Inhibition of Glucose Uptake by 2-Deoxy-D-Glucose in Chick Embryo Heart.* (27963)

RENATO LITTA MODIGNANI† AND PIERO P. FOÀ

Departments of Physiology and Pharmacology, Chicago Medical School, Chicago, and Wayne State University College of Medicine and Division of Research, Sinai Hospital of Detroit, Mich.

Measurements of glucose uptake, performed under various experimental conditions, have led to the suggestion that the chick embryo heart is freely permeable to glucose up to the fifth day of embryologic development. In this preparation, which is not sensitive to insulin, glucose uptake appears to be limited by the rate of intracellular phosphorylation. An insulin sensitive "membrane," regulating glucose uptake, begins to develop between the seventh and tenth day of embryologic development and, at this stage, glucose transport becomes the limiting factor of glucose uptake(1,2). Since these properties of the chick embryo heart may prove to be useful for studying cell permeability and insulin action, they were investigated further by measuring glucose uptake in presence of 2-deoxy-D-glucose (2-DG). The compound, structurally similar to glucose, penetrates the cell where it is phosphorylated to 2-deoxy-glucose-6-phosphate (2-DG-6-P), but is not metabolized further. The resulting accumulation of 2-DG-6-P in the cell blocks glucose transport non-competitively, thus inhibiting its uptake and utilization in a variety of organs and tissues(3-9).

Methods. Tissue preparation and 10 minutes' preliminary incubation in buffer were performed as described previously(1). All experiments were carried out in a Dubnoff metabolic shaker at 75 cycles per minute and at 37.5°C in an atmosphere of 95% O_2 and 5% CO_2. The incubation medium was Krebs-Henseleit bicarbonate buffer(10).

Six to eight 9-day-old hearts, or twelve to fourteen 5-day-old hearts, were incubated for 30 minutes in 1 or 2 ml of buffer containing 40×10^{-3}M 2-DG, a concentration previously found effective(6). When indicated, glucagon-free insulin (Lilly Batch No. 466368; 26 units per mg)‡ was added at a concentration of 0.4 unit per ml. Longer incubation periods did not change the results significantly, in agreement with the observation that 2-DG enters the cells rapidly and has lasting effects(5). After exposure to 2-DG or 2-DG plus insulin (first incubation period), the hearts were rinsed twice and incubated for 15 minutes in fresh buffer to eliminate any 2-DG still present in the extracellular space. Finally, the hearts were incubated in buffer containing D-glucose at a concentration of 8×10^{-3}M, or 16×10^{-3}M, for 1 hour (second incubation period). These concentrations had been previously found to cause maximal glucose uptake by 5-day-old hearts and easily measurable uptake by 9-day-old hearts(1). In a few exploratory experiments with 5-day as well as 9-day-old hearts, insulin was added during the second incubation period. This procedure did not alter the results and, therefore, was discontinued.

Glucose was determined in 50 microliter samples, deproteinized according to Somogyi, using a glucose oxidase-peroxidase method previously described(11). Glucose uptake was calculated from its disappearance from the incubation medium and expressed as micromoles per gram of dry tissue per hour. The hearts were weighed on a Cahn electrobalance as described previously(1).

Results. Tables I and II show that pretreatment with 2-DG causes a marked inhibition of glucose uptake at both glucose concentrations used. This inhibition is approximately the same in 5- and 9-day-old hearts and is not appreciably modified by addition of insulin.

Discussion. The results obtained with 2-

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† Present address: Clinica Med. dell' Università, Milano, Italy.

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TABLE I. Effect of 2-Deoxy-D-Glucose and 2-Deoxy-D-Glucose Plus Insulin on Glucose Uptake by 5-Day-Old Chick Embryo Heart.

1st incubation period Additions	2nd incubation period Glucose	Glucose uptake μM/g dry tissue/hr	Inhibition uptake
None	8×10^{-3}M	271 ± 12.2 (5)	—
2-DG	8×10^{-3}M	155 ± 10.3 (10)	43%, p<.01
2-DG + insulin	8×10^{-3}M	170 ± 10.4 (5)*	37%, p<.01
None	16×10^{-3}M	319 ± 24.0 (4)	—
2-DG	16×10^{-3}M	185 ± 12.6 (6)	42%, p<.02
2-DG + insulin	16×10^{-3}M	179 ± 4.9 (3)†	44%, p<.05

2-DG concentration 40×10^{-3}M. Insulin concentration 0.4 U/ml. No. of experiments in parentheses.

* Insulin effect (2-DG + insulin *vs* 2-DG): 9.7%, p>.05.

† Insulin effect (2-DG + insulin *vs* 2-DG): none.

TABLE II. Effect of 2-Deoxy-D-Glucose and 2-Deoxy-D-Glucose Plus Insulin on Glucose Uptake by 9-Day-Old Chick Embryo Heart.

1st incubation period Additions	2nd incubation period Glucose	Glucose uptake μM/g dry tissue/hr	Inhibition uptake
None	8×10^{-3}M	100 ± 4.5 (5)	—
2-DG	8×10^{-3}M	65 ± 4.4 (11)	35%, p<.02
2-DG + insulin	8×10^{-3}M	70 ± 2.6 (7)*	30%, p<.02
None	16×10^{-3}M	199 ± 11.2 (5)	—
2-DG	16×10^{-3}M	108 ± 5.2 (8)	45%, p<.02
2-DG + insulin	16×10^{-3}M	105 ± 2.6 (3)†	47%, p<.01

2-DG concentration 40×10^{-3}M. Insulin concentration 0.4 U/ml. No. of experiments in parentheses.

* Insulin effect (2-DG + insulin *vs* 2-DG): 7.7% p>0.8.

† Insulin effect (2-DG + insulin *vs* 2-DG): none.

DG are in agreement with those obtained with other tissues by the investigators cited above. In addition, the fact that 2-DG inhibits glucose uptake, not only in 9-, but also in 5-day-old chick embryo hearts and that this inhibition is not modified by insulin, suggests that the action of 2-DG is independent of the presence of a cell membrane. This conclusion is compatible with the generally held view that the inhibition of glucose uptake is due to intracellular accumulation of 2-DG-6-P and consequent inhibition of glucose phosphorylation.

Conclusions. 2-deoxyglucose inhibits glucose uptake by 5- as well as 9-day-old chick embryo heart. This inhibition is not modified by addition of insulin. The lack of insulin effect, as well as the previously demonstrated facts that the 5-day-old chick embryo heart is freely permeable to glucose and is not sensitive to insulin, are compatible with the belief that 2-DG acts by inhibiting the intracellular phosphorylation of glucose. These results constitute further evidence for the usefulness of the chick embryo heart in studies of glucose transport and insulin action.

Summary. 2-deoxy-D-glucose (2-DG) inhibits glucose uptake by 5- and 9-day-old hearts *in vitro*. Insulin does not modify the effect of 2-DG. The results are compatible with the generally held beliefs that 2-DG inhibits the intracellular phosphorylation of glucose and, confirming the results obtained with other tissues, demonstrate the potential usefulness of chick embryo hearts at various stages of development for studies of glucose transport and insulin action.

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Effects of Long-Term Feeding of Vegetable Fats on Atherosclerosis.* (27964)

HUGH B. LOFLAND, JR. AND THOMAS B. CLARKSON
Department of Biochemistry, and The Vivarium, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.

The relationship between degree of unsaturation of dietary fat and extent of coronary and aortic atherosclerosis has been studied in experimental animals. Furthermore, such relationships have exerted a significant influence on the dietary habits of human populations. This subject has been reviewed by Katz, Stamler, and Pick(1). Many experiments have been done with cholesterol-induced atherosclerosis. However, relatively few studies have been carried out in species of animals which develop atherosclerosis spontaneously. For this reason, the studies described here utilized the White Carneau pigeon, which has been shown previously to develop atherosclerosis spontaneously while maintained on cholesterol-free grain diets (2,3). We have studied the prophylactic effects of 2 vegetable fats of different degrees of unsaturation (Crisco† *vs.* safflower oil‡), as well as the effects of safflower oil on regression of cholesterol-aggravated atherosclerosis in these pigeons.

Materials and methods. Seven groups of 6-week-old White Carneau pigeons§ were housed in growing batteries and were kept in a temperature and humidity controlled room (72 ± 2°F, 50% ± 5% relative humidity), lighted from 7:00 a.m. to 5:00 p.m. The birds were divided into dietary groups as shown in Table I, and kept on experiment for the periods specified. The supplementary dietary fats were added by allowing the fat to be absorbed into the basal ration (commercial pigeon pellets,‖ composed of pelleted grains) to the extent of 10% by weight of the diet (in the case of Crisco, the fat was first liquified by heating). For those groups receiving a cholesterol supplement, cholesterol was first dissolved in a small amount of Crisco by heating, then added to the pellets.

A sample of blood was drawn from the alar vein of each bird at beginning of the experiment for determination of initial levels of serum cholesterol. The birds were weighed initially and at intervals during the experiment. As initial levels of serum cholesterol or body weights at any time during the experiment did not differ among the various groups, these data are not presented in detail.

Groups I, II, and III were kept on the diets for 26½ months, as shown in Table I. Group IV was sacrificed after 6 months on the cholesterol-containing diet, and these birds were used to establish the extent to which cholesterol aggravates development of gross atherosclerosis (previous studies have shown

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† Procter and Gamble Co., Cincinnati, O.

‡ Generously supplied by Pacific Vegetable Oil Co., Richmond, Cal.

§ Obtained from Palmetto Pigeon Plant, Sumter, S. C.

‖ "Pigeon Checkers," Ralston-Purina Co., St. Louis, Mo.