefore appear to be different in nature from that considered here. Further, Lusky and Nelson (*31*) obtained negative results on testing a particular dextran. Finally, the negative outcome of the experiments now recorded in respect to the dextran of low-molecular weight used in the preparation of the iron complex, agrees with the experience of Richmond. Hence, present indications tend to exclude the carbohydrate component of iron-dextran, at least *per se*, as responsible for the carcinogenicity of the complex.

The present experiments show that the mere administration of iron itself in various forms, though not entirely negative, is certainly insufficient to account for the overwhelming carcinogenicity of iron-dextran; and hence it is necessary to consider whether this activity is dependent on

the complex in its entirety. If this were so, the complex could operate in one of two ways: first, as a colloid that produces reticuloendothelial blockade and damage, and perhaps interference with, or even abrogation of, immune processes; and, second, as a peculiarly favorable means of introducing iron within the cell. It is clear that the role of the metal in the former mechanism could well be nonspecific or indifferent, but specific in the latter. Considering the former, it is of interest that the cellular uptake of iron-dextran is similar in its distribution to that of various antigens (*e.g.*, γ-globulin) and viruses (*e.g.*, influenza virus) in the Kupffer and reticuloendothelial cells. As to the site of action, information of some interest has been obtained by Dr. and Mrs. O. G. Fahmy, from experiments to test the mutagenicity of iron-dextran in *Drosophila*. The product was administered by intra-abdominal injection into adult males of various doses ranging from 1.5×10^{-2} to 1.5×10^{-4} mg. Fe, and the offspring were tested for sex-linked recessive lethals by the Muller-5 technique. Only 10 lethals were recovered from 5,998 treated chromosomes. Since this is within the control range, it was concluded that iron-dextran is nonmutagenic in *Drosophila*, and that, while this result does not altogether exclude the possibility of its mutagenicity in mammals, its primary and major site of cellular damage probably lies outside the nuclear genes. So far as its carcinogenicity is concerned, it is perhaps relevant that Lindegren, Nagai, and Nagai (*32*), in studies of the induction of respiratory deficiency in yeast by manganese, copper, cobalt, and nickel, point out that many effects ascribed to gene mutation may be due to damage to autonomous cytoplasmic particles, and that physical carcinogenic influences such as ultraviolet radiation and X rays have often been demonstrated to produce stable variations in the extrachromosomal apparatus.

Although clearly the question at this stage cannot be decided, equally can it by no means be excluded that the action is specifically dependent upon iron. This at once raises a host of intriguing possibilities, bearing upon the mechanisms of carcinogenic action, and the disturbances of iron metabolism in the cancer cell and tumor-bearing organism. Further, the involvement of metals in carcinogenesis, through the complexing and catalytic power of their ions, and their directive activity in many biosyntheses, may have more fundamental implications than have hitherto been suspected. We have already been reminded of the metalloid quality of the carcinogenic hydrocarbons, arising from the electron mobilities in their aromatic rings. It may also prove that chelation processes have greater importance than has been realized, as is now seen in the fields of pharmacology (*33*), chemotherapy (*34*), and plant growth substances (*35*, *36*). In the last connection must be considered a general principle which has emerged over the last few years from the work of Clayson and others (*37–39*), concerning the possible importance of *o*-hydroxy derivatives as key metabolites of the carcinogenic amines. These substances could function as chelating agents, and the avidity of various *o*-aminophenols for metallic ions has recently been investigated by Sims (*40*). Although the same

would not, however, apply in the case of certain methoxy homologues which are equally carcinogenic, nevertheless the possibility must still be explored, as Boyland has suggested, of the possible role and significance of mixed chelate formation, as a stage in the action of particular carcinogens at least.

Although the situation is by no means clear or simple, certain restricted associations are already known between iron deposition and the incidence of specific types of malignant disease—as in Argyll Campbell's early description of the effects of precipitated silica and of iron oxide on the incidence of primary tumors of the lung in mice (*2*), the liability to carcinoma of the liver in hemochromatosis (*41*), and the frequency of cancer of the lung in hematite miners (*3*, *4*). Although siderosis might be suspected as contributory in the etiology of cancer of the liver in the South African Bantu, in this case there does not, however, appear to be any convincing evidence (*42–45*).

While the carcinogenicity of iron-dextran is obviously of much interest in its own right, it also recalls many well-founded observations in the literature, suggesting interference by carcinogenic agents with iron metabolism, hematopoiesis, and the hemoprotein enzymes. A few examples are:

Warren (*46*) and Parsons and Warren (*47*) studied the liberation of iron by treatment with carcinogens. Strong (*48–50*), in work which was largely supported by Goulden and Warren (*51*, *52*), described an anemia occurring in mice of mammary cancer-susceptible lines prior to tumor development, (but not in nonsusceptible pure lines), and reported a contrast in the iron content of the tissues of high- and low-cancer strains. Such examples would appear to have their counterparts in certain aspects of the anemia of cancer and the Plummer-Vinson syndrome (*53–56*). Concerning the iron content of tumor cells themselves, Rawlinson (*57*) made the observation that, on malignant transformation, the epithelial cells of the mammary gland of C3H mice appeared very abruptly to lose the property of accumulating iron. In a study of the distribution of various trace elements (zinc, molybdenum, manganese, chromium, tin, copper, nickel, aluminium, silver, lead, cobalt, and iron), as between normal and malignant human liver, Olson and others (*58*) recorded that in all the tumor tissues these elements were markedly decreased.

Next there is the striking so-called "hemolymph" change produced by many different kinds of carcinogens, almost diagnostically, which was studied by Lasnitzki and Woodhouse (*59*, *60*), Haddow and coworkers (*61*), and more recently by Elson (*62*), but whose underlying meaning still escapes us. There is also the propensity of certain carcinogens to produce methemoglobinemia, *e.g.*, various aminostilbenes (*61*) and azo-dyestuffs (*63*). Apart from such changes during the course of carcinogenesis, well-recognized and perhaps related alterations may also accompany the course of tumor growth, *e.g.*, variable reduction in the amount or activity of such hemoprotein enzyme systems as catalase (*64*) and cytochrome *c*, and an almost complete deficiency of porphyrins (*65*) (for which last it may be noted, however, tumors are markedly avid). Since not only the catalase activity but also the hemoglobin concentration is markedly lowered in tumor-bearing animals, Greenstein had suggested that this interference with catalase and hemoglobin synthesis is concerned with the formation of the hematoporphyrin nucleus. In their studies of the respiratory pigments of tumor cells, Chance and his colleagues (*66*, *67*) have found that while the concentration of cytochrome in the intact cell may

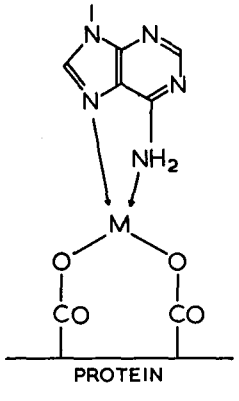
be adequate, the respiratory system is not used to the extent of its capabilities. Marked inconsistencies may be found especially affecting cytochrome *c*, a finding supported by preliminary observations carried out in the course of the present work, on our behalf, by Dr. E. F. Hartree. Yet the possibility is still not excluded, that an iron-mediated competitive or other interference with the cytochrome system could lead to the imposition of a respiratory change or mutation. Much interest has also developed (*68*) in a possible association between malignancy and the porphyrin content of tissues, the apparently frequent inability of tumor cells to synthesize the tetrapyrrole system, and the ability of neoplasms, and also perhaps rapidly growing normal tissues (*69*), to concentrate injected porphyrin. Of particular interest in the present connection is an iron-incorporating enzyme, described by Lochhead and Goldberg (*70*), transferring iron to protoporphyrin for the biosynthesis of heme. Yet another metalloflavoprotein closely involved in iron metabolism is xanthine oxidase (*71*); thus Mazur and his coworkers (*72*) have provided data suggesting that the mechanism of ferritin-iron reduction involves a transfer of electrons from the iron atoms of xanthine oxidase to those of ferritin, each iron atom in association with sulfydryl groups. Further, the physiological stimulus which results in accelerated release of iron from hepatic ferritin to the plasma appears to be tissue hypoxia (*73*, *74*). This produces an increase in xanthine oxidase substrates and a consequent reduction and release of ferritin iron to the plasma. Hence, the ferritin-xanthine oxidase system constitutes part of a homeostatic mechanism for the regulation of iron levels. The significance of these observations is obvious, in relation to the possible importance of xanthine oxidase as a limiting enzyme in certain tumors.

Apart from these observations, many others attest to the possibly key importance of iron metabolism in neoplasia—altogether omitting the views and contributions of such pioneers as Warburg and de Hevesy. So far as concerns deficiencies in iron metabolism, it is not always clear to what extent these are specific or merely secondary. In a recent study, Dorothy Ley (personal communication) has observed such defects in 80 percent of cases of advanced malignant disease with metastases, *e.g.*, inefficiency in iron-binding, and a reduced level of serum iron, together producing a marked reduction in the total iron-carrying capacity.

Lastly must be mentioned a relationship which is certainly intriguing and which may be important, namely, that between iron deposition and the aging of tissues and cells, a subject studied by Zondek and Karp (*75*) 25 years ago when they observed, at a definite middle period in life, that the iron content of epithelial organs is increased up to 200 percent; this value is maintained until death. It had also been observed by Warren and Goulden (*52*) that, in some species, aging is accompanied by an increase in the nonheme iron content of the tissues.

If the carcinogenicity of the iron-dextran preparation were indeed a function of the metal, either alone or in its complexed form, it would then be tempting to speculate as to possible mechanisms. Introduction of excess metal into what is certainly a delicately balanced system of metals and metal enzymes could undoubtedly result in maldistribution (*see 76*) and so interfere with function, and with what Drabkin (*77*) has called "the precarious homeostasis of iron"—"the tight squeeze of iron between the evils of impoverishment and overabundance . . . ." Such interference could certainly and very readily impinge on the respiratory chain, either through competitive interference with the cytochrome system, leading to the imposition of a respiratory mutation as has already been considered, or, as Elson has suggested, through the

combination of iron with protein, resulting in an abnormal hemoglobin-like compound and competitive anoxia. It might also proceed via nucleotide metabolism, by many different routes of which one is, however, perhaps especially worthy of attention, namely, the withdrawal of diphosphopyridine nucleotide (DPN) from its functional form through the introduction of a metal with which it will readily chelate. In any such studies, much could be learned from what is already known of the physicochemical role of iron in histological staining, and especially its mordant action (*78*, *79*). According to Wigglesworth (*80*), the substrates mainly responsible for the uptake of iron are the nucleic acids and proteins. The staining capacity of different proteins runs parallel with the number of free carboxyl groups which they contain, and it is concluded that iron forms nonionizing complexes with carboxyl and phosphoric acid groups, the intensity of staining being a measure of the relative abundance of such groups in the proteins and nucleic acids present, and of the concentration density of these substances. In the staining of nuclei and chromosomes with iron, the proteins are at least as important as the nucleic acids. The iron taken up is mostly in the ferric state but is partially converted to ferrous iron by reducing substances, chiefly SH groups. It is of some related interest that Kirby (*81*) has described a possible means whereby DNA could form a complex (I) through a metal (M) with a protein containing aspartic or glutamic acid residues—in a paper suggesting the importance of metal bonding as well as ionic bonding, in the DNA structure. Incidentally, Zamenhof (*82*) has warned of the importance of the avoidance of traces of iron or rust ($Fe^{++} \rightarrow Fe^{+++}$) in the preparation of DNA, where it leads to rapid degradation.



(I)

Lastly, there must not be neglected the known effects of iron in the inactivation of vitamin E. In their studies of the changes associated with the accumulation of excessive amounts of iron in various organs of the rat, Golberg and Smith (*23*) point out that the sequelae closely resemble

many of the characteristic pathological and biochemical changes observed in rats deficient in vitamin E—for example, ceroid formation, the production of fat peroxides ("brown fat") hemolysis *in vitro*, "brown" uterus, testicular atrophy, renal autolysis, reduced liver vitamin A, and incisor depigmentation: Ceroid formation appears to be favored by such prooxidants as the iron porphyrins and other chelates, and by Fe from ferritin, and inhibited by the antioxidants vitamin E, glutathione, cysteine, and ascorbic acid.

Before concluding, it must again be stressed that the carcinogenicity of iron-dextran in the mouse and rat has been demonstrated thus far only through the use of massive doses. The body content of iron is approximately 2 mg. in the mouse and about 10 mg. in the adult rat (*83*), while a single dose of 0.2 cc. Imferon will itself introduce 10 mg. Fe. Although such overloading certainly entails systemic changes, there is no reason to believe that the carcinogenic reaction is dependent on these, or that it is other than a preponderantly local action. It may indeed mainly be determined by that fraction of the dose which is poorly absorbed or locally retained. In studies of the absorption of radioactive iron-dextran in pregnancy, Evans and Ramsey (*84*) showed that about 80 percent of a dose of Fe^{59}-dextran, given intramuscularly in the gluteal mass, was absorbed within 3 weeks after injection, but that recorded activity then diminished at only a reduced rate, over a period which might be as long as 6 months. On the basis of body weight, the "clinical dose" in the mouse would be only one one-hundredth or one one-hundred fiftieth of the dose administered in the present experiments. Tests are already under way to examine doses of this order in the mouse and rat, but are as yet incomplete. Meantime, however, Golberg and his colleagues (personal communication) have administered repeated doses to a total of 80 times the clinical dose to rats, rabbits, mice, guinea pigs, and dogs, without any apparent effect on health. In a series of 414 stock mice receiving repeated injections by various routes up to a total of 170 times the clinical dose during a period of 88 weeks, 9 tumors were observed, of which 8 were regarded as spontaneous and only 1 was a spindle-cell sarcoma at the injection site. In a series of 527 albino and hooded rats receiving up to a total of 210 times the clinical dose by repeated intramuscular injection, 11 sarcomas appeared at the injection site after 82 to 118 weeks. In 5,200 stock mice receiving up to 170 times the clinical dose, no tumors were observed up to 48 weeks, though the experiment continues. While Golberg has therefore obtained a small yield of tumors attributable to iron-dextran, in certain of his experiments, this in no way compares with the much greater incidence reported in the present paper, and by Richmond. In considering questions of hazard, it would appear most probable that the carcinogenic response is decided primarily by the absolute amount administered, and only secondarily, if at all, by the relation of this to the total-body weight. Even if quite negative results were to be obtained in mice, with doses that bear the same relation to those used in man, as do the body weights of mice and men, this would not necessarily

in any way diminish a hazard attaching to the absolute amounts that are in fact administered to humans.

In conclusion, we are still ignorant of the real significance of the carcinogenic action of metals, whether this is particular or relatively nonspecific, or whether it possesses wider and more fundamental meaning. So far as the iron-dextran complex is concerned, it has already been emphasized that we know no more than that it is carcinogenic for the mouse and rat. The specificity of, or necessity for, the iron component is still quite undecided, but in view of the unique functions of iron the observation could clearly be of profounder significance. In any event it has been thought not only desirable but necessary to draw attention to Richmond's recent and important discovery, and to confirm and extend it.

Note Added in Proof, December 12, 1959

Since this paper was submitted, additional tumors (all at the injection site) have been observed as follows: 1 rat sarcoma in the Imferon series, 1 sarcoma in the series of Chinese hamsters receiving 0.1 cc. Imferon weekly, and 1 sarcoma each in the series of mice treated with Imferon batches 145Z and 107/59 (*see* page 114). It is of much interest that 2 further sarcomas have appeared in the mice treated with aluminium-dextran batch 6438/7 (*see* table 1) and no fewer than 5 in a similar series (batch 6463/2) not referred to above. Lastly, in experiments to test successively lower dosages, 1 sarcoma has appeared after 8 months, in a series of mice receiving 0.05 cc. Imferon weekly by subcutaneous injection. These experiments continue.

REFERENCES

(*1*) HUEPER, W. C.: Experimental and histological studies of metal cancers of the lung. (Abstract.) *In* Seventh Internat. Cancer Congress, London, 1958, pp. 74–75.

(*2*) CAMPBELL, J. A.: Effects of precipitated silica and of iron oxide on the incidence of primary lung tumours in mice. Brit. M. J. 2: 275–280, 1940.

(*3*) FAULDS, J. S., and STEWART, M. J.: Carcinoma of the lung in haematite miners. J. Path. & Bact. 72: 353–366, 1956.

(*4*) BONSER, G. M., FAULDS, J. S., and STEWART, M. J.: Occupational cancer of the urinary bladder in dyestuffs operatives and of the lung in asbestos textile workers and iron-ore miners. Am. J. Clin. Path. 25: 126–134, 1955.

(*5*) RICHMOND, H. G.: Induction of sarcoma in rats by an iron-dextran complex. Scottish M. J. 2: 169, 1957.

(*6*) ———: Induction of sarcoma in the rat by iron-dextran complex. Brit. M. J. 1: 947–949, 1959.

(*7*) ———: The carcinogenicity of an iron-dextran complex. *In* Cancer Progress, I. London, Butterworth & Co., 1959. In press.

(*8*) HADDOW, A.: The possible role of metals and of metal chelation in the carcinogenic process. *In* Ciba Foundation Symposium on Carcinogenesis: mechanisms of action. London, Churchill, 1959, pp. 300–306.

(*9*) GILLMAN, J., and GILLMAN, T.: The pathogenesis of experimentally produced lymphomata in rats (including Hodgkin's-like sarcoma). Cancer 5: 792–846, 1952.

(*10*) MARTIN, L. E., BATES, C. M., BERESFORD, C. R., DONALDSON, J. D., MCDONALD, F. F., DUNLOP, D., SHEARD, P., LONDON, E., and TWIGG, G. D.: The pharmacology of an iron-dextran intramuscular haematinic. Brit. J. Pharmacol. 10: 375–382, 1955.

(*11*) CAPPELL, D. F., HUTCHINSON, H. E., HENDRY, E. B., and CONWAY, H.: A new carbohydrate-iron haematinic for intramuscular use. Brit. M. J. 2: 1255–1257, 1954.

(*12*) MCDONALD, F. F., DUNLOP, D., and BATES, C. M.: An effective treatment for anaemia of piglets. Brit. Vet. J. 111: 403–407, 1955.

(*13*) BROWNLIE, W. M.: The treatment of piglet anaemia. Vet. Rec. 67: 350–354, 1955.

(*14*) BARBER, R. S., BRAUDE, R., and MITCHELL, K. G.: Studies on anaemia in pigs. I. The provision of iron by intramuscular injection. Vet. Rec. 67: 348–349, 1955.

(*15*) KERNKAMP, H. C. H.: A parenteral hematinic, 'Imferon' for the control of iron-deficiency anemia in baby pigs. N. Amer. Vet. 38: 6–9, 1957.

(*16*) Intramuscular iron therapy. Brit. M. J. 2: 1281, 1954.

(*17*) NISSIM, J. A.: Deposition of iron in the testes after administration of an iron-dextran complex. Lancet 1: 701–702, 1955.

(*18*) ———: Evidence of a spermatocytotrophic hormone produced by the interstitial cells of the testis. J. Physiol. 131: 27P–28P, 1956.

(*19*) ———: The nature and chemical affinities of the interstitial cells of the testis. (Abstract.) J. Endocrinol. 13: 36–37, 1956.

(*20*) GOLBERG, L., SMITH, J. P., and MARTIN, L. E.: Effects of massive iron overload in the rat. Nature, London 179: 734, 1957.

(*21*) ———: The effects of intensive and prolonged administration of iron parenterally in animals. Brit. J. Exper. Path. 38: 297–311, 1957.

(*22*) BERESFORD, C. R., GOLBERG, L., and SMITH, J. P.: Local effects and mechanism of absorption of iron preparations administered intramuscularly. Brit. J. Pharmacol. 12: 107–114, 1957.

(*23*) GOLBERG, L., and SMITH, J. P.: Changes associated with the accumulation of excessive amounts of iron in certain organs of the rat. Brit. J. Exper. Path. 39: 59–73, 1958.

(*24*) NORDÉN, Å.: Studies of iron-59 labeled iron-dextran complex following intramuscular injection. Nord. med. 58: 1216–1221, 1957.

(*25*) KARLEFORS, T., and NORDÉN, Å.: Studies on iron-dextran complex. Acta med. scandinav. Supp. 342, 1958, 54 pp.

(*26*) TURNER-WARWICK, R. T.: Intravital techniques for the demonstration of lymphatics. Ann. Roy. Coll. Surgeons England 24: 101–109, 1959

(*27*) MACKENZIE, A., and TINDLE, J.: Determination of plasma-volume using intravenous iron dextran. Lancet 1: 333–335, 1959.

(*28*) RICHTER, G. W.: The cellular transformation of injected colloidal iron complexes into ferritin and hemosiderin in experimental animals. A study with the aid of electron microscopy. J. Exper. Med. 109: 197–216, 1959.

(*29*) HUEPER, W. C.: Experimental carcinogenic studies in macromolecular chemicals. I. Neoplastic reactions in rats and mice after parenteral introduction of polyvinyl pyrrolidones. Cancer 10: 8–18, 1957.

(*30*) ———: Carcinogenic studies on water-soluble and insoluble macromolecules. A.M.A. Arch. Path. 67: 589–617, 1959.

(*31*) LUSKY, L. M., and NELSON, A. A.: Fibrosarcomas induced by multiple subcutaneous injections of carboxymethylcellulose (CMC), polyvinylpyrrolidone (PVP), and polyoxyethylene sorbitan monostearate (Tween 60). (Abstract.) Fed. Proc. 16: 318, 1957.

(*32*) LINDEGREN, C. C., NAGAI, S., and NAGAI, H.: Induction of respiratory deficiency in yeast by manganese, copper, cobalt, and nickel. Nature, London 182: 446–448, 1958.

(*33*) CHENOWETH, M. B.: Chelation as a mechanism of pharmacological action. Pharmacol. Rev. 8: 57–87, 1956.

(*34*) ALBERT, A.: Metal-binding agents in chemotherapy: the activation of metals by chelation. *In* The Strategy of Chemotherapy. Society for General Microbiology Symposium, 8th. London, Cambridge Univ. Press, 1958, pp. 112–138.

(*35*) HEATH, O. V. S., and CLARK, J. E.: Chelating agents as growth substances. Nature, London 178: 600–601, 1956.

(*36*) ———: Chelating agents as plant growth substances. A possible clue to the mode of action of auxin. Nature, London 177: 1118–1121, 1956.

(*37*) CLAYSON, D. B.: A working hypothesis for the mode of carcinogenesis of aromatic amines. Brit. J. Cancer 7: 460–471, 1953.

(*38*) BRADSHAW, L., and CLAYSON, D. B.: Metabolism of two aromatic amines in the dog. Nature, London 176: 974–975, 1955.

(*39*) CLAYSON, D. B., JULL, J. W., and BONSER, G. M.: The testing of orthohydroxyamines and related compounds by bladder implantation and a discussion of their structural requirements for carcinogenic activity. Brit. J. Cancer 12: 222–230, 1958.

(*40*) SIMS, P.: The stability constants of some metal chelates of *ortho*-aminophenols. J. Chem. Soc. 3648–3649, 1959.

(*41*) WILLIS, R. A.: Haemochromatosis, with special reference to supervening carcinoma of liver. M. J. Australia 2: 666–669, 1941.

(*42*) HIGGINSON, J., GERRITSEN, T., and WALKER, A. R. P.: Siderosis in the Bantu of Southern Africa. Am. J. Path. 29: 779–815, 1953.

(*43*) HIGGINSON, J.: Pathogenesis of liver cancer in the Johannesburg area. (S. Africa). Part I. Acta Unio internat. contra cancrum 13: 590–598, 1957.

(*44*) BUTT, E. M., and HIGGINSON, J.: Trace element pattern in liver disease and liver carcinoma. Part II. Acta Unio internat. contra cancrum 13: 599–601, 1957.

(*45*) HIGGINSON, J., and OETTLE, A. G.: The incidence of liver cancer in South Africa. Part III. Acta Unio internat. contra cancrum 13: 602–605, 1957.

(*46*) WARREN, F. L.: Estimations of iron in the lymph glands of mice during treatment with a carcinogenic compound. Biochem. J. 33: 729–733, 1939.

(*47*) PARSONS, L. D., and WARREN, F. L.: Cellular changes in the spleen and lymph glands in mice used for carcinogenic and related experiments, with special reference to giant cells of spleen. J. Path. & Bact. 52: 305–321, 1941.

(*48*) STRONG, L. C.: Hemoglobin levels in various degrees of susceptibility to spontaneous tumors. Am. J. Cancer 27: 500–509, 1936.

(*49*) FRANCIS, L. D., and STRONG, L. C.: Hemoglobin studies on blood of female mice of CBA strain: effects of age, diet, strain, and reproduction. Am. J. Physiol. 124: 511–516, 1938.

(*50*) STRONG, L. C., and FRANCIS, L. D.: Differences in hemoglobin values in the blood of breeder female mice; a comparison between cancer-susceptible and cancer-resistant strains. Am. J. Cancer 38: 399–403, 1940.

(*51*) GOULDEN, F., and WARREN, F. L.: The hemoglobin content of the blood of mice of the RIII and CBA strains. Cancer Res. 4: 421–424, 1944.

(*52*) WARREN, F. L., and GOULDEN, F.: The non-heme iron content of the tissues of mice of high-cancer and low-cancer strains. Cancer Res. 4: 417–420, 1944.

(*53*) PRICE, V. E., and GREENFIELD. R. E.: Anemia in cancer. Advances Cancer Res. 5: 199–290, 1958.

(*54*) GREENFIELD, R. E., GODFREY, J. E., and PRICE, V. E.: Studies on the anemia of tumor-bearing animals. I. Distribution of radioiron following the injection of labeled erythrocytes. J. Nat. Cancer Inst. 21: 641–656, 1958.

(55) GELLHORN, A., HYMAN, G., and ULTMANN, J.: The mechanism of the anemia of cancer. New York Med. 13: 614–626, 1957.
(56) VON HEVESY, G.: Die Krebsanämie. Naturwiss. Rdsch. 11: 247–252, 1958.
(57) RAWLINSON, H. E.: The iron content of the resting mammary glands of normal and tumour-bearing C3H mice. Acta Unio internat. contra cancrum 12: 711–717, 1956.
(58) OLSON, K. B., HEGGEN, G., EDWARDS, C. F., and GORHAM, L. W.: Trace element content of cancerous and noncancerous human liver tissue. Science 119: 772–773, 1954.
(59) LASNITZKI, A., and WOODHOUSE, D. L.: Formation of haemolymph nodes in rats treated with 1:2:5:6-dibenzanthracene. Nature, London 150: 660, 1942.
(60) ———: Effect of 1:2:5:6-dibenzanthracene on lymph-nodes of rat. J. Anat. 78: 121–129, 1944.
(61) HADDOW, A., HARRIS, R. J. C., KON, G. A. R., and ROE, E. M. F.: The growth-inhibitory and carcinogenic properties of 4-aminostilbene and derivatives. Phil. Trans. A. Roy. Soc. London 241: 147–195, 1948.
(62) ELSON, L. A.: Hematological effects of the alkylating agents. Ann. New York Acad. Sc. 68: 826–833, 1958.
(63) NEISH, W. J. P.: Effect of size and age of female rats on their response to the methaemoglobinogenic action of 3′-methyl-4-dimethylaminoazobenzene. Nature, London 178: 1350–1351, 1956.
(64) GREENSTEIN, J. P.: Further studies of the liver catalase activity of tumor-bearing animals. J. Nat. Cancer Inst. 3: 397–404, 1943.
(65) FIGGE, F. H. J.: The relationship of pyrrol compounds to carcinogenesis. *In* A.A.A.S. Research Conf. Cancer (1944), pp. 117–128, 1945.
(66) CHANCE, B., and CASTOR, L. N.: Some patterns of the respiratory pigments of ascites tumors of mice. Science 116: 200–202, 1952.
(67) CHANCE, B., and HESS, B.: Spectroscopic evidence of metabolic control. Science 129: 700–708, 1959.
(68) RIMINGTON, C.: Haem pigments and porphyrins. Ann. Rev. Biochem. 26: 561–586, 1957.
(69) KENNEDY, G. Y.: The chemistry and metabolism of some porphyrins and porphyrin derivatives. Univ. Sheffield, Ph.D. Thesis, 1954.
(70) LOCHHEAD, A. C., and GOLDBERG, R.: Transfer of iron to protoporphyrin for haem biosynthesis: role of ascorbic acid and glutathione. Lancet 2: 271–272, 1959.
(71) BERGEL, F., and BRAY, R. C.: The role of metals in oxidations catalysed by xanthine oxidase and by other metalloflavoproteins. Symp. Biochem. Soc. 15: 64–75, 1958.
(72) MAZUR, A., and GREEN, S.: Relation of iron to sulphydryl groups in ferritin. *In* Sulphur in Proteins. (Benesch, R., *et al.*, ed.) New York, Academic Press, Inc., 1959, pp. 189–196.
(73) MAZUR, A., GREEN, S., SAHA, A., and CARLETON, A.: Mechanism of release of ferritin iron *in vivo* by xanthine oxidase. J. Clin. Invest. 37: 1809–1817, 1958.
(74) GREEN, S., SAHA, A. K., CARLETON, A. W., and MAZUR, A.: Release of storage iron to the plasma by xanthine oxidase after purine administration. (Abstract.) Fed. Proc. 17: 233, 1958.
(75) ZONDEK, S. G., and KARP, J.: The relationship of iron with the ageing of cells. Biochem. J. 28: 587–591, 1934.
(76) GOLDBLATT, M. W., and GOLDBLATT, J.: Some Aspects of Industrial Toxicology. *In* Industrial Medicine and Hygiene. (Merewether, E. R. A., ed.). London, Butterworth & Co., Ltd., 1956, vol. 3, p. 473.
(77) DRABKIN, D. L.: Metabolism of the hemin chromoproteins. Physiol. Rev. 31: 345–431, 1951.
(78) BAKER, J. R.: Principles of Biological Microtechnique. London, Methuen & Co., Ltd., 1958.

(*79*) VICKERSTAFF, T.: Physical Chemistry of Dyeing. 2d ed., Edinburgh, Oliver & Boyd, Ltd., 1954.

(*80*) WIGGLESWORTH, V. B.: The role of iron in histological staining. Quart. J. Micr. Sc. 93: 105–118, 1952.

(*81*) KIRBY, K. S.: A new method for the isolation of deoxyribonucleic acids: Evidence on the nature of bonds between deoxyribonucleic acid and protein. Biochem. J. 66: 495–504, 1957.

(*82*) ZAMENHOF, S.: Deoxyribonucleic acid (DNA). Biochem. Prep. 6: 8–12, 1958.

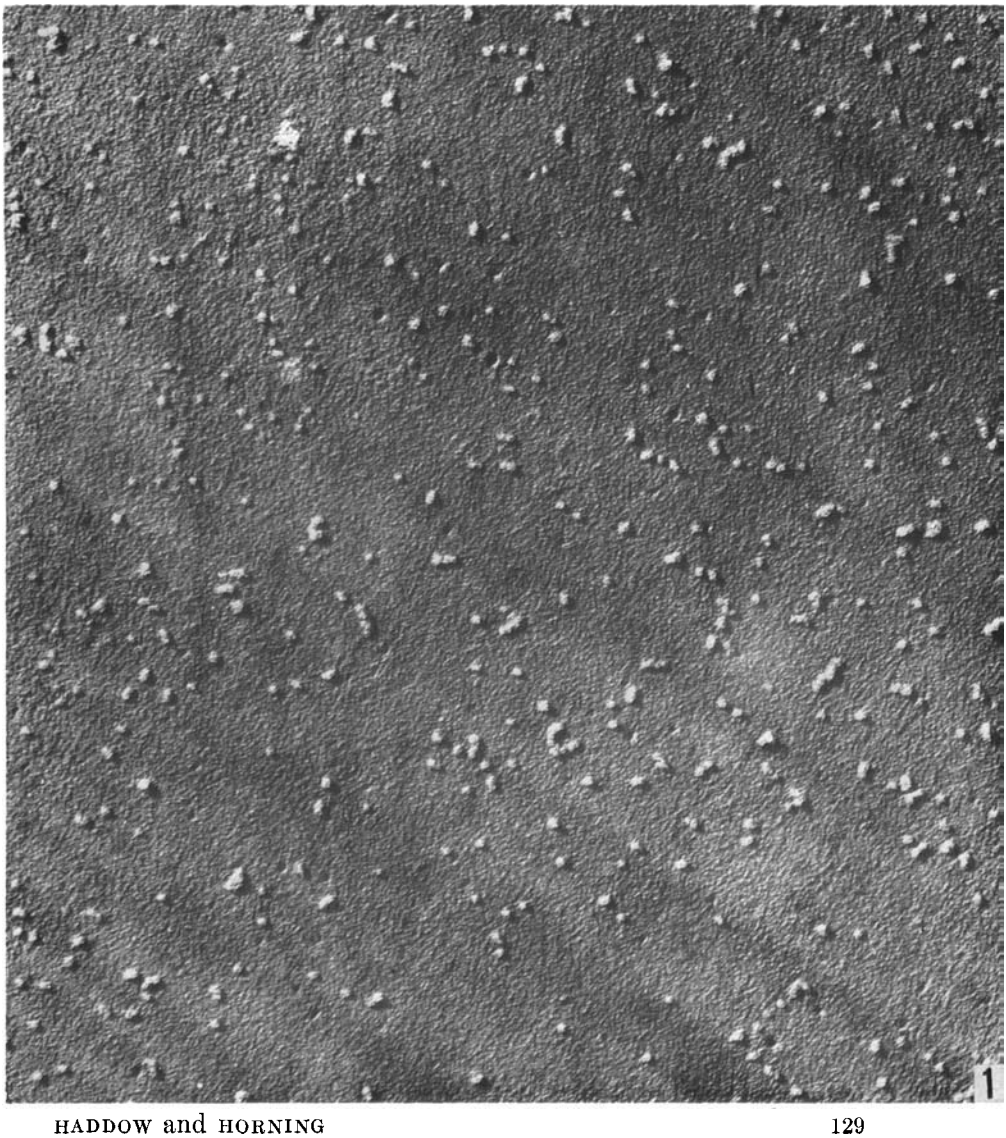
(*83*) UNDERWOOD, E. J.: Trace Elements in Human and Animal Nutrition. New York, Academic Press, Inc., 1956.

(*84*) EVANS, L. A. J., and RAMSEY, N. W.: Absorption studies on radioactive iron-dextran in pregnancy. Lancet 2: 1192–1196, 1957.

PLATES

PLATE 2

FIGURE 1.—Electron micrograph (tungsten-shadowed) of iron-dextran particles and clusters. × 160,000



HADDOW and HORNING 129

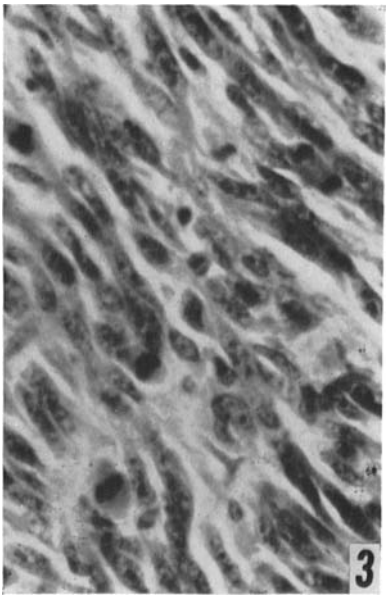
528860—60——12

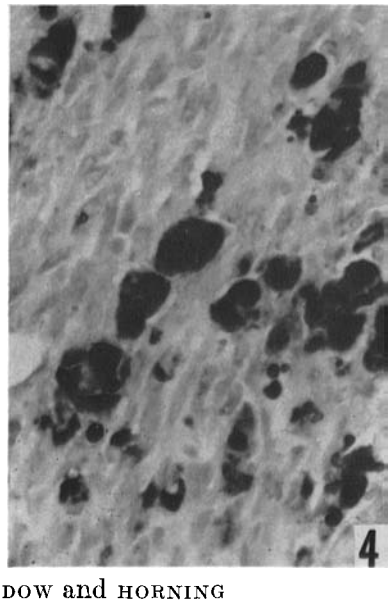
PLATE 3

FIGURE 2.—Epilation as a result of repeated subcutaneous injection of iron-dextran.

FIGURE 3.—Spindle-cell sarcoma in the mouse, induced by iron-dextran 0.2 cc. subcutaneously on 13 occasions. × 480

FIGURES 4 AND 5.—Examples of iron-dextran-induced sarcomas in the first series in the mouse. × 320





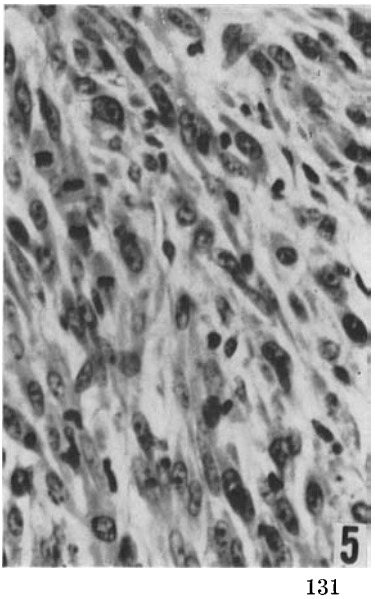


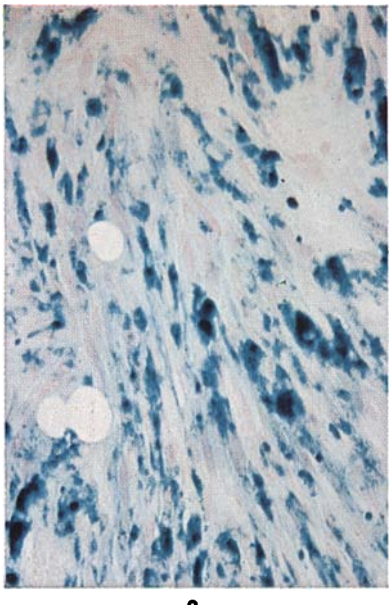
PLATE 4

FIGURE 6.—The Prussian-blue reaction in same tumor shown in figure 3. × 240

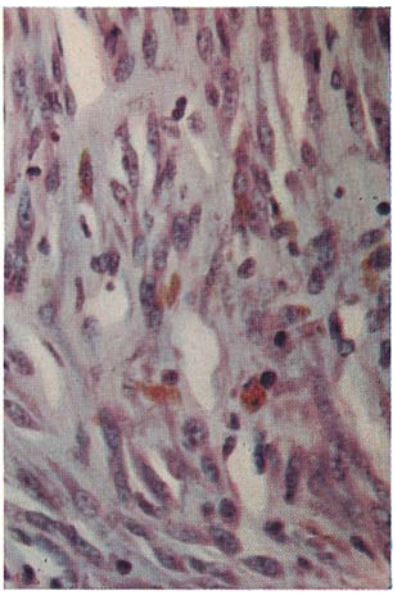
FIGURE 7.—Example of iron-dextran-induced sarcoma in the first series in the mouse. × 320

FIGURE 8.—Coincident hypertrophic areas in liver. × 60

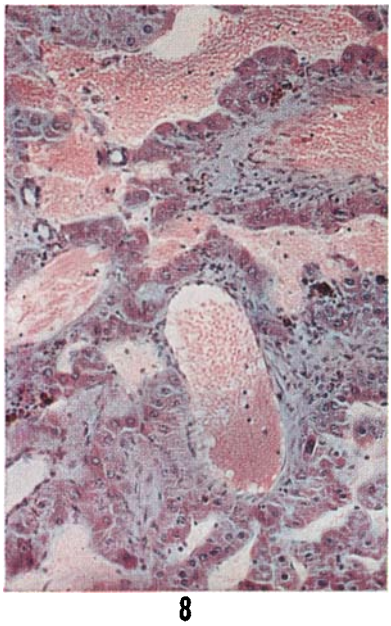
FIGURE 9.—Seventh primary iron-dextran-induced sarcoma in the second mouse series. × 158



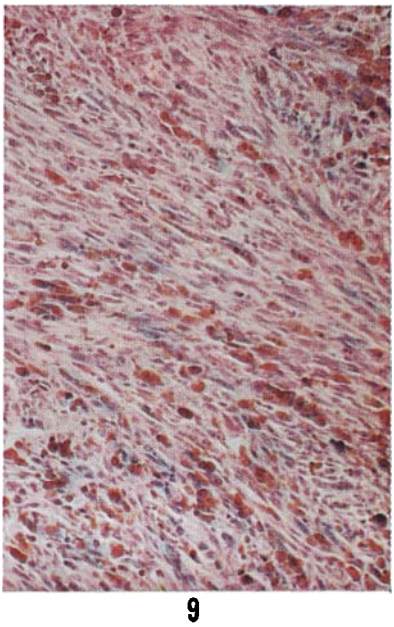
6



7



8



9

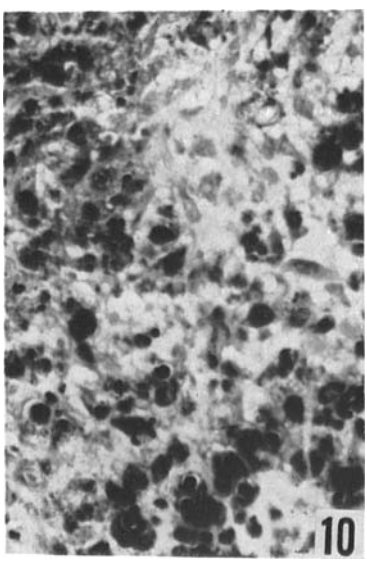
PLATE 5

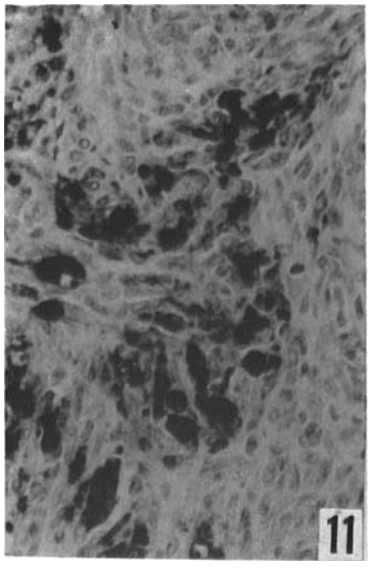
FIGURE 10.—Prussian-blue reaction in iron-dextran-induced sarcoma. × 200

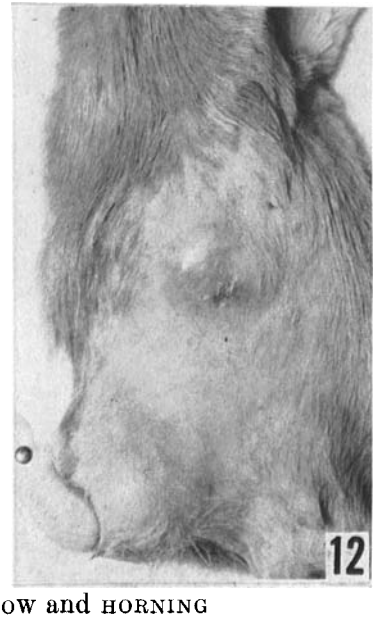
FIGURE 11.—Primary iron-dextran-induced mouse sarcoma. × 240

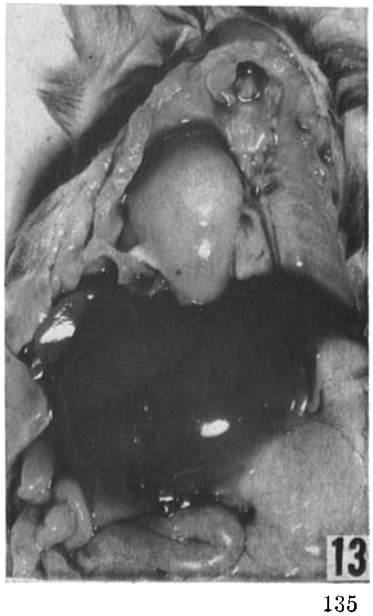
FIGURE 12.—Primary induced sarcoma with tumor mass in hind limb.

FIGURE 13.—Coincident bronchogenic carcinoma.









528860—60——13

PLATE 6

FIGURE 14.—Primary iron-dextran-induced mouse sarcoma: islets of ferritin granules in cytoplasm.

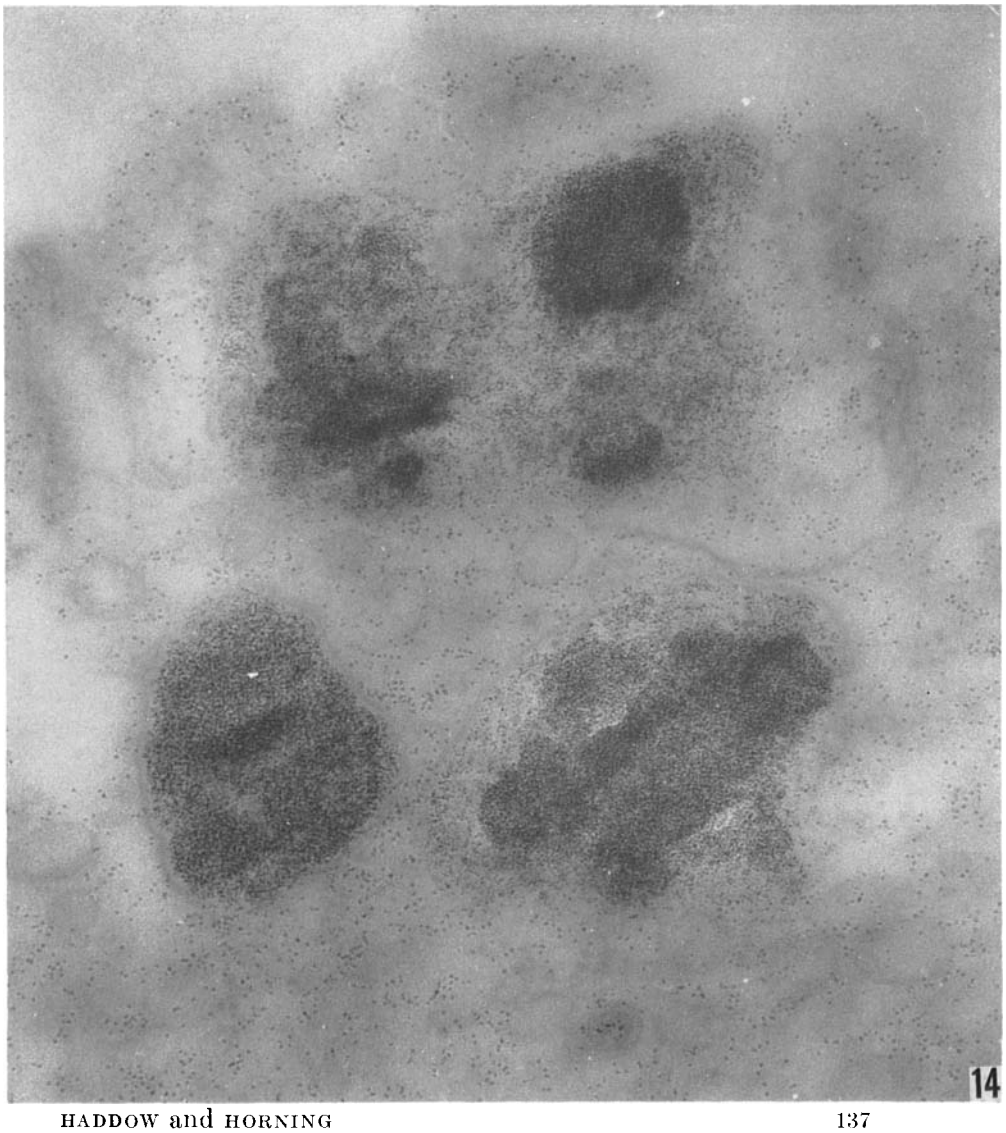


PLATE 7

FIGURE 15.—Ferritin granules in splenic macrophage (18-month-old rat). × 160,000

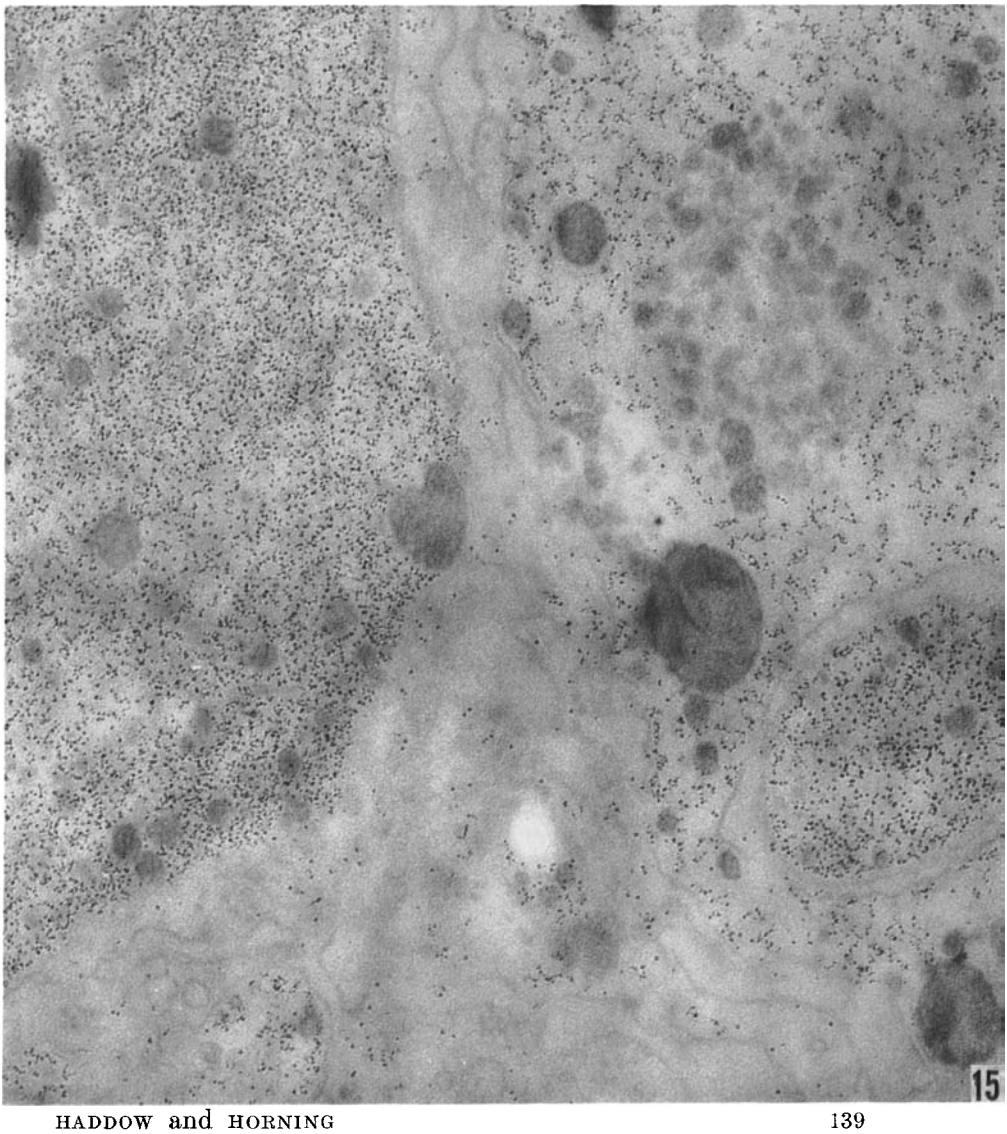
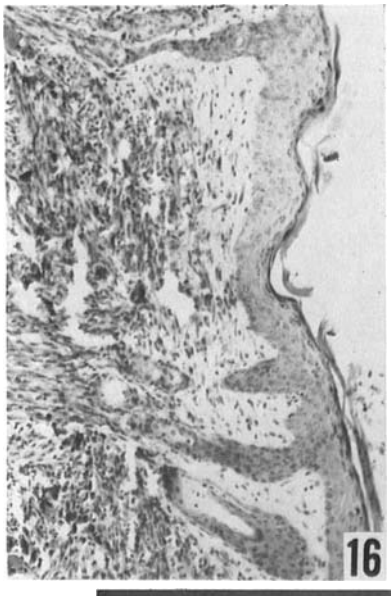
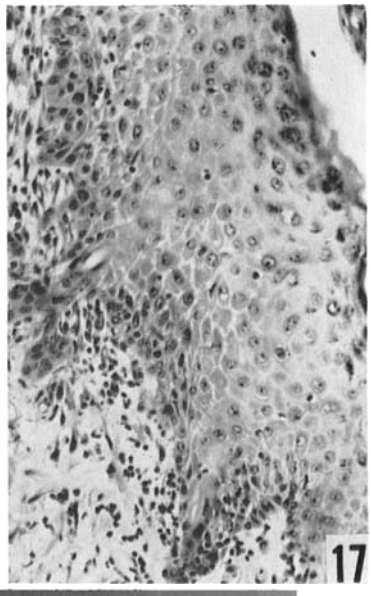


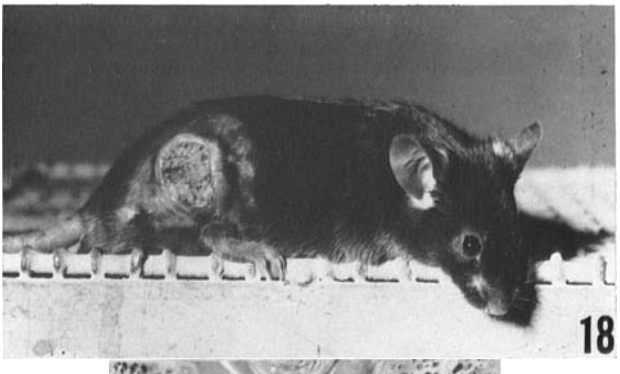
PLATE 8

FIGURES 16 AND 17.—Epithelial downgrowth overlying primary induced sarcomas. (Fig. 16 × 158; fig. 17 × 80.)

FIGURES 18 AND 19.—Epithelioma induced at site of injection of a poorly absorbed specimen of iron-dextran (fig. 19 × 90).







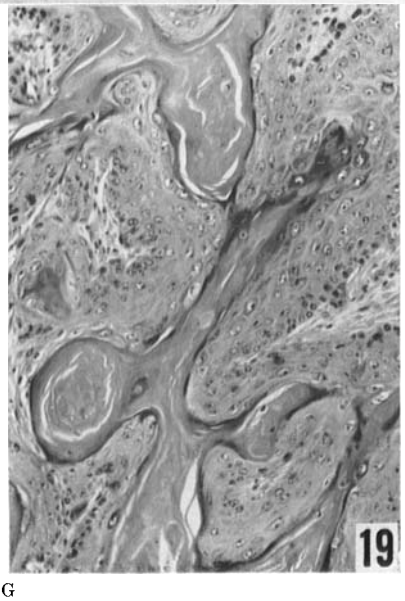


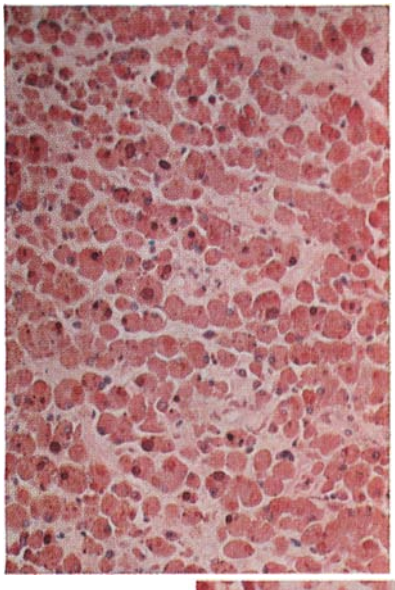
PLATE 9

FIGURE 20.—Induced histiocytoma of mouse. × 158

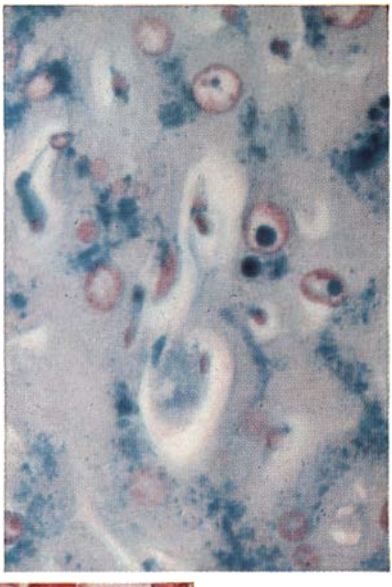
FIGURE 21.—Spherical intranuclear iron-containing inclusion bodies in hepati parenchyma. × 480

FIGURE 22.—Benign iron-dextran-induced rat histiocytoma. × 260

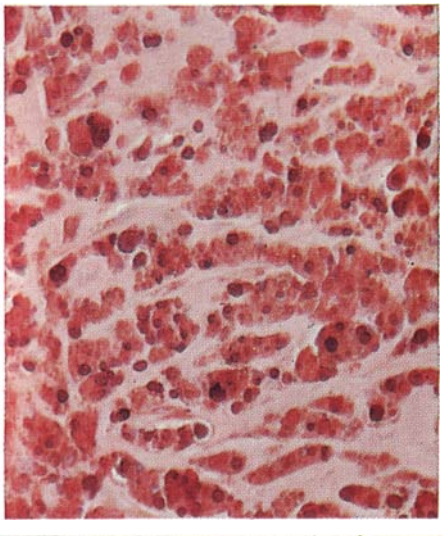
FIGURE 23.—The distribution of iron in intestinal mucosa. × 160



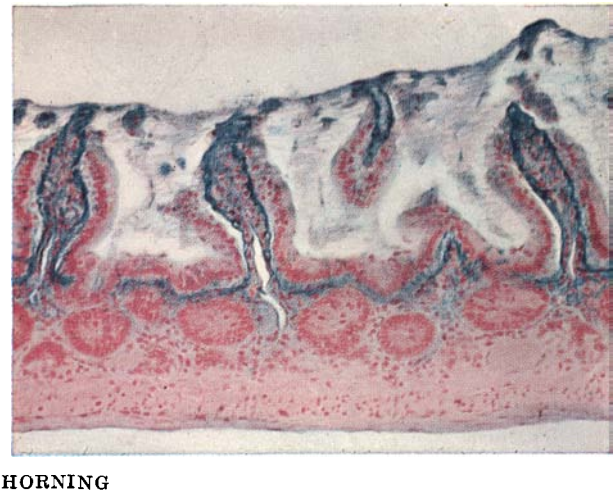
20



21



22



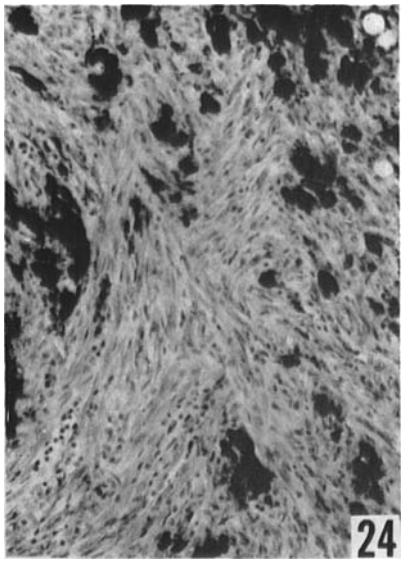
23

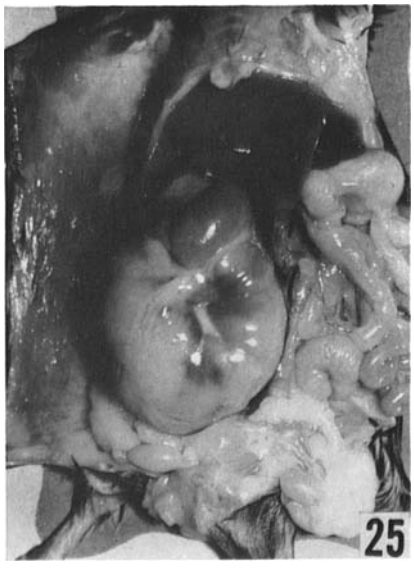
PLATE 10

FIGURE 24.—Primary fibrosarcoma induced by repeated subcutaneous injection of a poorly absorbed specimen of iron-dextran. This tumor had invaded the abdominal wall, with widespread lymphatic metastasis. × 160

FIGURE 25.—Intra-abdominal lymphosarcoma.

FIGURE 26.—Inhibition by iron-dextran of regrowth of hair, with tuft stimulated by injection trauma.





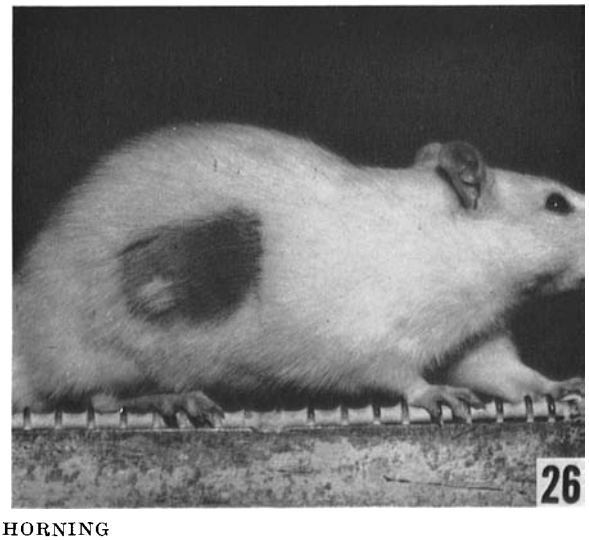


PLATE 11

FIGURE 27.—Adenocarcinoma of mammary gland in a male rat forced-fed with oral iron-dextran.

FIGURE 28.—Iron-dextran-induced hypertrophy of liver in male Chinese hamster.

FIGURE 29.—Iron-dextran-induced spindle-cell sarcoma in male rat, with dispersed pigment-laden macrophages. × 300

FIGURE 30.—Pleomorphic mouse sarcoma induced by 8-hydroxyquinoline copper complex. × 216