Evaluation of participation of glucose in elevation of glycemia of scorpion *(Heterometrus fulvipes)* caused by the hyperglycemic principle of the scorpion and crab[1]

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Summary. A single injection of the hyperglycemic principle obtained from scorpion cephalothoracic ganglionic mass causes an elevation of glycemia in the scorpion *H. fulvipes* in which little glucose is involved (10.8%). With the crustacean hyperglycemic principle, the elevation of glycemia is initially almost exclusively contributed by glucose (glucose is 92% of total carbohydrate at 2 h post injection); 6 h after treatment, non-glucose carbohydrates appear to participate in the elevation of glycemia.

The local scorpion, *Heterometrus fulvipes*, shows hyperglycemic response to injection of extracts of its own cephalothoracic ganglionic mass (CTGM)[2,3], and to crustacean hyperglycemic hormone[4,5]. The two principles have been found to cause hyperglycemia through different mechanisms with regard to the mobilization of reserves of the central metabolic organ, the hepatopancreas[3,5]. The present communication is intended to provide further information about these mechanisms.

Materials and methods. Adult scorpions (8–10 g) were caught in September. Since the sex and the molt cycle affect hemolymph sugar level[6,7], only intermolt, male scorpions were used. They were kept for at least 7 days in the laboratory at 28 °C with a daily light period of 8 h. The scorpions were fed ad libitum daily with cockroaches; 1 day prior to experimentation feeding was stopped to avoid prandial effects.

The mode of isolation and extraction of hyperglycemic principles from scorpion CTGM (SHGP) and crab *(Oziotelphusa senex senex)* eyestalks (CHGP) were described earlier[2,4]. Hyperglycemic principle was administered by a single injection of either 2 eyestalk equivalents or 1 CTGM equivalent in 50 µl of solution containing 15 µg of protein with a microsyringe. Controls were injected with 50 µl of scorpion Ringer's salts[8] or muscle extract (2% w/v). 2 and 6 h after injection the levels of glucose[9] and total carbohydrates (TCHO)[10] were estimated in hemolymph samples of scorpions subjected to different treatments (Ringer-injected controls; muscle extract-injected controls; SHGP-injected animals; CHGP-injected animals).

Results and discussion. The hyperglycemic effect, i.e. the elevation of total carbohydrates (TCHO) of the hemolymph, is shown by both hyperglycemic principles (table 1). The smaller increase in the glucose level under SHGP-treatment, as compared with the increase caused by CHGP, agrees well with the conclusions of Raghavaiah et al.[3] and Streenivasula Reddy et al.[11]. The authors found that CHGP-induced hyperglycemia results from phosphorolysis of glycogen of the hepatopancreas or central metabolic organ (CMO). It may therefore involve greater participation of glucose. The SHGP, on the other hand, causes hyperglycemia without appreciable involvement of glycogen reserves of the CMO[3] and this is reflected in a smaller participation of glucose in the hyperglycemia (table 1).

The present data, besides corroborating the former conclusions of Sreenivasula Reddy et al.[11], reveal one more point of interest with regard to the mode of action of CHGP: The contribution of glucose to the elevation of glycemia at different periods after administration of the hyperglycemic principles (table 2) shows that glycemia caused by CHGP is initially almost entirely due to glucose; as much as 92% of glycemia elevation in the first 2 h is accounted for by glucose alone. Subsequently, the contribution of glucose to the elevation of glycemia declines to 56.4%, which seems to show that the CHGP begins to exert an effect on some non-

Table 1. The effect of hyperglycemic principles obtained from scorpion and crab on the levels of hemolymph glucose and TCHO in the South Indian scorpion *(H. fulvipes)*

Treatment	Time post injection (h)	Glucose[1]	TCHO	Glucose % TCHO
Normal		7.70±0.78[2]	84.0± 9.1	9.17
Injection of distilled water	2	7.18±1.38	79.0± 8.8	9.09
Control-1	6	7.82±1.22	85.0±10.2	9.20
Muscle extract injection	2	7.12±1.28	78.0± 8.3	9.10
Control-2	6	7.32±1.06	83.0± 9.2	8.82
SHGP	2	9.18±1.00	98.0± 9.3	9.36
Injection	6	13.12±0.78*	128.0±10.1*	10.3
CHGP	2	26.0 ±3.18*	102.0± 6.8*	24.5
Injection	6	38.0 ±3.69*	136.0±13.2*	27.9

[1] Values expressed as mg glucose per 100 ml of hemolymph.
[2] Values are means ± SD of 8 individuals.
* Values are significantly ($p < 0.001$) different from normal ones.

Table 2. Percent participation of glucose in glycemia-elevation at different times after SHGP and CHGP administration

Source	Time post injection (h)	Elevation of Glucose (mg)	TCHO (mg)	Glucose % TCHO
SHGP	2	1.75	16.20	10.8
	6	5.69	46.20	12.3
CHGP	2	18.57	20.20	91.9
	6	30.57	54.20	56.4

Elevation of the levels of glucose/TCHO were calculated by way of difference of the levels in experimental and the average control value (for glucose 7.43 and for TCHO 81.80).

glycogen sources of carbohydrates of the CMO to sustain the hyperglycemic action for longer periods. Detailed examination of this phase of hyperglycemia may prove worthwhile. Work is in progress.

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Cyclic AMP accumulation in human fibroblast cultures: diabetics compared with normals

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Summary. Isoproterenol-stimulated accumulation of adenosine 3′,5′-monophosphate (cyclic AMP) in skin fibroblast cultures from type 2 diabetics was twice as high as in fibroblasts from control subjects.

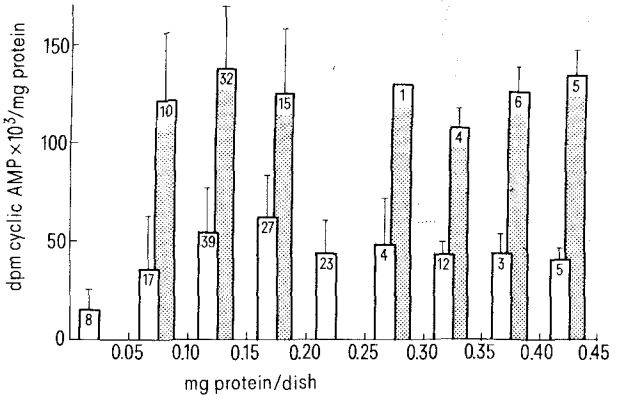
Differences observed between cultured fibroblasts from different donors are supposed to reflect intrinsic characteristics, rather than hormonal or metabolic conditions at the time of biopsy. Previously it has been shown that in fibroblast cultures, cells from diabetics have a reduced capacity for cell proliferation[1-4]. It is also known that cells in which growth is arrested in some way tend to have higher levels of intracellular adenosine 3′,5′-monophosphate (cyclic AMP)[5].

The purpose of this study was to find out if fibroblasts from diabetics have an alteration in cyclic AMP metabolism which could be associated with the aberration in growth properties which has previously been reported.

Patients and methods. Skin fibroblasts were cultured from punch biopsies taken from the medial aspect of the left upper arm of 4 healthy subjects aged 35–52 and 4 subjects 46–57 years old with maturity onset (type 2) diabetes. Cells were grown in humidified air at 37 °C in Eagle's minimal essential medium (MEM) containing 10% fetal calf serum, 2% L-glutamine, 1% non-essential amino acids, and 0.2% gentamicin. Cells at passage 2–6 were used, i.e., cells at an average of 4–12 population doublings in vitro. The fibroblasts were cultured in 3-cm dishes in 2 ml growth medium. Cells were allowed to settle for 48 h. Subsequently medium was changed every 24 h. Studies were made on day 3–6 after subcultivation.

Cyclic AMP accumulation in the medium was determined with a prelabeling technique using [^{3}H]-adenine (Amersham) for subsequent determinations of [^{3}H]-cyclic AMP[6]. Immediately after the growth medium had been removed 3 ml MEM containing 30 µCi [^{3}H]-adenine was added to each dish. Attempts were made to make concomitant determinations of 5′-nucleotidase according to Belfield and Goldberg[7]. For that reason adenosine deaminase, adenosine monophosphate, and glycerophosphate were added. These results will be presented elsewhere. After 60 min of prelabeling, cells were washed twice with 3 ml MEM and finally with another 3 ml MEM for 20 min at 37 °C. Cells were then incubated for 10 min with a medium which contained MEM supplemented with 1 mmole/l cyclic AMP (Sigma Chemical Co), 12 µmoles/l isoproterenol, 1 mmole/l thiourea, and 0.2 mmoles/l ascorbate. Thiourea and ascorbate were included to minimize catecholamine oxidation[8]. Incubations were terminated after 10 min by transferring the medium to tubes containing 0.1 ml 2% sodium dodecyl sulfate. [^{14}C]-cyclic AMP (approximately 1200 disintegrations per min, DPM) in 50 µl was then added to monitor cyclic AMP recovery. [^{3}H]-cyclic AMP liberated by the fibroblasts into the medium was determined according to Salomon et al[9]. Aquasol-2 was used for liquid scintillation at 10 °C using a Packard B2450 liquid scintillation spectrometer. Cyclic AMP was expressed as dpm per mg protein after individual recovery and quench corrections. Protein was determined with the Fluram method[10] using serum albumin as standard. Cells were scraped out of the dishes in water with a rubber policeman and carefully disintegrated with a motor-driven micro glass homogenizer, or dissolved in situ with 0.2 moles/l NaOH for 15 min at 37 °C.

Results. The figure illustrates that up to a certain level of cell density (0.2 mg protein/dish) cyclic AMP accumulation



Isoproterenol-stimulated (12 µmoles/l) cyclic AMP accumulation in fibroblast cultures from 4 normals (open bars) and 4 diabetics (solid bars). Numbers of observations are indicated.