Effect of Insulin, Fasting and Tolbutamide on Dextran Edema in Rats.* (25050)

GAÉTAN JASMIN† AND PIERRE BOIS
Depts. of Pathological Anatomy, University of Montreal, and Histology and Embryology, University of Ottawa, Canada

Insulin has been shown by Adamkiewicz to increase the rat's sensitivity to dextran(1). As a rule, dextran causes no reaction when injected subcutaneously, except after several hours(2), but when given by this route conjointly with insulin, it produces a prompt characteristic edematous reaction in paws, ears and snout. While confirming these observations, Goth further reported that insulin promotes histamine release induced by dextran(3). However, the rather high dosages of insulin used make it difficult to interpret the mechanism of this sensitizing phenomenon. The present communication describes experiments in which insulin was given in decreasing doses to rats treated with dextran; changes in blood sugar were recorded. The role of hypoglycemia as possible contributing factor to dextran susceptibility was also investigated in intact and adrenalectomized fasted rats and following treatment with tolbutamide.

Methods. One hundred and sixty-eight female rats (Holtzman) weighing 100 to 120 g were used in 4 experiments. They were kept on Purina Fox Chow and received tap water *ad lib*. Whenever food was withheld, animals had access to water. Bilateral adrenalectomies were performed 24 hours before start of experiments and rats received, as maintenance therapy, a single subcutaneous injection of 1 mg of desoxycorticosterone acetate in the form of aqueous suspension of its microcrystals. Dextran, a commercial 6% solution (Abbott), was administered at dose of 0.3 ml, subcutaneously in scapular region. Zinc-insulin (Connaught) was also injected at same time, subcutaneously, in the inguinal region. Tolbutamide (Horner) was given by stomach tube at dose of 30 mg in 0.5 ml of physiologic saline, 30 minutes prior to injection of dextran. Degree of edema of paws and snout was appraised according to arbitrary scale of + to ++++. Readings were made 1 hour after dextran injection and scores were alloted to each degree so that + (traces) = 1; ++ = 2; +++ = 3; and ++++ = 4. Our results are expressed as percentages of maximal possible effect (++++). Values shown in Tables represent means of degrees of edema when present. Blood samples were withdrawn one hour and 15 minutes after dextran injection. They were taken with heparinized syringe from jugular vein, under ether anaesthesia; sugar determinations were made according to method of Folin-Wu.

Results. Table I lists results of 2 experiments in which rats received increasing doses of insulin. Both experiments were identical, except that in the second, animals were fasted for 12 hours before receiving insulin and dextran. In both cases, there was a maximal sensitizing effect to dextran when 0.5 unit of insulin was used. Higher doses did not increase the intensity of edema reaction; on the contrary, there was a decrease in response proportional to lowering of blood sugar. This is more evident in Exp. 2, where the 12-hour fast so increased the hypoglycemic effect of insulin that all animals receiving 2 units were dead after 4 hours. However, one fasted rat not treated with insulin showed a slight increase in sensitivity to dextran.

The third experiment was devised to evaluate the influence of fasting alone on dextran edema. Twelve to 48 hours of fasting did not promote sensitivity to dextran in intact animals and blood sugar levels remained unaffected (Table II). This situation was totally

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† Medical Research Associate of Nat. Research Council of Canada.

TABLE I. Effects of Insulin on Edema Reaction to Dextran Injected Subcutaneously in Rats.

Insulin (I.U.)	Exp. I: No fasting (10 rats/group) Edema* Incidence	Edema* Degree (%)‡	Glycemia, mg %	Exp. II: 12 hr fasting (8 rats/group) Edema* Incidence	Edema* Degree (%)‡	Glycemia, mg %
.0	0			1	25	98 ± 3.4
.1	8	25		8	32	67 ± 4.4
.25	8	50		8	38	63 ± 3.6
.5	10	72	120 ± 5.7	7	56	45 ± 3.9
1.	10	65	89 ± 3.9	7	50	46 ± 3.4
2.	9	56	57 ± 4.7	8	38	39† ± 1.6

* Mean readings 1 hr after inj. of dextran (0.3 ml 6% s.c.).
† All rats died within 4 hr after insulin inj.
‡ Mean percentage of degrees of edema when present.

reversed in adrenalectomized animals; prolonged fasting was particularly efficient in increasing susceptibility of rats to dextran. The 48-hour fasted adrenalectomized rats remained edematous as long as they were kept without food even though they had received only a single injection of dextran; this is in contrast with usual fleeting appearance of dextran edema, which usually lasts no more than a few hours.

Table III summarizes our observations on the influence of tolbutamide upon dextran reaction. Hypoglycemic sulfonamide had a more potent sensitizing action on dextran edema than insulin. This effect was produced without much alteration of blood sugar level. Here, the fasting period was purposely restricted to 6 hours before administration of tolbutamide, followed half an hour later by dextran injection.

TABLE II. Effect of Fasting on Edema Reaction to Dextran Injected Subcutaneously in Intact and Adrenalectomized Rats.*

Treatment	Fasting period, hr	Edema† Incidence	Edema† Degree, %‡	Glycemia, mg %
	12	0		110 ± 5.4
	48	0		108 ± 6.1
Adrenalectomy + DCA	12	2	25	85 ± 4.3
Idem	48	10	100	66 ± 4.2

* 4 groups, 10 rats each.
† Mean readings 1 hr after inj. of dextran (0.3 ml 6% s.c.).
‡ Mean percentage of degrees of edema when present.

Discussion. Our study reveals that the influence of insulin on dextran reaction in rats is not necessarily related to hypoglycemia. Furthermore, sensitivity to dextran may be augmented by fasting in adrenalectomized rats. Whether the latter condition is attributable to a higher level of endogenous insulin or merely the result of adrenal insufficiency remains to be demonstrated. The action of tolbutamide on dextran edema is probably mediated by insulin(4); it occurs without significant change in blood sugar values. Should variations in glycemia be involved in this process of sensitization, they would fall within the physiologic range. In this regard, let us recall that, according to Edlund, the inhibitory effect of alloxan upon dextran edema is also demonstrable in absence of blood sugar changes(5).

It is still controversial whether the increase in capillary permeability produced by dextran is attributable to direct vasotoxic action or secondary to histamine release(6). Goth recently reported that insulin augments release of histamine produced by dextran, but not that produced by 48/80(3). This accords with our own experiments (unpublished) in which insulin exerted no promoting action on

TABLE III. Effect of Tolbutamide on Edema Reaction to Dextran Injected Subcutaneously in Rats.*

Treatment	Fasting period, hr	Edema† Incidence	Edema† Degree, %‡	Glycemia, mg %
	6	0		125 ± 5.3
Tolbutamide (30 mg orally)	6	10	97	97 ± 3.9

* 2 groups, 10 rats each.
† Mean readings 1 hr after inj. of dextran (0.3 ml 6% s.c.).
‡ Mean percentage of degrees of edema when present.

production of edema in rats treated with 48/80. However, the increase of histamine liberation by dextran with insulin may be secondary to augmentation of edema reaction *per se*.

Goth's hypothesis that insulin may promote entry of certain large molecules of a histamine releasing agent(3) is perhaps the most valid one. It would be in accord with known effect of insulin on cell membrane permeability for glucose. Besides, the influence of insulin on dextran edema consists more in precipitating than in augmenting the reaction. Consequently, the sensitizing action of insulin may be explained either by increased rate of penetration of dextran into cells capable of liberating histamine or through accelerated systemic absorption of dextran with resulting toxic effects.

Summary. Insulin in doses that are not hypoglycemic increases the rat's sensitivity to dextran reaction, characterized by edema. A comparable effect was observed in adrenalectomized fasted rats and following treatment with tolbutamide.

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Absence of Gene Interaction in Mouse Hybrids, Revealed by Studies of Immunological Tolerance and Homotransplantation.* (25051)

CARLOS MARTINEZ, FRED SHAPIRO AND ROBERT A. GOOD
Depts. of Physiology and Pediatrics, University of Minnesota Medical School

It has recently been suggested(1,2) that the well-established phenomenon of immunological tolerance and homotransplantation immunity be used to ascertain whether or not genic interaction in antigen production occurs in inbred strains of mice. Both Haldane and Fox suggested that the hypothesis of genic interaction in mice might be tested as follows. Animals of one inbred strain of mice may be made tolerant of another inbred strain by injection of hematopoietic cells at birth, then skin from F_1 hybrids grafted to tolerant representatives of parent strain. In addition, animals of one parent strain made tolerant by injection of hybrid cells at birth could be tested by homotransplantation with skin from homologous parent strain. Under these conditions, if the tolerant parent rejects the F_1 transplant, this might be taken as convincing evidence that the F_1 hybrid possesses histocompatibility antigens not present in either of parent strains. If, however, the graft is accepted by the tolerant parent, this would be evidence that the F_1 individual carries all antigens present in both parent strains and would lend support to the one-gene-one-antigen theory previously formulated by Haldane. The observations here reported appear to be compatible with the latter hypothesis.

Method. Highly inbred mice of A and C3H strains and their reciprocal F_1 hybrids were used. In the first experiments, tolerance of A tissue in C3H mice was induced by injecting C3H animals at birth with approximately 4 million viable spleen cells taken from an adult A donor. The technic for spleen cell injection has been previously described(3). Approximately one month following weaning, treated mice were submitted to skin grafts taken from adult donors of either A strain or (A x C3H) F_1 animals by the method previously described(3). Both donor and recipient animals were always of the same sex and approximately of same age. Grafts were made when recipient mice were

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