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LOCAL EFFECTS AND MECHANISM OF ABSORPTION OF IRON PREPARATIONS ADMINISTERED INTRAMUSCULARLY

BY

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An attempt has been made to correlate factors involved in the absorption of iron-polysaccharide complexes administered intramuscularly. Different complexes varied greatly in degree of retention in muscle and in diffusibility in agar; these two characteristics were not closely related. The local changes in the muscle produced by the iron complexes consisted of an acute inflammatory reaction at the site of injection, with degenerative changes. Subsequent regeneration was rapid and complete. The major proportion of the absorption occurred during the initial 72 hr. and appeared to be mediated partly by the inflammatory reaction evoked, with enhancement of lymphatic transport of the iron complex. Rapid fixation by tissue macrophages impeded absorption and, with some complexes, this factor may make much of the injection inaccessible.

Intramuscular injection of drugs has been practised for over 70 years, but the factors concerned in the mechanism of absorption of the injected substances have aroused little attention. At first sight absorption from muscle would appear to involve merely a process of passive diffusion through the extracellular fluid: this process if delayed would result in formation of a depot with gradual entry of the drug into the circulation. Drinker and Field (1933) showed that the path of absorption depends upon the molecular size of the injected substance, small molecules being absorbed directly into the blood stream whilst larger ones entered the lymphatics. The molecular weight range was more precisely characterized by Barnes and Trueta (1941), who showed that venoms and toxins of molecular weight 20,000 or more are absorbed by the lymphatics whilst cobra venom of molecular weight 5,000 enters the blood stream directly. Another factor which could influence absorption is the local effect of the injected substance on the muscle, a topic on which the literature is relatively silent, though there are many reports of nerve palsies and other mishaps arising from the intramuscular administration of certain drugs. With the object of throwing some light on the mechanism of absorption of iron, a study was undertaken of a number of iron polysaccharide complexes, including commercial and laboratory preparations.

METHODS AND MATERIALS

Quantitative Study of Absorption of Iron from Muscle.—The following four preparations of iron-polysaccharide complexes were studied: the iron-dextran complex "Imferon" (IDA), an iron-dextran complex known to possess poor absorption characteristics (IDB), an iron-polysaccharide complex marketed for human use (IPH), and an iron-polysaccharide complex marketed for veterinary use (IPV).

All four preparations contained 5% (w/v) of elemental iron. Each of the iron-dextran complexes was polydisperse, but it was likely that a proportion of each lies within the molecular weight range 10,000 to 20,000. Retention of iron in muscle was studied after doses equivalent to 20 mg. Fe/kg. were injected deep into the right gluteal muscles of rabbits weighing approximately 2 kg. At varying periods of time after the injection the animals were killed, the skin was removed from the leg, and the soft tissues were dissected away from the bones and placed in an acid-washed beaker. The tissue was wet-oxidized with sulphuric and nitric acid (Middleton and Stuckey, 1954). The solution after oxidation was diluted to 250 ml., and a suitable aliquot taken for determination of iron by the thiocyanate method (Ventura and Klopper, 1951). The left (uninjected) leg was treated in a similar manner to obtain the control iron value.

In vitro *Study of the Diffusion of Iron.*—These experiments were intended to compare the rates of diffusion *in vitro* of various iron-dextran complexes prepared by different methods. Serum-agar plates were prepared containing 0.7% agar in saline and 0.01%

merthiolate, with 20% (v/v) of horse serum. The iron complex (0.1 ml.) was added to a Whatman 3MM paper disc (18 mm.) placed on the agar. After keeping at room temperature overnight, the dishes were inverted and incubated at 37° C. for 6 days. Diffusion took place symmetrically outwards from the paper disc, and usually two zones were discernible; a dark inner zone immediately around the disc, and a lighter "halo" beyond. The diameters of the total zone of diffusion and of the dark inner zone were measured.

Histological Study of the Local Changes at the Injection Site.—The four iron polysaccharide complexes used in the first experiment were studied. Each was mixed with a small amount of Indian ink to assist identification of the injection site, and 0.1 ml. containing 5 mg. of iron was injected into the left soleus muscles of 28 adult rats (112 animals in all). In the case of rats given iron-dextran complex, the right soleus muscles were injected with 0.1 ml. of 20% dextran as controls. Seven animals injected with saline were also employed as controls. The animals were killed at 1, 8, 24, and 48 hr., 1 week, 1 month, and 3 months after the injection. At autopsy the leg musculature was divided into four blocks (Fig. 1) and the histological changes in each block were studied. Formalin-fixed tissues were stained by haematoxylin and eosin and by the Prussian blue reaction, and alcohol-fixed tissues were stained also by the alcoholic periodic acid-aqueous Schiff (P.A.S.) technique (Mowry and Millican, 1953).

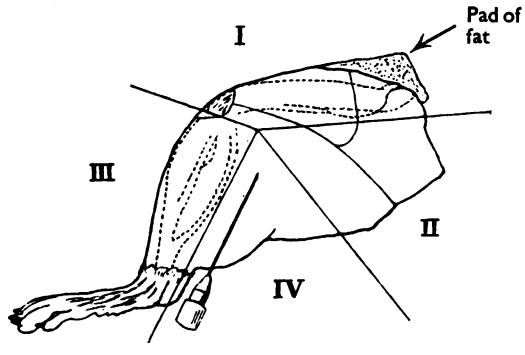


FIG. 1.—Diagram of rat hind limb showing the site of injection and the division into sectors for histological study.

RESULTS

Retention in Muscle.—The uptake of iron from the tissues at the injection site at varying times after the injection is shown in Fig. 2. Such absorption of iron as occurred from all four preparations took place largely during the 72 hr. immediately following the injection, and very little was absorbed subsequently. The iron-dextran complex IDA showed a greater initial rate of absorption and a smaller retention of iron in the muscle at 8 weeks than the other three preparations studied.

Agar Diffusion Experiments.—It was interesting to compare the above observations with measurements of the diffusion zones of the iron polysaccharide complexes in a protein-containing medium *in vitro* (Table I). There was a striking difference between the homogeneity of the diffusion zone in the case of IDA and the dark central zones of the other preparations. In the case of saccharated oxide of iron, and most probably with IPV and IPH also, the dark inner zone indicates interaction of the complex with plasma protein. The homogeneity of IDA is a reflection of the

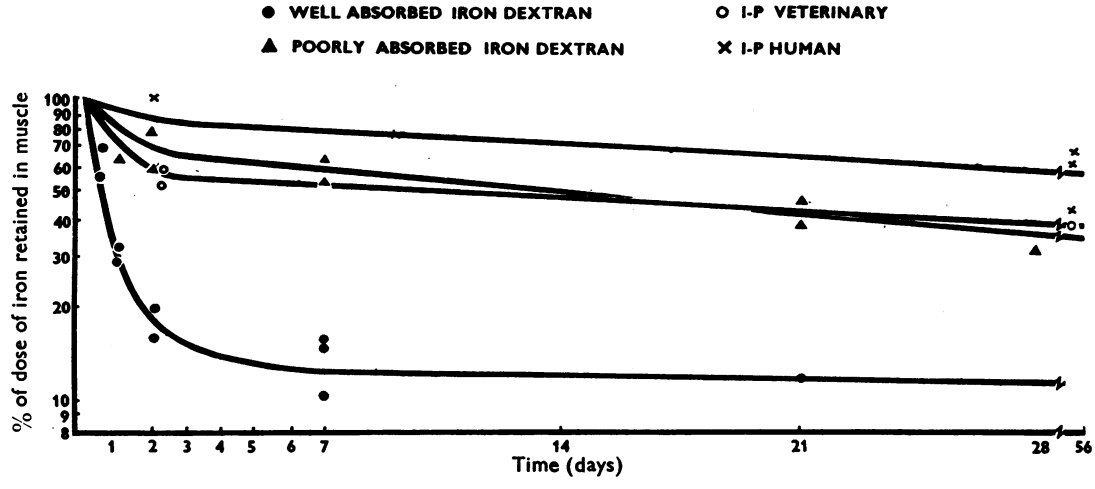


FIG. 2.—Graphs relating the retention of the iron four iron polysaccharide complexes in rabbit muscle at various times following injection.

TABLE I

IN VITRO STUDY OF DIFFUSION OF VARIOUS IRON PREPARATIONS IN SERUM-AGAR AT 37° C. FOR 6 DAYS

Iron Preparation	% Iron Content	Diameters of Diffusion Zones (mm.)	
		Total Zone	Dark Inner Zone
Saccharated oxide	2	22	22
IPV	5	32	24
IPH	5	34	26
IDA	5	37	Nil

stability of this complex (Martin, Bates, Beresford, Donaldson, McDonald, Dunlop, Sheard, London, and Twigg, 1955).

The divergence between diffusion and intramuscular absorption is seen in Fig. 3, in which % retention at the injection site at 48 hr. is compared with diffusion *in vitro* for a variety of preparations of iron-dextran. It is obvious that the diameter of the diffusion zone is not always closely correlated with the degree of absorption; in fact, the same diffusion zone may be observed with preparations of widely differing absorption characteristics. It is clear that the determinants of intramuscular absorption include factors other than passive diffusibility.

Histological Observations. — Sections were examined of the muscles in all four sectors of the leg (Fig. 1), but significant changes were found only in area IV, where the main mass of the injection was deposited. Some seepage occurred occasionally into area II, when minor changes were seen similar to those which will be described in area IV. Little of any injection spread into areas I and III, and in no case was tissue damage seen in these.

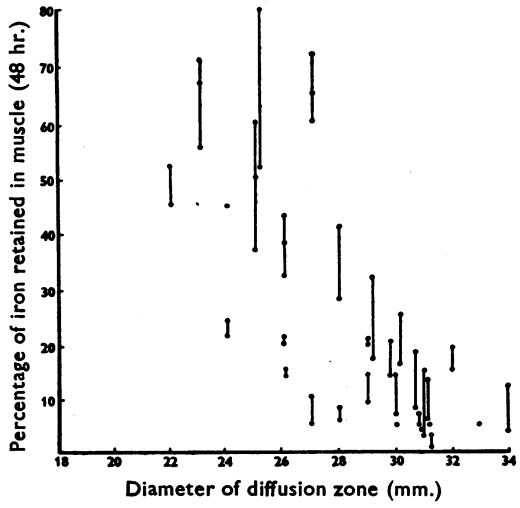


FIG. 3.—The relation between diffusion *in vitro* and muscle retention *in vivo* observed in various preparations of iron-dextran complex. *In vitro* measurement of the extent of diffusion of each complex in serum-agar was made after 6 days at 37° C.; *in vivo* measurement of the proportion of iron retained in rabbit muscles was made 48 hr. after injection. The vertical lines join points representing an individual preparation.

Histological Changes Following Injection of the Complex IDA

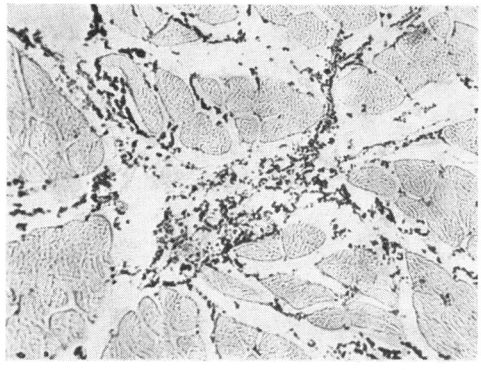
One Hour After the Injection.—There was a linear focus of damaged muscle along the line of the needle track, the damaged muscle fibres being swollen, eosinophilic, and often vacuolated. The mass of injection was lying free in the stroma between the muscle bundles and in the larger septa (Fig. 4*a*); some, however, had already entered the lymphatics and had been fixed in the littoral cells of the popliteal lymph nodes (Fig. 4*b*).

Eight Hours After the Injection.—A small focal area of muscle about 2 to 3 mm. across, presumably the precise locus of the injection, showed acute muscle damage. There were swollen eosinophilic fibres, sometimes hyalinized, sometimes vacuolated. Occasionally the sarcolemmal sheath appeared to have ruptured and amongst the fibres there was a mild infiltration of polymorphs, some being inside the sarcolemmal sheath (Fig. 4*c* and *d*). Practically no iron was detected by the Prussian blue reaction in the area of damaged muscle, and only small amounts were found where it had seeped away from the injection site along the septa. Here it produced no tissue reaction, but a little had already been taken up in the histiocytes.

Twenty-four Hours After the Injection.—At the locus of injection the polymorphs were almost completely replaced by macrophages (Fig. 4*e*), and some of the damaged muscle fibres showed a granular basophilia. No iron could be stained in the injection site at this time, either free or within macrophages, and the iron in the surrounding septal tissues was now partly taken up in the tissue histiocytes (Fig. 4*f*).

Forty-eight Hours After the Injection.—Macrophage infiltration of the focus of damaged muscle was intense, the macrophages being found both inside and outside the sarcolemmal sheaths, which were usually collapsed (Fig. 5*a*). Proliferation of sarcolemmal nuclei could also be recognized. The iron in the surrounding stroma was now almost entirely intracellular.

One Week After the Injection.—The inflammatory reaction had almost disappeared and a



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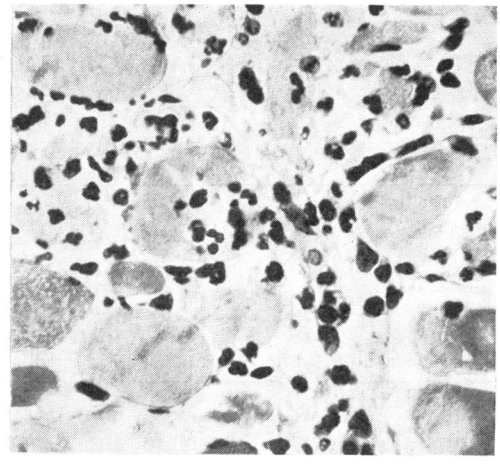

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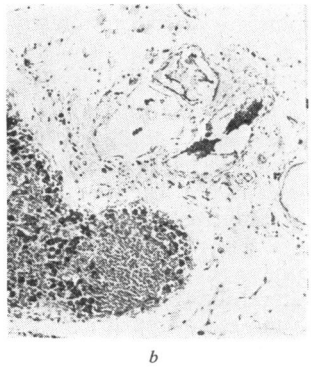

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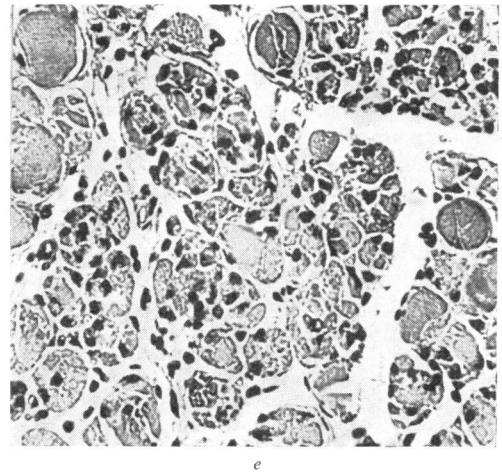

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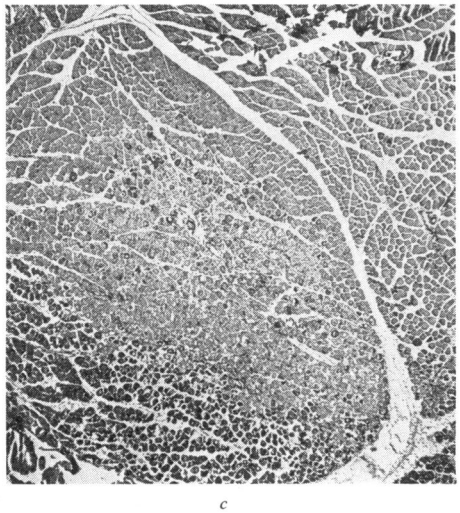

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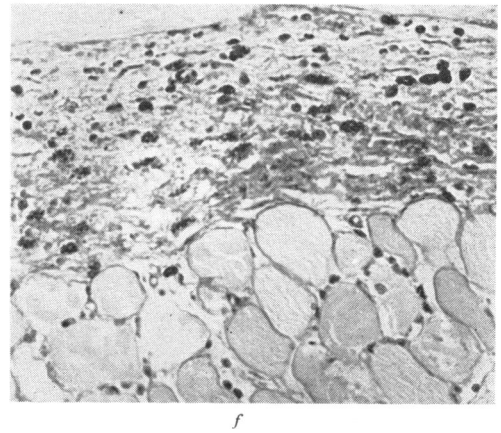

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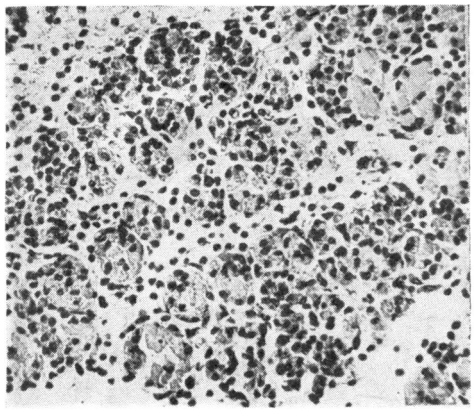
FIG. 4.—(*a*) One hr. after the injection of IDA. Prussian blue ×120. Intact muscle fibres with the injection material free in the stroma. (*b*) One hr. after the injection of IDA. Prussian blue ×42. Iron is seen in the lymphatics and in the lymph gland. (*c*) 8 hr. after injection of IDA. H. and E. ×22. The focus of degeneration and inflammation at the injection site. (*d*) 8 hr. after the injection of IDA. H. and E. ×480. Swollen, necrotic muscle fibres at the injection site with polymorphs in the stroma and inside the sarcolemmal sheaths. (*e*) 24 hr. after the injection of IDA. H. and E. ×250. The macrophage response at the injection site. (*f*) 24 hr. after the injection of IDA. Prussian blue ×200. The injection material in the septa away from the injection site is partly intracellular and partly extracellular.



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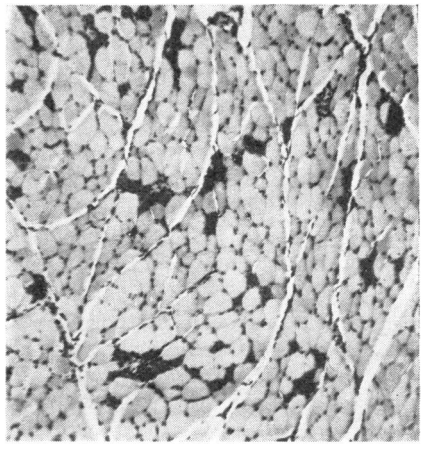

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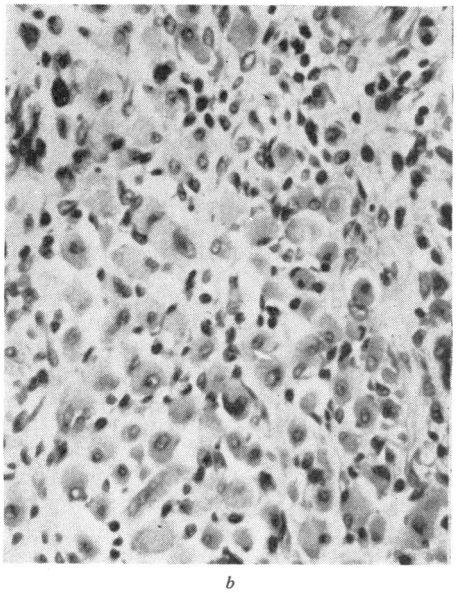

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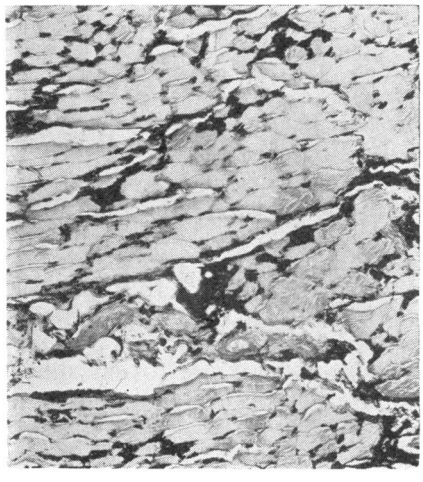

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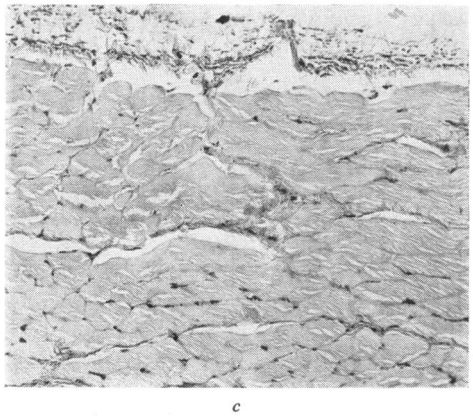

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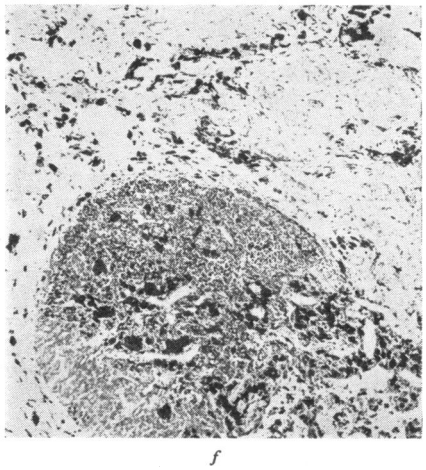

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FIG. 5.—(*a*) 48 hr. after the injection of IDA. H. and E. ×180. Collapsed muscle fibres with proliferation of sarcolemmal nuclei and intense macrophage infiltration. (*b*) One week after the injection of IDA. H. and E. ×240. Regenerating muscle fibres at the injection site. (*c*) One month after the injection of IDA. Prussian blue ×80. Residual injection material in macrophages in the stroma. (*d*) 48 hr. after the injection of IDB. H. and E. ×65. The macrophage response at the many small scattered foci of muscle damage. (*e*) One month after the injection of IDB. Prussian blue ×80. Very heavy residual injection material in macrophages in the stroma. (*f*) Three months after the injection of IDB. Prussian blue ×75. Much residual injection material in regional lymph nodes and in the stroma.

process of active regeneration of muscle was seen at the injection site. The regenerating fibres were small, with basophilic cytoplasm and central nuclei (Fig. 5*b*). Prussian-blue-stained sections showed small amounts of iron in stromal histiocytes outside the zone of regeneration.

One Month After the Injection.—The area of muscle damage could only be recognized by finding an occasional small muscle fibre with a central nucleus. There was no residual cellular reaction or fibrosis. Iron could still be detected in histiocytes in the septa (Fig. 5*c*) in amounts little less than those seen at one week.

Three Months After the Injection.—The muscle now appeared entirely normal. Small amounts of iron were stainable, about as much as was seen at the end of one month.

Histological Changes Following the Injection of IDB, IPH, and IPV

All three iron preparations produced an inflammatory reaction at the site of injection, the pattern being identical with that described for IDA. The amount of necrosis, however, varied. The two iron polysaccharides IPH and IPV produced areas of necrosis equal or greater in extent to that described above, but the iron-dextran IDB showed a different appearance. No single large focus of damage developed; instead scattered foci of muscle damage involving groups of only two or three muscle fibres were seen (Fig. 5*d*). In all three cases regeneration of the damaged muscle was complete within one month.

Large and important differences were seen between the four preparations when sections stained for iron were examined. These differences are summarized in Table II. The iron-dextran IDB was less completely absorbed from the foci of muscle damage and was more quickly taken up by the tissue macrophages than was the product IDA. The iron polysaccharide IPV was as rapidly and completely removed from the injection site as was IDA, but there was a much more rapid take-up by the tissue macrophages of the iron which had diffused away from the injection site, so that very little free iron was seen after 24 hr. The iron polysaccharide IPH, whilst producing large areas of damaged muscle, showed considerable residual injection material in these damaged areas even after 48 hr. Little of it had been taken up by the tissue macrophages, and much of it appeared still to be extracellular after 48 hr. Prussian-blue-stained sections one and three months after the injection showed apparently undiminished numbers of iron-laden macrophages in the stroma amongst the muscle bundles (Fig. 5*e* and *f*).

Controls.—At the sites of injection of both dextran and saline no lesions were seen apart from those attributable to needle trauma, but occasionally a mild polymorph infiltration was observed around the larger particles of Indian ink.

Discussion

Histological Features

At the sites of intramuscular injection of the iron polysaccharides studied, an acute inflammatory reaction developed with local degenerative changes in the muscles. Regeneration rapidly occurred; repair was complete in less than one month, and left no residual damage in muscle, nerves, or other near-by tissues. Similar changes in injected muscles have been seen to a much more striking degree with other drugs. Lüthy (1955) and von Hochstetter (1955) have quoted Shallock's observations on the histological changes in the gluteal muscles of rabbits injected with a variety of drugs, including camphor, bismuth, liver extract, depot penicillin, and "Irgapyrin." In all

TABLE II

HISTOLOGICAL ASSESSMENT OF IRON DISTRIBUTION IN RAT MUSCLES AT VARIOUS TIMES AFTER INJECTION

Time After Injection	Iron Complex											
	IDA			IDB			IPV			IPH		
	Injection Site	Surrounding Stroma		Injection Site	Surrounding Stroma		Injection Site	Surrounding Stroma		Injection Site	Surrounding Stroma	
		Extra-cellular	Intra-cellular		Extra-cellular	Intra-cellular		Extra-cellular	Intra-cellular		Extra-cellular	Intra-cellular
8 hr. ..	±	++++	±	+++	++++	+	±	+++	++	+++	++++	+
24 ,, ..	0	+++	±	+++	++	+	0	±	+++	+++	+++	++
48 ,, ..	0	±	+	++	±	++	0	0	+++	++	++	+++
1 week ..	0	0	+	+	0	++	0	0	+++	++	0	++++
1 month ..	0	0	+	+	0	++	0	0	++	++	0	++++
3 months	0	0	+	+	0	++	0	0	+++	++	0	++++

0=No iron; ±, +, ++, +++, and ++++ indicate increasing tissue response.

cases, haemorrhages, thromboses, necrosis, and disintegration of muscle fibres occurred at the injection site, and were followed by the development of granulation tissue and subsequent formation of a fibrous scar. Glückert and Benoit (1952) reported similar damage following the injection of sulphonamide into the thigh muscles of guinea-pigs near the sciatic nerve. Considerable damage was also caused to the nerve.

The quantitative experiments showed great differences between the four iron preparations in the rate of their absorption from the injection site, but in all cases absorption was greatest during the first 72 hr., and little more occurred subsequently. These results were probably largely attributable to the changes developing in the muscle at the injection site, which suggested that active intervention by the tissue itself was essential for the absorption of iron-polysaccharide complexes deposited within it.

In their extensive study of the pharmacology of parenteral iron preparations, Brownlee, Bainbridge, and Thorp (1942) put forward the view that, "while necrosis is commonly seen with some compounds, this is regarded as indicating an unsuitable compound which is badly absorbed and therefore likely to give a poor haemoglobin response." No evidence in support of these statements was provided, nor was the nature of the "necrosis" to which they referred made clear. Nevertheless, the type of compound tested by these authors differed significantly from the iron-polysaccharide complexes to make it likely that their absorption mechanisms were quite distinct.

Relation to Absorption

Everett, Garrett, and Simmons (1954) showed that ionic iron was absorbed from a subcutaneous site primarily into the blood vessels, whereas a subcutaneous injection of plasma-bound iron passed almost exclusively into the lymphatics. The molecular size of the iron complexes studied here made it likely that absorption would take place by a lymphatic route. In fact, uptake into the lymphatics was rapid, stainable iron being found in the regional lymph nodes within an hour of injection. Clinical studies using Fe^{59}-labelled iron-dextran showed curves of absorption from the muscle similar to those given in Fig. 2, corresponding to IDA. These studies revealed, as might be expected, a considerable amount of individual variation. There is a suggestion from this and other work on intramuscular absorption that absorption is delayed if lymphatic drainage is retarded by immobilization of the injected limb (Grimes and Hutt, personal communication).

In considering the mechanism of absorption of the iron polysaccharide complexes a distinction must be made between the concentrated mass of material deposited at the injection site and the portion which diffuses into the surrounding tissues and is diluted by the extracellular fluid. Lymphatic absorption is dependent upon the rate of lymph flow and upon lymphatic capillary permeability; both of these are increased in an acute inflammatory reaction. The inflammatory focus at the injection site of the iron polysaccharide complexes might therefore be expected to enhance their absorption. Indeed, the central masses of the complexes IDA and IPV which appear histologically to be the best absorbed produced a much brisker inflammatory reaction than that which developed around the poorly absorbed complex IDB. Paradoxically, the central mass of the complex IPH was poorly absorbed, despite a very extensive inflammatory reaction. It can only be surmised that its molecular weight lay beyond the range capable of penetrating easily into the lymphatics: it also had a higher viscosity than the other polysaccharides, and this fact, together with other unknown physico-chemical properties, may have adversely affected its rate of absorption.

The macrophage also has an effect upon the rate of absorption of the iron complexes. Uptake by local macrophages renders the iron no longer immediately available to the body. In the present experiments, tissue macrophages accumulated abundantly in and around the injection site within 24 hr. In the central concentration of injected material the local inflammatory reaction was usually sufficient to remove the bulk of the injection before the macrophages appear. That portion of the injection which had diffused more widely was not removed before the arrival of the macrophages; its rapid uptake by these macrophages, as occurs with the complex IPV, must seriously limit its general availability to the body. This probably accounts for the lower absorption figures for IPV recorded in the quantitative experiment. The persistence of iron-laden macrophages in undiminished number in the stroma up to three months after the injection suggests that this iron may be permanently fixed. Thus the concept of an effective intramuscular depot, a familiar feature of many well-known drugs, is not applicable to the iron-polysaccharide complexes. Prompt absorption from the muscle or prolonged retention within it appear to be the only alternatives.

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