Streptozotocin-induced diabetes in Syrian hamsters: New model of diabetes mellitus[1]

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Summary. Syrian golden hamsters can 'recover' from single diabetogenic doses of alloxan and return to normoglycemia. Induction of chronic diabetes was accomplished by multiple injections of streptozotocin.

Syrian golden hamsters *(Mesocricetus auratus)* have been widely used in biological research, especially in studies of viral disease, oncology and toxicology. However, this species has not been extensively used in diabetes research as there are problems in producing diabetes in Syrian hamsters. The most serious problem is that a significant percent of hamsters which appear to become diabetic after injections of alloxan 'recover' from the treatment and return to normal glucose concentrations after a few days to a few weeks[2]. Therefore, this work was undertaken to produce and characterize a chronic diabetic syndrome in this species that can be maintained for extended periods without insulin therapy.

Materials and methods. Hamsters: Male Syrian hamsters, approximately 40 days of age (Sasco, Inc., Omaha, NE) were kept on a 12-h light period at 23 °C, and, except when noted, allowed free access to Wayne Lab Blox and water.

Serum glucose was determined with a Beckman Glucose II Analyzer (Beckman Co., Fullerton, CA). Serum concentrations of insulin were determined in a double-antibody radioimmunoassay (Amersham-Searle, Chicago, IL). Human insulin was used as the standard; therefore, the hamster insulin concentrations are expressed in terms of the human standard. Aliquots of serum were assayed for triglyceride content by the method of Neri and Frings[3].

Induction of a chronic diabetic syndrome was attempted using both alloxan (Ax) (Eastman Co.) and streptozotocin (Sz) (courtesy of Dr W.E. Dulin, Upjohn Co., Kalamazoo, MI). Only hamsters with fed serum glucose concentrations > 300 mg/dl at each sampling time over a 1-month period were considered to be chronically diabetic. Ax was dissolved in 0.9% saline and injected within 1 min. Sz was similarly injected after being dissolved in acidified (pH 5.0) 0.9% saline. Results are expressed as the means of the groups ± SEM. Values of $p < 0.05$, as determined by Student's t-test are considered to represent statistically significant differences.

Results. Alloxan: Intraperitoneal (i.p.) as well as intracardiac (i.c.) injection routes at several dose levels were utilized. The data in table 1 show that i.p. injections of Ax were not effective in producing diabetes, regardless of whether the animals were fed or fasted. I.c. injections of Ax were diabetogenic but the high doses required were poorly tolerated by the animals and subsequently high numbers of fatalities occurred. Table 1 indicates that at a dose of 60–75 mg/kg, approximately 50% of the hamsters (≅ 60% of survivors) were diabetic after 1 week. However, only a small percentage (11%) of the total number of treated hamsters remained diabetic after 1 month.

Streptozotocin: After treating over 350 hamsters with Ax, it became obvious that the number of animals required to produce chronic diabetes would be prohibitive; therefore, Sz was tried in both fed and fasted hamsters. Table 1 indicates that doses of 60–80 mg/kg of Sz injected either i.p. or i.c. in fasted hamsters were not tolerated well. In light of the limited success with single large doses of both Ax and Sz in producing chronic diabetes in an acceptable proportion of hamsters, it was decided to try multiple small injections of Sz[4].

As the data in table 1 show, 40 mg/kg of Sz injected i.p. daily for 5 days was tolerated well. In addition, a significant percentage of the treated animals were diabetic after 1 week. However, after 1 month, some of the diabetic hamsters had 'recovered'. With a dose of 50 mg/kg of Sz injected i.p. daily for 3 days, the results were much more satisfactory. In several separate experiments involving 229 hamsters, 81% (range 62–98%) of the hamsters were diabetic 1 week after the final Sz injection. Approximately 84% of these animals remained diabetic after 1 month. Furthermore, we have monitored these diabetic hamsters for 4 months and have observed that essentially 100% of the hamsters diabetic after 1 month remain diabetic.

While we have monitored diabetic hamsters for as long as 4 months after Sz treatment, the characteristics of this model given in table 2 were accumulated from several groups of diabetic hamsters sampled at 1 month after Sz treatment.

Discussion. We have reaffirmed the ability of Syrian hamsters to 'recover' from chemically-induced diabetes. Nace et al.[2] reported an extremely narrow range (45–50 mg/kg) for an effective diabetogenic dose of Ax in hamsters, with dose

Table 1. Chemical induction of chronic diabetes[a] in Syrian hamsters

Treatment	N	Route of administration	Dosage (mg/kg b.wt)	Fed or fasted[b]	Survival 1 week after treatment	Diabetic 1 week after treatment
Alloxan	6	i.p.[c]	250	Fed	100%	0
Alloxan	5	i.p.	250	Fasted	80%	0
Alloxan	5	i.c.[d]	50	Fasted	100%	0
Alloxan	117	i.c.	60	Fasted	52%	21%
Alloxan	237	i.c.	75	Fasted	49%	20%
Streptozotocin	10	i.p.	60	Fasted	0	–
Streptozotocin	16	i.c.	60	Fasted	6%	6%
Streptozotocin	17	i.c.	80	Fasted	12%	12%
Streptozotocin (5 days)	104	i.p.	40	Fed	87%	54%
Streptozotocin (3 days)	229	i.p.	50	Fed	91%	81%

[a] Diabetic = serum glucose > 300 mg/dl; [b] fasted 18 h from 15.30 to 09.30 h; [c] intraperitoneal; [d] intracardiac.

Table 2. Serum glucose, triglycerides and insulin concentrations of normal and diabetic Syrian hamsters[a]

	Fed Serum glucose (mg/dl)	Fasted[c] Serum glucose (mg/dl)	Serum triglycerides (mg/dl)	Insulin[d] (μU/ml)
Normal	106±4 (N=34)	109±3 (N=52)	126±89 (N=29)	60±7 (N=48)
Diabetic[b]	412±7 (N=58) p<0.001	329±11 (N=40) p<0.001	478±181 (N=29) p<0.01	25±3 (N=57) p<0.001

[a] Untreated normal and diabetic age-matched male hamsters. [b] All serum values of both groups determined from blood samples collected one month after initiation of streptozotocin injections in the diabetic group. All values are the means of the number of samples given in parentheses ± SEM. [c] Fasted 18 h prior to collection of blood by orbital sinus puncture. [d] Serum insulin concentrations determined by radioimmunoassay as described in the materials and methods section.

levels higher than 55 mg/kg being lethal. In a later report, House and Tassoni[5] found that most (70%) of their 'diabetic' hamsters reverted to normoglycemia after a few days to several weeks even though their definition of diabetes was very liberal (blood glucose > 130 mg/dl = diabetic). Sak and Beaser[6] were able to induce diabetes in hamsters with a much higher i.c. dose (100 mg/kg) of Ax but experienced difficulties with fatalities.

However, by giving multiple injections of Sz, we have been able to consistently induce diabetes in a majority (≅ 80%) of our hamsters. In addition, most of the hamsters (≅ 84%) which fell into the diabetic range 1 week after treatment remained diabetic after 1 month.

To our knowledge, this is the first report of the successful induction of chronic diabetes in a predictable high percent of Syrian hamsters. The value of the hamster cheek pouch for microcirculation studies has long been appreciated and the diabetic Syrian hamster model should gain considerable utility.

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The transfer of juvenile hormone from male to female during mating in the Cecropia silkmoth[1]

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Summary. The juvenile hormone (JH) stored in the accessory sex glands (ASG) of adult male *Hyalophora cecropia* (L.) originates both from sequestration of circulating hormone and from JH synthesized de novo in the ASG from JH acid taken up from the hemolymph. The secretions present in the lumina of the ASG contain most of the accumulated JH. During mating, endogenous JH, labeled biosynthetically via injected [^{3}H-methyl]-methionine, is transferred along with the other seminal material to the bursa copulatrix of the female. The physiological significance of the JH transfer remains unknown.

The storage of large quantities of juvenile hormone (JH) in adult male Cecropia silkmoths[4] represents an atypical endocrine phenomenon which is known to occur in only 2 other closely related saturniids[5,6]. This phenomenon is based on the ability of the male accessory sex glands (ASG) to act as a repository for JH[7]. These glands contain a JH acid methyltransferase which facilitates the transfer of the methyl group of S-adenosyl methionine to a JH acid, thus forming the respective JH[8-11]. In addition to this process, the ASG are also able to take up JH intact. Once in the ASG, most of the JH stored is found in association with the secretions contained in the lumina of the glands[9]. Since the ASG contribute material to the formation of the spermatophore[12,13], we investigated the possibility of JH transfer to the female during copulation.

Cecropia were obtained from commercial suppliers as diapausing pupae and kept at 4 °C for a minimum of 90 days. Adult development was initiated by exposing the pupae to 27 °C, 70–80% relative humidity and 16–8 h light-dark cycle. [^{3}H-methyl]-methionine (3.7 Ci/mmole) was purchased from Schwarz/Mann. Ether was anhydrous, analytical reagent grade (Mallinckrodt).

Freshly eclosed adult male Cecropia were injected with [^{3}H-methyl]-methionine in Weevers' saline[14] (1 μCi/μl). After 24 h the radiolabeled males were placed with untreated freshly eclosed females. Adult Cecropia mate about 30 min before lights on, continue throughout the light period, and separate shortly after the lights go off again. Mating of all pairs occurred during the following dark phase and the day of copulation is referred to as day 0. The copulation of pair 1 was disturbed prior to the next dark phase and both animals were sacrificed, removing the male ASG and the female bursa copulatrix (BC) separately for processing. Pairs 2 and 3 were allowed to complete copulation; the males were sacrificed at the beginning of day 1 after copulation while the females were sacrificed on day 3 after copulation. The ASG and BC were processed for JH identification by extraction with ether/ethanol (6:1), TLC on silica gel HF_{254} (0.25 mm, methanol washed and activated) with a hexanes/ethyl acetate/acetic acid (70:25:5) solvent system, and high pressure liquid chromatography on μ Porasil with a hexanes/ethyl acetate/2-propanol (96.48:3.5:0.02) solvent system. A mixture of cold JH-I and JH-II was added to the ether/ethanol extracts as an internal standard.

As is evident in the table, JH accumulated in the ASG of adult males is transferred to the BC during copulation. When interrupted during mating, all of the labeled JH was found in the BC (pair 1). When the male was sacrificed after copulation, a small amount of radiolabeled JH was detected in the ASG (pair 2, pair 3), which suggests some post-coital accumulation of JH. It is also interesting to note that even 2 days after mating radiolabeled JH was present in the BC.