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.

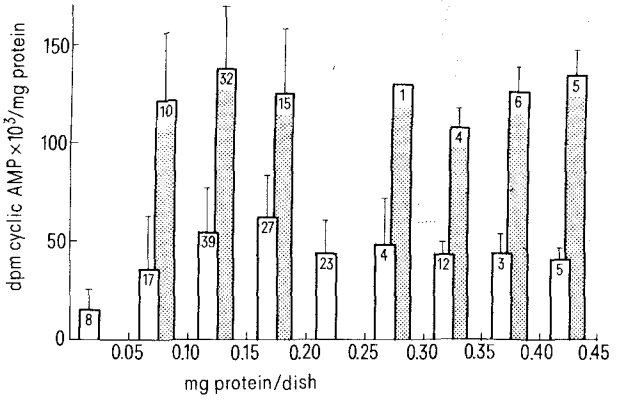
Differences observed between cultured fibroblasts from different donors are supposed to reflect intrinsic characteristics, rather than hormonal or metabolic conditions at the time of biopsy. Previously it has been shown that in fibroblast cultures, cells from diabetics have a reduced capacity for cell 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.

Isoproterenol-stimulated cyclic AMP accumulation in fibroblast cultures from normal and diabetic subjects

| Donor | Age | Years after diagnosis | Therapy | Culture passage No. | DPM [$^3$H]-cyclic AMP × $10^3$ / mg protein | Group mean |
|---|---|---|---|---|---|---|
| MOD | 46 | 6 | Sulphonylurea | 2 | 114 ± 13 (n = 5) | |
| MOD | 49 | 5 | Sulphonylurea | 6 | 125 ± 18 (n = 22) | |
| MOD | 54 | 6 | Sulphonylurea | 3,5 | 125 ± 44 (n = 15) | |
| MOD | 57 | 3 | Diet | 2,4 | 141 ± 28 (n = 31) | 126 ± 11 |
| N | 35 | | | 4,6 | 42 ± 15 (n = 59) | |
| N | 36 | | | 2 | 53 ± 27 (n = 32) | |
| N | 43 | | | 3 | 44 ± 6 (n = 20) | |
| N | 50 | | | 6 | 79 ± 20 (n = 19) | 55 ± 17 |

Difference between groups significant by Mann-Whitney rank sum test ($p = 0.05$). MOD, Maturity onset diabetics; N, normals.

per mg protein in cells from healthy subjects tended to increase in parallel with protein. Cells from maturity onset diabetics were consistently above control cells in cyclic AMP release.

In the range of 0.05–0.45 mg protein per dish a total of 203 observations were made on fibroblasts from 4 diabetics and 4 healthy subjects. Cells from the diabetic subjects displayed cyclic AMP levels significantly higher $(126 \pm 11) \times 10^3$, than those exhibited by the normal cells, $(55 \pm 17) \times 10^3$ (table).

*Discussion.* Two different methods of detaching cells from the culture dishes were used. Similar results for protein concentration were obtained. Dissolving the protein with 0.2 moles/l NaOH was less time-consuming than the mechanical method and was therefore preferred in most experiments. In the present study, differences in cyclic AMP accumulation could be the result of alterations in adenylate cyclase or phosphodiesterase activity. Phosphodiesterase inhibitor was not used, in order to avoid restriction of nucleotide efflux from cells[11]. Strict comparison of net cyclic AMP release by the [$^3$H]-adenine prelabeling method requires that uptake of tracer is the same in both cell groups and that cell content of ATP is likewise similar. Thus, the adenine prelabeling technique entails some limitations in the interpretation of the cyclic AMP data. However, it is evident that a difference in nucleotide metabolism exists between the two fibroblast groups.

In previous studies abnormalities in cell growth have been noticed in fibroblast cultures from diabetics. Cumulative number of fibroblast doublings have been decreased[2,3], and reduction in growth rate and cell density at confluence has also been noticed[12]. An association between increased cyclic AMP levels and growth cessation in cells has also been recognized[5]. The results in this study showing an increased cyclic AMP accumulation in fibroblast cultures from diabetics agree with what would be expected in cells which have a reduced capacity for cell proliferation. The results also indicate the presence of an intrinsic difference in nucleotide metabolism between 'normal' and 'diabetic' human cells. If present in other tissues as well, this deviation from the normal state may well be relevant for the understanding of diabetic metabolism.

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## Effect of lectins from leguminous seeds on rat duodenal enterokinase activity

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*Summary.* Enterokinase activity from rat duodenal brush borders was assayed in vitro in the presence of purified lectins from 3 leguminous seeds. Noncompetitive inhibition of the enzyme was observed in each case. *Phaseolus* hemagglutinin was the most potent inhibitor among the 3 lectins tested.

Protein malnutrition observed in rats fed raw leguminous seeds is the result of imbalance in the amino acid pattern of the proteins and, above all, of the presence of numerous antinutritional and toxic components, which impair the protein digestibility[1,2]. Lectins may be responsible for this effect[3], just as trypsin inhibitors are[4]; the question still remains controversial[5]. Lectins could act by damaging enterocytes[6,7] or more specifically, by impairing the digestion of proteins and/or the absorption of amino acids[8]. Concerning protein digestion, every pancreatic protease activity we tested remained unaffected by lectins from leguminous seeds. But the enzyme required for the activation of pancreatic zymogens has not previously been tested. Enterokinase inhibitors have recently been found in numerous tubers and seeds[9], among which are several *Phaseolus* seeds. The only information reported about their nature is