Effect of Desferrioxamine Methansulphonate (DFOM) on Glucose Uptake by Rat Epididymal Adipose Tissue. (30958)

A. Ghidoni, E. Sanesi and G. Pozza
Institute of Clinical Medicine, University of Milan, Italy

Desferrioxamine Methansulphonate (DFOM) is an iron chelating agent used for treatment of hemochromatosis. The formula of Desferrioxamine B, which represents its active basis, appears in Fig. 1.

It has been observed that during DFOM treatment of patients with diabetes mellitus and hemochromatosis, glucose tolerance improves, together with increased iron excretion. This improvement often requires the insulin dosage to be lowered(1). To investigate whether this change in glucose tolerance is related to increased peripheral glucose utilization, the effect of DFOM on glucose uptake by rat epididymal fat pad has been studied.

Materials and methods. Male albino rats of the Wistar strain, weighing 160-200 g, fed *ad libitum,* were used. For each experiment 8 rats were killed, and the epididymal fat pads were cut into 4 pieces each; the pieces were dropped into 8 flasks, so that each flask contained a fragment of adipose tissue of each rat. As incubation medium, Ringer's Bicarbonate Buffer with gelatine (200 mg%) and glucose (300 mg%) were used. Two ml of incubation medium were present in each flask.

Crystalline insulin (Hoechst) 26 U/mg was prepared as a concentrated solution once a month, kept at 0.4°C and diluted on the day of experiment.

DFOM,* freshly prepared, was used at concentrations of 0.75, 1.5 and 3 μM/ml.

Incubation was carried out for 2 hours, 5% CO_2 and 95% O_2 being used as gas phase.

TABLE I. DFOM Effect on Glucose Uptake by Rat Epididymal Adipose Tissue.

Exp No.	Glucose uptake (mg/g/h) Control	DFOM (1.5 μM/ml)	Difference
1*	.59	1.05	+.46
2*	1.29	1.64	+.35
3	1.23	1.43	+.20
4†	.82	1.11	+.29
5	.96	1.14	+.18
6	.81	1.34	+.53
7	.64	.82	+.18
8	.98	1.10	+.12
9	1.07	1.64	+.57
Mean	.932	1.252	+.320
S.E.			.0555

$t = 5.768$ $P < .001$.
* Mean of 2 replications.
† Mean of 4 replications.

Glucose uptake was measured by the glucose-oxidase method, according to Hugget and Nixon(2). The data are expressed as mg of glucose uptake per g net weight per hour of incubation.

Results. Table I shows the results obtained when DFOM was added to the incubation medium (1.5 μM/ml); increased glucose uptake is evident in the presence of the drug.

Table II shows the results obtained when DFOM was present in the incubation medium at different concentrations (0.75, 1.5 and 3 μM/ml), with and without insulin (100 μU/ml), and in Table III the statistical analysis

Chemical Formula of Desferrioxamine

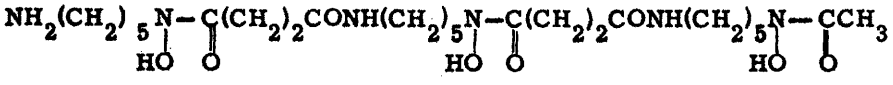

FIG. 1

* Kindly supplied by Dr. P. E. Lucchelli, CIBA, Milan.

TABLE II. Effect of Increasing Concentrations of DFOM on Glucose Uptake by Rat Epididymal Adipose Tissue, With and Without Insulin Added to the Medium.

Exp No.	Glucose uptake (mg/g/h)							
	Buffer alone	Buffer + DFOM, .75 μM/ml	Buffer + DFOM, 1.5 μM/ml	Buffer + DFOM, 3 μM/ml	Buffer + insulin, 100 μU/ml	Buffer + insulin, 100 μU + DFOM, .75 μM/ml	Buffer + insulin, 100 μU + DFOM, 1.5 μM/ml	Buffer + insulin, 100 μU + DFOM, 3 μM/ml
11	.96	1.26	1.14	1.47	1.66	1.94	2.22	2.30
12	.81	1.18	1.34	1.46	1.42	1.86	1.72	1.88
13	.64	.59	.82	.83	.96	1.03	1.05	1.10
14	.98	1.22	1.10	1.43	1.94	1.82	1.79	2.18
15	1.07	1.67	1.64	1.72	2.06	2.42	2.51	2.45
Mean	.89	1.19	1.21	1.38	1.61	1.81	1.85	1.98
Mean effect of DFOM	—	+.30	+.32	+.49	—	+.20	+.24	+.37

TABLE III. Statistical Analysis of Data Contained in Table II.

Sources of variability	Degrees of freedom	F	P
Effect of insulin	1	138.25	<.001
Total effect of DFOM (presence *vs* absence)	1	25.42	<.001
Between doses of DFOM			
Linear term	1	5.50	<.05
Quadratic term	1	.72	n.s.
Interaction			
Insulin × DFOM	3	.20	n.s.
Experiments	4	39.52	<.001
Error	28		
Total	39		

of the data of Table II is shown. It appears that the effect of DFOM is related to the dosage of the drug, although the data show only a slight significance as a linear term. When DFOM and insulin are both present in the incubating medium, no interaction between the two substances is apparent. Glucose uptake, markedly enhanced by insulin, shows a further increase in the presence of DFOM.

Discussion. Leonards *et al*(3) demonstrated that EDTA, which like DFOM, is a chelating agent, increases glucose uptake by rat adipose tissue. Recently, Dixit and Lazarow(4) showed that EDTA increases glucose oxidation by epididymal fat pads only at high concentrations (10^{-2} M), lower concentrations having no metabolic effect when used alone but producing a 2-fold potentiation of the effect of insulin. On the basis of these results, the authors advance several hypotheses, namely:

a) EDTA, as a chelating agent, is capable of removing a metal ion from the cell membrane, which controls the entry of glucose; b) EDTA prevents insulin from being inactivated or destroyed during incubation in the medium; c) EDTA depolymerizes insulin complexes with zinc; d) EDTA alters the tertiary structure of insulin and unmasks "reactive centers."

Since DFOM is a chelating agent, it seems reasonable to suggest that it has a mechanism of action similar to that advanced for EDTA.

From the results, it appears that DFOM alone, even if used at much lower concentrations than EDTA, always increases glucose uptake by the epididymal fat pad, with and

TABLE IV. Stability Constants (K) for Complexes of DFOM with Several Metallic Ions.

Ion	K
Mg^{++}	10^4
Ca^{++}	10^2
Sr^{++}	10
Co^{++}	10^{11}
Ni^{++}	10^{10}
Cd^{++}	10^3
Zn^{++}	10^{11}
Fe^{+++}	10^{31}

General equation: metallic ion of valance n : M^{+n} DFOM as tribasic acid : AH_3 : $M^{(+n)} + AH_3 = MAH_{3-n} + nH^{(+1)}$

$K = ([M \cdot AH_{3-n}])/([M^{(+n)}] \cdot [AH_{3-n}{}^{(-n)}])$

without insulin. This fact rules out 3 of the above hypotheses, *i.e.*, those assuming its effect on the insulin molecule. If the first hypothesis were correct, that the effect of the drug is due to the removal of a metal ion from the membrane, the high affinity of DFOM for iron in comparison with other metal ions, as shown in Table IV(5) and the low dosage necessary to detect its activity has led to the assumption that iron might be the ion metal removed from the cell membrane in order to increase glucose permeability.

These data also suggest that the increased glucose tolerance observed in patients with hemochromatosis treated with DFOM is related to increased peripheral glucose utilization induced by the drug.

Summary. DFOM increases glucose uptake by the rat epididymal fat pad *in vitro*. This effect is also evident when insulin is present in the incubation medium. It seems reasonable to assume that the improved glucose tolerance shown by patients with diabetes and hemochromatosis during DFOM treatment is due to increased peripheral utilization.

1. Frey, F., in Roundtable-Discussion, Schweiz. Med. Wschr., 1962, v92, 1295.

2. Hugget, A. S. A., Nixon, D. H., Biochem. J., 1957, v12, 66.

3. Leonards, J. R., Landau, B. R., Bartsch, G., J. Lab. and Clin. Med., 1962, v60, 552.

4. Dixit, P. K., Lazarow, A., Proc. Soc. Exp. Biol. and Med., 1965, v118, 368.

5. Schwarzenbach, G., Schwarzenbach, K., Helv. chim. acta, 1963, v46, 1390.

Received July 19, 1965. P.S.E.B.M., 1966, v121.

Persistence of Active Interferon in Cells Washed After Treatment With Interferon. (30959)

SEYMOUR LEVINE* (Introduced by H. R. Cox)

Viral and Rickettsial Research Section, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

Experiments performed at 37°C in which puromycin(1-3) was used to block the action of interferon suggested that interferon could not be removed from cells by extensive washing and led to the work described below to demonstrate its persistence in treated cells.

Materials and methods. Virus. The western equine encephalitis (WEE) virus used was grown at 37°C in an atmosphere of 4% CO_2 in air in monolayers prepared from trypsinized chicken embryo, as previously described(1), and was assayed by the plaque method. The virus growth medium consisted of 0.1% lactalbumin hydrolysate, balanced salts and 2% calf serum. The same medium supplemented with 0.9% agar was used for virus assay. Each cell monolayer was inoculated with 0.5 ml of virus which contained 9×10^7 plaque forming units (pfu), providing a virus-to-cell ratio of approximately 25. Virus was harvested after cells had been disrupted by a cycle of freezing and thawing.

Interferon. The interferon used was prepared from allantoic fluid infected with influenza B virus, and was partially purified by 2 cycles of zinc precipitation followed by dialysis for 24 hours against 0.9% NaCl and for another 24 hours against 0.01 M phosphate

* Present address: Research Division, Upjohn Co., Kalamazoo, Mich.