The low molecular weight of iron-sorbitol complex resulted in a rapid excretion in the urine. This was maximal in the first twelve hours. The urinary excretion for the 48-hour period after injection ranged from 18% to 53%.

The red-cell utilization at ten days ranged from 27% to 66%. Utilization in the iron-deficient group was greater than in the control group. The utilization curve in the iron-deficient group showed that maximal utilization had not occurred by T+10 days.

The surface counting data suggested that the retained fraction of iron not utilized for haemoglobin synthesis by T+10 days (16% to 40%) was stored predominantly in the liver.

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REFERENCES

Andersson, N. S. E. (1961). *Brit. med. J.*, **2**, 275.
Barnes, J. M., and Trueta, J. (1941). *Lancet*, **1**, 623.
Beresford, C. R., Golberg, L., and Smith, J. P. (1957). *Brit. J. Pharmacol.*, **12**, 107.
Bothwell, T. H., and Mallett, B. (1955). *Biochem. J.*, **59**, 599.
Dacie, J. V. (1956). *Practical Haematology*, 2nd ed. Churchill, London.
Eriksson, F. R. (1961). Quoted by Svärd and Lindvall (1961).
Grimes, A. J., and Hutt, M. S. R. (1957). *Brit. med. J.*, **2**, 1074.
Ledlie, E. M., and Baxter, C. F. (1954). *Proceedings of 2nd Radio-active Isotope Conference*, p. 97. Butterworths, London.
Lindvall, S., and Andersson, N. S. E. (1961). *Brit. J. Pharmacol.*, **17**, 358.
Loeffler, R. K., Rappoport, D. A., and Collins, V. P. (1955). *Proc. Soc. exp. Biol. (N.Y.)*, **88**, 441.
Mollison, P. L. (1960). *Recent Advances in Clinical Pathology*, edited by S. C. Dyke, p. 223. Churchill, London.
Svärd, P. O. (1961). *J. Pharm. Pharmacol.*, **13**, 641.
—— and Lindvall, S. (1961). Ibid., **13**, 650.
Swedberg, B. (1962). *Proceedings of 8th Congress of European Society of Haematology*, p. 254. Karger, Basel.
Wetherley-Mein, G., Hutt, M. S. R., Langmead, W. A., and Hill, M. J. (1956). *Brit. med. J.*, **1**, 1445.

SARCOMA INDUCTION BY IRON-CARBOHYDRATE COMPLEXES

BY

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[WITH SPECIAL PLATE]

In order to administer iron parenterally without incurring the toxic effects of the ionized metal, ferric hydroxide has been bound to carbohydrates to form complexes of fairly high molecular weight. These complexes simulate the binding of iron by the beta-globulin transferrin, the physiological carrier of iron in the circulation. The first clinically useful carbohydrate complex was made with saccharose, later with dextrans and dextrins, and more recently with sorbitol. In 1959 Richmond reported that iron–dextran ("imferon") induced sarcomas in rats after intramuscular injection in massive doses. Haddow and Horning (1960) confirmed this in both rats and mice. This unexpected observation restimulated interest in the role which metals may play in carcinogenesis; it also demanded reconsideration of the use of iron–carbohydrate complexes in man for the parenteral treatment of iron deficiency.

The discussion has centred mainly on the massive dose of iron complex used to induce sarcomas in experimental animals. Golberg and his co-workers emphasized the systemic effects of massive iron overload, which include reticulo-endothelial stimulation, altered tissue enzyme activity, increased tissue peroxide and lipofuscin polymer formation, indicative of defective vitamin-E metabolism. They propose that the iron dosage given to experimental animals should be compared to the dosage used in treatment on a body-weight basis in order to assess the possible tumour risk in man. They point out that similar biochemical changes occur at the site of injection, and postulate that the bulk of tissue available for injection relative to the amount injected is also relevant (Golberg *et al.*, 1960; Baker *et al.*, 1961).

Haddow and Horning (1960) concluded that sarcoma induction by iron–dextran is essentially a local action. This implies that the absolute amount of iron injected is the determinate factor rather than the body-weight/dose ratio (Haddow, 1960).

Most experimental work so far has been with mice and rats, which are evidently highly susceptible species. Haddow and Horning record a single induced tumour in a hamster; they failed to produce tumours in guinea-pigs, but of three surviving rabbits in a group of six treated with massive iron–dextran one has produced a tumour after a latent period of 39 months (Haddow, 1961). Golberg and his co-workers (1960) failed to induce tumours in dogs.

Previously published experimental findings in rats are summarized in Table I. Total dosages of 250 mg. or more of iron as iron–dextran given intramuscularly or subcutaneously in divided doses into a single site produce high yields of sarcomas varying from 55% to 92% of animals at risk. At such dose levels the sarcogenic action of iron–dextran is evidently easily reproducible in different laboratories with different strains of animal. The results of Golberg and his co-workers (1960) are of particular interest, since their experiments differed in design from others: the doses were given alternately into two limbs instead of into a single site. Injecting 116 mg. of iron as iron–dextran into each limb —that is, a total of 232 mg. Fe—they obtained only a 5% yield of tumours, compared with the much higher yields obtained when a similar total dose is given into a single site. There is perhaps evidence here which favours the "local action" hypothesis rather than the systemic effect of iron overload as the significant factor in iron sarcogenesis. Table I also shows a comparison made by Lundin (1962) of three iron complexes. He found that both iron–dextrin and iron–dextran in high dosage produced high yields of tumours in rats, whereas iron-sorbitol produced a single tumour, which he described as a fibroma rather than a sarcoma. The significance of these results is discussed below.

The object of the experiment described here was to test the effect in mice of subcutaneous injection of a

lower dosage of iron–dextran than had been previously described, and at the same time to compare sarcoma induction by three iron–carbohydrate complexes with differing properties.

Methods and Materials

Male albino mice of a Tuck strain were used, weighing 30 to 35 g. at the beginning of the experiment. They were fed "oxoid" S.G. 1 pellet diet.

Iron–dextran was used as "imferon"; iron–dextrin as "astrafer"; iron–sorbitol as "jectofer."

The dose of iron complex per injection was limited by the largest amount of iron–sorbitol complex which could be given at one time without excessive mortality.

Each animal was given 1 mg. iron as undiluted iron complex subcutaneously into the left flank at weekly intervals, using a micrometer syringe. In the case of iron–dextran and of iron–sorbitol each dose was contained in 0.02 ml.; a single dose of iron–dextrin was contained in 0.05 ml. Treatment of the iron–dextran group was started two weeks later than that of the other groups, and all injections were stopped at the same time when 28 mg. iron as iron–dextran and 30 mg. as iron–dextrin or iron–sorbitol had been given.

Results

Trophic Effects at Injection Site

The animals given iron–dextran and iron–dextrin all showed a marked loss of hair over the injection site developing three months or more after the injections began. Haddow and Horning described a similar failure of hair to regrow at the injection site after preliminary depilation. In the present experiment the skin was not depilated, but an area of complete alopecia resulted nevertheless. The skin revealed by the loss of hair was deeply brown-pigmented and lacking in elasticity and tone. These effects were not seen in the iron–sorbitol-treated animals.

Lymphatic Obstruction of Injection Region

Many of the iron–dextran- and iron–dextrin-treated animals developed a persistent unilateral soft-tissue swelling of the skin and abdominal wall on the treated side. Some of these in both groups showed the effect in marked degree, developing a unilateral abdominal swelling sharply limited by the mid-abdominal line, large enough to reach the ground on the affected side, and clearly the result of lymphatic obstruction of the region drained by the injection site. None of the iron–sorbitol-treated animals showed such evidence of lymphatic obstruction.

Residual Iron at Injection Site

A striking feature of the skin and subcutaneous tissues at the injection site has been the contrast between the massive amounts of residual iron in the iron–dextran- and iron–dextrin-treated animals compared with the relatively small amounts of iron remaining in the iron–sorbitol-treated group. The difference is seen grossly in skin staining mentioned above. Section of the skin and subcutaneous tissues at the injection site demonstrates this more clearly. Special Plate, Fig. 1 shows typical injection-site sections stained for iron in the three groups in animals which had not suffered malignant change 17 months after the beginning of the experiment. The iron–dextrin group showed the largest amounts of residual iron; there were also massive residual deposits of iron–dextran, while iron–sorbitol was retained in relatively small amounts.

Tumour Induction (Table II)

Of 20 animals given a total of 28 mg. iron as iron–dextran, 17 survived 12 months. In two animals a tumour developed at the injection site 11 and 13 months respectively from the beginning.

Among 20 animals given iron–dextrin in doses containing 1 mg. iron 12 survived 12 months. In three

TABLE I.—*Tumour Induction by Iron Complexes in Rats*

Author	Iron Complex	Route	Dose (mg. Fe)	Total Fe Given (mg.)	Length of Treatment (Months)	Animals at Start	Animals at Risk	Tumours
Richmond, 1959 ..	Iron–dextran	Intramuscular	20 5–20	880–1,250 475	11–16 3	40 40	23 40	16 (70%) 22 (55%)
Haddow and Horning, 1960	,,	Subcutaneous	50	1,200	6	30	30	24 (80%)
Golberg *et al.*, 1960 ..	,,	Intramuscular	10 10	436+436* 116+116*	13 5	20 93	20† 66†	12 (60%) 3 (5%)
Lundin, 1961	,, Iron–dextrin	,, ,,	5–20 5–20	510 510	4 4	39 39	27 30	25 (92%) 25 (83%)
	Iron–dextran Iron–dextrin Iron–sorbitol	,, ,, ,,	2·5–10 2·5–10 2·5–10	255 255 255	4 4 4	39 39 53	31 31 30	26 (84%) 16 (51%) 1 (3%)

* Into each hind limb. † Survivors at 1 year.

TABLE II.—*Tumour Induction by Iron Complexes in Mice*

Author	Iron Complex	Route	Dose (mg. Fe)	Total Fe Given (mg.)	Length of Treatment (Months)	Animals at Start	Survivors at 0 Months	Tumours
Haddow and Horning, 1960	Iron–dextran	Subcutaneous	15 10	400 130	7 3	30 25	23 (6) 14 (12)	18 10
Golberg *et al.*, 1960 ..	,,	Intramuscular	10 10 5 1	50+50* Continuous weekly dose	2½	50 50 50 50	25 (12) 0 (12) 2 (12) 22 (12)	0 0 1† 0
Present experiment ..	,, Iron–dextran Iron–sorbitol	Subcutaneous ,, ,,	1 1 1	28 30 30	6½ 7 7	20 20 40	17 (12) 12 (12) 28 (12)	2 3 0

* Into each hind limb. † At 47 weeks.

injection-site tumours developed; one tumour occurred six months after the first injection, by which time only 26 mg. iron had been given; two other tumours occurred at 12 and 13 months respectively from the start, after a total of 30 mg. iron.

Of 40 mice given 30 mg. iron as iron–sorbitol, 28 survived 12 months. None had produced an injection-site tumour when the experiment was terminated 17 months from the start.

Histology

Several descriptions of the tumours induced by iron–dextran have now appeared (Richmond, 1959, 1960; Muir and Golberg, 1961; Lundin, 1961). The study by Muir and Golberg is particularly detailed and amplified by electron-microscopy. From the consistent pattern of cellular morphology which emerges, these tumours evidently form a well-defined entity.

The five injection-site tumours encountered in the present experiment conform to those previously described. The features of the iron–dextran- and iron-dextrin-induced tumours are similar. A prominent histological feature is the presence of large rounded histiocytes laden with iron and other pigment inclusions. The presence of these inclusion-laden histiocytes does not itself characterize the malignancy, since they are also found in large numbers at injection sites when no tumours develop. Many contain multiple and aberrant nuclei, and Richmond (1959) suggests that malignancy begins in these cells. In the zones around these large histiocytes are seen gradations in form from histiocytes to fibroblasts containing progressively smaller amounts of iron pigment. The main tumour mass consists of interlacing bundles and whorls of spindle cells showing considerable pleomorphism and many mitotic figures. There is a notable absence of intracellular iron in these tumour cells. The earliest identifiable indication of tumour formation is seen as a focal proliferation of fibroblasts. Such foci are seen in Special Plate, Fig. 2 in a subcutaneous nodule from an iron–dextrin-treated animal 17 months after the beginning of treatment. This nodule had shown no change in size for several months; it has not been included in the numbers of induced tumours. Collagen formation is variable, but quite considerable in some tumours. One tumour consisted mainly of polygonal cells with abundant eosinophilic cytoplasm, a feature also noted by Richmond (1959).

The degree of malignancy, as judged by the rate of growth, infiltration of skin and muscle, the number of mitoses, and the degree of collagen formation, varies from tumour to tumour. No metastases were found in the affected animals in these experiments, though they have been described elsewhere (Haddow and Horning, 1960; Richmond, 1959).

Discussion

A comparison of the sarcoma yield in rats following intramuscular injection of iron–dextran in experiments by various authors (Table I) suggests that there is a dose–response relationship. In mice Haddow and Horning (1960) showed that 130 mg. or more iron as iron–dextran injected subcutaneously into a single site gave a high yield of injection-site sarcomas. On a body-weight basis this is about 150 times the total amount given in the treatment of a severe iron-deficiency in man. Golberg and his co-workers (1960) in an extensive series of experiments in mice used the intramuscular route, and their results are thus not comparable with those of Haddow and Horning; they obtained only one sarcoma in an animal given 235 mg. of iron as iron–dextran. Unfortunately a high mortality among their animals obscured the significance of their results.

In the present experiment two injection-site sarcomas were seen in 20 animals given a total of 28 mg. iron as iron–dextran into a single subcutaneous site. There thus appears to be a dose–tumour-induction relationship in mice also, and the dose of iron–dextran used here is evidently at or near the threshold dose for sarcoma induction in mice by the subcutaneous route. This amount—that is, 28 mg. of iron—is about 1/35 of the average total human dose; it was given in divided amounts of 1 mg. iron in 0.02 ml., which is 1/250 of the usual single clinical dose. On a body-weight basis it represents about 40 times the human treatment dose. Table II summarizes these results.

Though the number of tumours produced is small, it would appear that the carcinogenicity of iron–dextrin is of the same order as that of iron–dextran. One of the three tumours produced in the iron–dextrin group appeared six months after the beginning of treatment, at which time 26 mg. iron had been given. This is the smallest tumour-inducing dose recorded for a carbohydrate complex.

In their detailed study of tissue changes, including sarcoma formation, following iron–dextran injections, Baker *et al.* (1961) concluded that the accumulation of large amounts of iron at the site of injection consequent on the breakdown of absorption mechanisms was an essential factor in the initiation of malignant change. They have moved in this towards Haddow and Horning's opinion that the action is essentially a local one.

The distribution of iron in these tumours is characteristic, and any view of pathogenesis should include an adequate explanation of it. The focal fibroblastic nodule, itself relatively free of iron, is seen before macroscopic or microscopic evidence of malignancy, set in the depths of a zone of densely packed iron-laden histiocytes. In the smaller tumours the bundles of spindle cells containing little iron interweave among small islands of similar iron-packed histiocytes (Special Plate, Fig. 3). In the larger tumours, sectioned after five or six weeks of obvious growth, the main tumour masses are notably free of iron, and only isolated iron-containing histiocytes are found irregularly scattered among them.

These appearances are consistent with a neoplastic origin in these fibroblastic foci, growing out among the dense histiocytic masses, splitting them into isolated groups and finally into isolated iron-laden cells. These tumours therefore appear to be fibrosarcomas originating in the depths of a densely packed histiocytic iron-bearing infiltration. Such a pathogenesis is more consistent with the appearances than a neoplastic change in the histiocytes themselves, which progressively diminish their iron content by sharing on division, as postulated by Richmond.

Iron–dextran has a relatively high average molecular weight of the order of 180,000 (Eriksson, 1961) and is dependent mainly, if not entirely, on lymphatic absorption (Beresford *et al.*, 1957).

The lymphatic route is evidently easily blocked by repeated injections. The lymphoedema shown by many of the iron–dextran- and iron–dextrin-treated animals was severe enough in some to be recognized as an elephantiasis of the abdominal wall. Baker *et al.* (1961) have similarly observed increased water content of the skin over the injection site after intramuscular injection of iron–dextran. This obstruction to the only absorption route available must clearly contribute in considerable measure to the fixation of injected iron at the site of deposition. It is of interest to recall that one of the few clinical toxic reactions resulting from intramuscular injection of iron–dextran consists of a regional lymphadenitis related to the injection site.

Iron–dextrin has many properties which distinguish it from iron–dextran—for instance, its faster rate of reticulo-endothelial uptake (Fielding, 1961). Both complexes, however, have a high mean molecular weight and are equally dependent on lymphatic absorption. The similarity of the two complexes in sarcogenic activity may therefore be an expression of a common failure of absorption. In the case of iron–dextrin the tendency to local accumulation may be enhanced by the greater avidity for it shown by the reticulo-endothelial system, and presumably, therefore, by tissue histiocytes. This view links high molecular weight of complex, lymphatic absorption, and histiocytic uptake with sarcoma induction by reason of the incomplete iron absorption which they entail. It is a view supported by the findings in the iron–sorbitol-treated animals.

The iron–sorbitol complex has a relatively low mean molecular weight in the region of 5,000 (Eriksson, 1961). It is absorbed mainly directly into the peripheral circulation as well as to a smaller extent by the lymphatic route (Svärd and Lindvall, 1961). None of the iron–sorbitol-treated animals showed evidence of lymphatic obstruction or local trophic effects, while the amounts of iron retained at the site of injection were comparatively trivial. In this group of iron–sorbitol-treated mice no sarcomas were observed. Lundin (1961), using iron–sorbitol in rats in higher dosage, which in the case of iron–dextrin and iron–dextran produced 16 and 26 sarcomas respectively, obtained one injection-site tumour in an iron–sorbitol-treated animal. He described this as a fibroma rather than a sarcoma; it apparently showed no malignant features.

Thus two iron–carbohydrate complexes which have in common high molecular weight, predominantly lymphatic absorption, and high residual iron are also sarcoma-inducing agents, while a third complex of low molecular weight, predominantly peripheral vascular absorption, and low residual iron is free from sarcoma induction in the dosage tested.

It is hypothesized, therefore, that iron complexes differ in their injection-site carcinogenic activity by virtue of differences in absorbability. It may be that all iron complexes or compounds are potentially carcinogenic in susceptible species, and their varying sarcogenic activity is an expression of varying degrees of fixation at injection sites.

The application of these findings to human therapy remains speculative. That man is a species susceptible to iron-induced malignancy is supported by two well-established observations: the association of hepatoma with haemochromatosis (Willis, 1953) and the high incidence of carcinoma of the bronchus in haematite miners (Faulds and Stewart, 1956). It would appear reasonable to suggest that iron complexes for human therapy should satisfy tests of absorbability from parenteral sites following repeated doses. Criteria for such tests are yet to be defined.

Summary

A comparison is made of injection-site sarcoma induction by three iron–carbohydrate complexes in mice following subcutaneous injection in dosages lower than previously described. Two tumours were observed in 20 animals after injection of 28 mg. iron as iron–dextran, after a latent period of about a year. Three tumours were observed in 20 animals treated with iron–dextrin: one tumour after 26 mg. Fe and a latent period of six months; two tumours after 30 mg. Fe. No tumours were noted after 17 months' observation in 40 animals given 30 mg. iron as iron–sorbitol.

It is suggested: (1) that dependence on lymphatic absorption is a characteristic predisposing to sarcoma induction by iron–carbohydrate complexes; and (2) that sarcoma induction by iron complexes in susceptible species is a function of the amount of iron retained at the site of injection.

REFERENCES

Baker, S. B. de C., Golberg, L., Martin, L. E., and Smith, J. P. (1961). *J. Path. Bact.*, **82**, 453.
Beresford, C. R., Golberg, L., and Smith, J. P. (1957). *Brit. J. Pharmacol.*, **12**, 107.
Eriksson, F. R. (1961). Cited by Svärd, P. O., and Lindvall, S.
Faulds, J. S., and Stewart, M. J. (1956). *J. Path. Bact.*, **72**, 353.
Fielding, J. (1961). *Brit. med. J.*, **2**, 279.
Golberg, L., Martin, L. E., and Smith, J. P. (1960). *Toxicol. appl. Pharmacol.*, **2**, 683.
Haddow, A. (1960). *Brit. med. J.*, **1**, 1734.
—— (1961). Personal communication.
—— and Horning, E. S. (1960). *J. nat. Cancer Inst.*, **24**, 109.
Lundin, P. M. (1961). *Brit. J. Cancer*, **15**, 838.
Muir, A. R., and Golberg, L. (1961). *J. Path. Bact.*, **82**, 471.
Richmond, H. G. (1959). *Brit. med. J.*, **1**, 947.
—— (1960). *Cancer Progress*, p. 24.
Svärd, P. O., and Lindvall, S. (1961). *J. Pharm. Pharmacol.*, **13**, 650.
Willis, R. A. (1953). *Pathology of Tumours*, 2nd ed. Butterworth, London.

The *Report on the Colonial Territories for 1961–62* has now been published. It states that malaria and tuberculosis remain the major health problems in many territories. Malaria-eradication programmes are in hand in a number of territories and are gradually making an impact on incidence, though in the African territories it has not been possible to control the infection adequately and it has been a significant cause of mortality in children. Tuberculosis is being tackled by vaccination, prophylactic treatment, mass miniature radiography, etc., and all these methods are clearly making an impression on the problem in, for example, Hong Kong, where the death rate fell from 208 per 100,000 in 1951 to 61.3 in 1961: but a great part of the available resources has still to be devoted to its treatment and control. There is still a shortage of doctors and nurses. Undergraduate medical education leading to degrees recognized by the General Medical Council for registration in Britain is available at four universities and university colleges. In addition, a number of students come to Britain: there were 478 studying medicine in the United Kingdom in January, 1962. Training of nurses in the territories continues to make progress, though there is a shortage of qualified tutors. In Uganda, for example, the number of locally trained nurses is expected to meet the needs of Government hospitals by 1963. Training in psychiatric nursing has been started in Kenya and Hong Kong. (H.M.S.O., Cmnd. 1751, price 8s. 6d.)

J. FIELDING: SARCOMA INDUCTION BY IRON-CARBOHYDRATE COMPLEXES

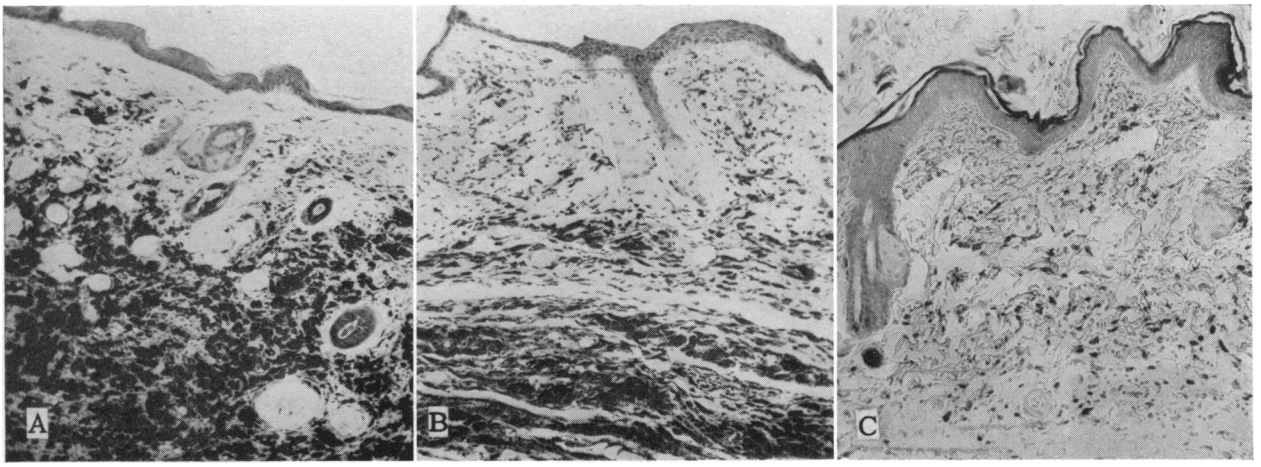

FIG. 1.—Skin and subcutaneous tissue at injection site stained for iron 10 months after injection of (A) 30 mg. iron-dextrin; (B) 28 mg. iron-dextran; and (C) 30 mg. iron-sorbitol. (Perl's stain. ×80.)

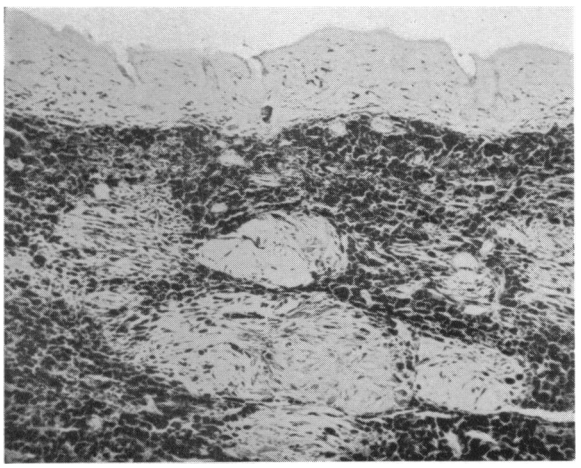

FIG. 2.—Subcutaneous focal fibroblastic proliferation among iron-laden histiocytes 10 months after injection of 30 mg. iron-dextrin. (Perl's stain. ×48.)

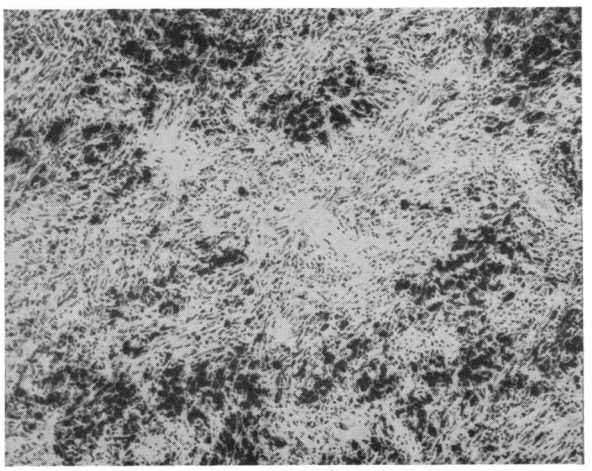

FIG. 3.—Tumour after three weeks' obvious growth, showing islands of iron-containing histiocytes in spindle-cell sarcoma. (Perl's stain. ×80.)

A. W. MORRISON: TREATMENT OF OTOSCLEROSIS BY STAPEDECTOMY

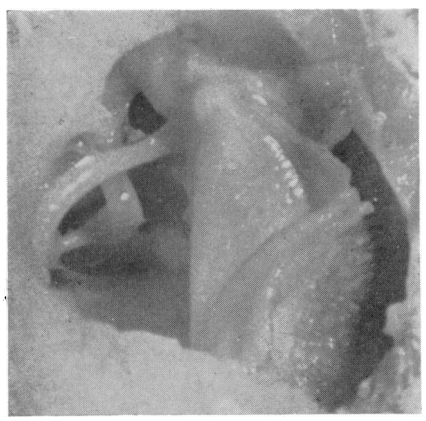

FIG. 1.—Middle ear opened, showing chorda tympani, long process of incus, stapes, and stapedius tendon.

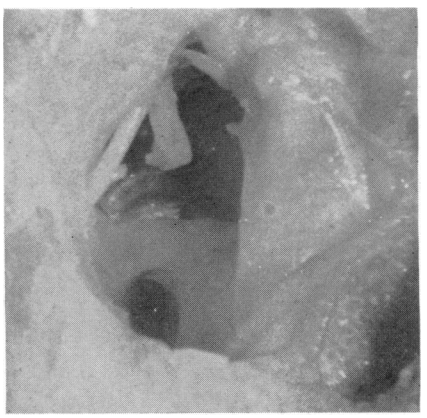

FIG. 2.—Stapes removed. Vein graft covering oval window. Round window clearly seen.

FIG. 3.—Polythene prosthesis in position, articulated with incus.