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Glucose Tolerance in Mildly and Moderately Diabetic Rats Exposed to Cold. (28496)

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Normal rats are able to acclimate and survive prolonged exposure to cold by maintaining sustained elevations in metabolic rate. This is accompanied by an increased consumption and utilization of their caloric intake. It has been noted that severely alloxan diabetic rats are able to survive extended cold exposure when sustained on a high fat regimen(1). When they were placed on a high carbohydrate diet, the elevation in metabolic rate after cold exposure was severely depressed and duration of survival sharply curtailed. However, administration of insulin to these rats was found to increase their metabolic utilization of the ingested carbohydrate and thereby extend their length of survival at 5°C.

In view of the demands for sustained increases in metabolic rates initiated by cold exposure, an attempt was made to evaluate the influence that chronic exposure to 5°C might have on the development of diabetes in rats with subnormal carbohydrate tolerance. Under conditions where the major caloric food is carbohydrate, it was anticipated that the metabolic demands of acclimation to cold would accelerate the progression of diabetes in such animals. To test this, rats in varying degrees of abnormal carbohydrate tolerance were placed on a moderately high carbohydrate diet and subjected to periodic evaluation of the progression of their diabetic state during cold confinement.

Methods. Male Sprague-Dawley rats ranging from 125-150 g in weight were made mildly to moderately diabetic by intravenous injection of alloxan monohydrate (30 mg/kg of 2% solution in physiological saline). Assessment of the diabetic state was determined by the response to periodically administered glucose tolerance tests (GTT) and expressed in terms of a diabetic index (Id) Coupland *et al*(2). Glucose was administered intraperitoneally as a sterile 7% solution in dosage of 3.5 g/kg to animals following a 16-17 hour fast. Blood sugars were determined in duplicate from blood obtained from the tip of the tail at one and 2 hours after administration of the glucose solution. The extent of deviation of experimental blood sugars from the response obtained in normal animals was calculated as follows:

$$\text{Id} = \frac{\text{1 hr exp blood sugar (mg \%)}}{\text{1 hr normal blood sugar (mg \%)}} \times \frac{\text{2 hr exp blood sugar (mg \%)}}{\text{2 hr normal blood sugar (mg \%)}}.$$

The variability of Id assessment in 12 normal rats acclimated to room temperature was observed to range from 0.75-1.40. The associ-

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ated standard deviation of the mean of 1.00 was 0.23. This is in agreement with Coupland and co-workers who have found normal rats to have an Id range less than 1.7, i.e., within 3 standard deviations of the mean. Animals in a mild to moderate diabetic state have been classified as having an index rating ranging from 1.8-12 and rats with a fully diabetic condition have indices from 12 to values in excess of 25. One week after administration of alloxan, the initial Id was determined on 23 animals. Eleven rats with indices ranging from 0.8-21 were placed in a constant temperature cold room (5°C) and 12 rats with a comparable Id range were maintained at room temperature (26 ± 1°C). Each animal was housed separately in a metabolism cage and sustained *ad libitum* on a mash of Purina Laboratory Meal, calculated to provide 60% of the caloric intake in the form of carbohydrate. The progression of the diabetic state in both the animals at 5°C and 26°C was jointly assessed by periodic determination of the Id. This was conducted every 2 weeks in 14 rats, 7 at 5°C and 7 at 26°C, and every 3 weeks in the 9 animals numbered from 57-65, of which 4 were assigned to the cold. Approximately 16 hours preceding determination of the Id, the animals maintained in the cold room were brought to room temperature at the beginning of the fast and returned to the cold room immediately after conclusion of the test.

Twenty-four hour sugar excretion was determined once a week under *ad libitum* feeding conditions employing the Somogyi carmelization procedure(3) for analysis of sugar concentration. Blood sugar was determined according to a micro-modification of the procedure of Roe(4).

Results. The carbohydrate tolerance of rats acclimated to cold and room temperature is illustrated in Fig. 1-4. For ease of comparison, the 23 test animals were subdivided into 4 groups, according to the initial Id. In the lowest, Group 1 (Fig. 1), are placed those animals whose initial Id rating would be considered as representative of that expected from a population of normal rats (Id of 0.8-1.8). Group 2 (Fig. 2) includes those animals mildly diabetic as characterized by an initial Id ranging from 1.8-3.0.

In Fig. 3 is illustrated the response of animals with an initially more severe diabetes (Group 3—Id range of 3.0-9.0) and in Fig. 4 those rats which were within the upper realm of a "subdiabetic" classification to those with a fully diabetic condition (Group 4—Id>9.0).

It was noted that both the animals acclimated to cold and those housed at room temperature reveal an aggravation of their diabetic state when maintained on the moderately high carbohydrate regimen. Of particular significance, however, was the observation that the imposition of chronic cold stress on mild to moderately diabetic rats favors a relative amelioration in carbohydrate tolerance when compared to those animals maintained at room temperature. Furthermore, no limitation on survival at 5°C for periods up to 3 months was observed in animals with a minimal insulin reserve, as reflected by those with an initial Id less than 12. This stands in contrast to the limited survival noted for rats #40 and #64 (Group 4). These animals were assessed as fully diabetic before exposure to cold by the presence of an initial Id>12, urine glucose excretion between 10-15 g/24 hours and the absence of weight gain when sustained on the moderately high carbohydrate meal. A similar distinction between the mild to moderately diabetic rats housed at 26°C and those exposed to 5°C was noted in their daily glucose excretion (Table I). Among normal animals, Group 1, glucose excretion is zero for both the cold-exposed and room-maintained animals. In Group 2, however, only the cold-exposed animals reveal a urine glucose excretion essentially zero. The animals maintained at room temperature exhibit substantial increases in glucose excretion. The zero glucose excretion observed in animal #27 is an exception; however, it is consistent with the concurrent depression in the rise of its diabetic index. Similar distinctions between cold exposed and room-acclimated animals, although less marked, are noted in the moderately diabetic Group 3.

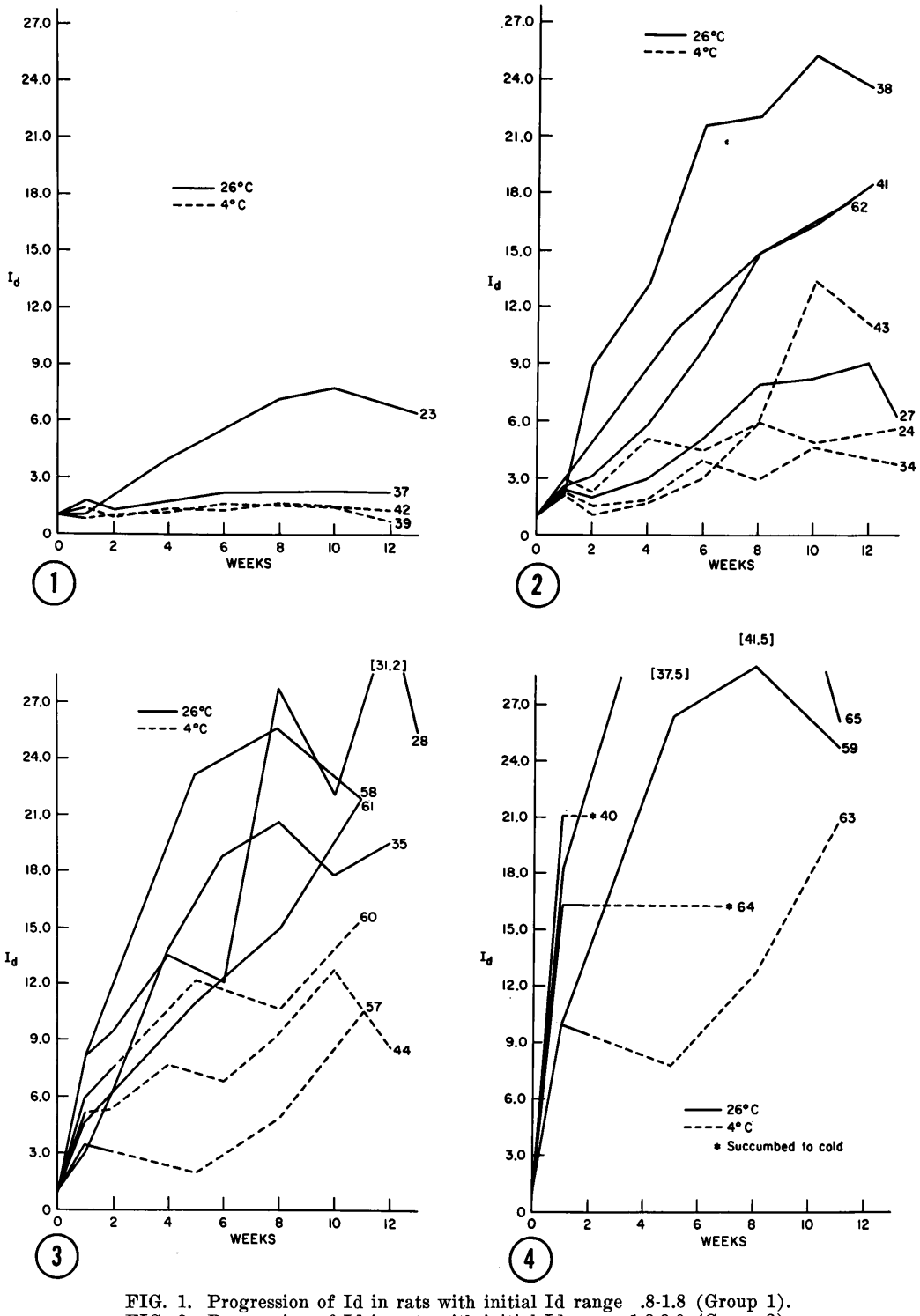

FIG. 1. Progression of Id in rats with initial Id range .8-1.8 (Group 1).
FIG. 2. Progression of Id in rats with initial Id range 1.8-3.0 (Group 2).
FIG. 3. Progression of Id in rats with initial Id range 3.0-9.0 (Group 3).
FIG. 4. Progression of Id in rats with initial Id range >9.0 (Group 4).

TABLE I. Urinary Glucose Loss of Rats Maintained at 26°C and at 5°C Cold After Week 1.

Rat No.	Temp C°	Urine glucose excretion (g per 24 hr) — Week 1	2	3	4	5	6	7	8	9	10	11	12
		Group 1. Initial Id assessment .8-1.8											
23	26	—	—	—	—	—	—	—	—	0	0	0	0
37	26	—	—	—	0	0	0	0	—	0	0	0	0
39	5	—	—	—	0	0	0	0	—	0	0	0	0
42	5	—	—	0	0	0	0	—	—	0	0	0	0
		Group 2. Initial Id assessment 1.8-3.0											
38	26	—	—	15	16	12	10	—	14	10	14	10	14
27	26	—	—	—	—	—	—	—	—	0	0	0	0
41	26	—	—	—	4	10	7	7	—	10	12	12	13
62	26	8	9	7	10	11	13	13	12	11	11	13	—
24	5	—	—	—	—	—	—	—	—	0	0	0	0
34	5	—	—	—	—	—	—	—	—	3	0	0	0
43	5	3	—	—	0	0	2	1	—	0	0	0	0
		Group 3. Initial Id assessment 3.0-9.0											
35	26	3	—	—	15	—	8	9	—	9	10	12	12
61	26	8	8	8	10	12	17	10	17	14	14	17	—
28	26	—	—	—	—	—	5	—	—	15	16	11	11
58	26	11	13	13	12	12	14	15	—	14	16	10	—
57*	5	6	2	0	0	0	0	0	0	0	0	0	—
44	5	6	—	—	14	20	14	10	—	8	9	7	9
60*	5	12	12	9	7	10	12	14	9	11	12	15	—
		Group 4. Initial Id assessment 9.0											
59	26	15	15	14	15	15	12	12	14	16	15	13	—
65	26	11	13	14	14	13	14	12	11	10	11	10	—
63*	5	10	14	8	10	8	9	14	13	16	14	18	—
64*	5	14	15	14	11	9	11	×					
40	5	—	×										

* Placed in cold room after week 2.

Discussion. The progression of a mildly alloxan diabetic state to a fully developed diabetic condition has been previously reported in rats maintained on a ration containing 42% carbohydrate(5). A similar tendency is indicated by the progressive rise in the diabetic index in both rats acclimated to room temperature and cold. However, the rate of progression toward a fully diabetic state was substantially lessened in those animals placed in the cold. Since acclimation and survival at 5°C is dependent on maintenance of an elevated caloric expenditure, prolonged survival of mild to moderately diabetic rats at low temperatures would require an increased consumption and utilization of the food provided. Under conditions where 60% of the caloric intake is derived from carbohydrate, an adequate utilization of glucose would be prerequisite for sustained cold survival. The effectiveness of glucose as a fuel for thermogenesis is demonstrated by the elevated rates of its oxidation and turnover in normal rats acclimated to 6°C(6,7). The association of the action of insulin with glucose utilization would suggest that under conditions where glucose metabolism is compensatorily increased, there would exist a requirement for increased insulin activity. On the other hand, it is quite evident that in the absence of administered insulin, severely diabetic rats on a similar regimen in the cold are unable to maintain metabolic rates at levels consistent with prolonged survival(1). Poe and Davis(8) have reported, as well, the short survival of diabetic rats in the cold. The fact that the mild to moderately diabetic rats are able to survive at 5°C for 3 months must be indicative of a retention of some insulin activity. Since it is reasonable to assume that the limited insulin reserves of such animals preclude a capacity for sustained increases in insulin secretion, the relative lowering of the Id suggests that these animals

exhibited an increased sensitivity to endogenous insulin during confinement at 5°C. Implications of an increased sensitivity to insulin during prolonged exposure to cold have similarly been inferred from the enhanced blood glucose lowering activity of exogenous insulin administered to normal cold-acclimated rats(9,10).

Summary. In the absence of administered insulin, severely alloxan diabetic rats are unable effectively to utilize a carbohydrate regimen at levels sufficient to sustain the elevated metabolic rate necessary for extended survival in cold. However, mild to moderately diabetic rats not only survive at 5°C for periods up to 3 months but show a substantial amelioration in the diabetic state in relation to control animals maintained at 26°C. This was attributed to the development of an increased sensitivity to endogenous insulin activity during confinement in cold.

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Proteins of Ovarian Cyst Fluid and Serum from Hypothyroid Gonadotrophin-Treated Rats.* (28497)

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Studies comparing follicular fluid, ovarian cyst fluid, and serum reveal similarities that suggest a common origin for their protein components. Human ovarian cyst fluid exhibits protein patterns of lower globulin content than serum(1), whereas no difference was noted in bovine follicular fluid and serum in protein patterns. Bovine follicular fluid, however, contains less total protein than serum and lacks beta lipoprotein(2). Changes in bovine serum electrophoretic patterns resulting from lung disease are similarly reflected in the follicular fluid(2).

Rat ovarian response to human chorionic gonadotrophin and pregnant mare's serum can be enhanced by feeding a diet containing 0.5% thiouracil. Chorionic gonadotrophin causes luteinization of the ovary in euthyroid rats, but in the hypothyroid rat response to this hormone is characterized by luteinization and formation of large follicular cysts(3).

Hypothyroidism increases total serum protein concentration, particularly the gamma globulin fraction of rat serum(4), which provides a basis for investigating the origin of cyst fluid protein. A preliminary study suggests that a major portion of cyst fluid protein may be of plasma origin(5). In an effort to establish the origin of cyst fluid, a comparison was made between this fluid and serum using protein and glycoprotein electrophoretic patterns, and incorporation of S^{35} from methionine as criteria.

Materials and methods. Long-Evans female rats (65-75 g) were fed a diet containing 0.5% thiouracil for 30 days. Ovarian cysts were induced by injecting 10 i.u. of human chorionic gonadotrophin (HCG) (Fol-

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