Growth hormone effect on accumulation of arterial basement membrane-like material studied on rabbit aortic myomedial cell cultures

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Summary. The effect of human growth hormone on arterial basement membrane-like (BM) material was studied. BM-like material was obtained from the cell layer of cultured aortic myomedial cells using a sonication-differential centrifugation technique. After the addition of small amounts of growth hormone (1 ng/ml) to the cultures, we observed a 26% increased incorporation of amino acids into BM-like material ($2p < 0.005$). However, further increase in the incorporation was not observed using either 3 ng or 10 ng growth hormone per ml. Growth hormone inhibited removal/degradation of BM-like material by 16% ($2p < 0.01$). However, pinocytosis rate and ac-

tivity of major lysosomal enzymes: cathepsin D, acid phosphatase and β -N-acetyl-glucosaminidase were unchanged. Incorporation of glycosaminoglycans as evaluated by [$^{35}\text{SO}_4$]-labelling was reduced by 8% when cells were exposed to growth hormone ($2p < 0.01$). The present study demonstrates an effect of growth hormone on the turnover and composition of BM-like material in cultured arterial myomedial cells.

Key words: Basement membrane degradation, diabetic angiopathy, arterial glycosaminoglycans, atherosclerosis.

Growth hormone has been suggested to be a causative or permissive factor in the development of diabetic microangiopathy and perhaps also in large vessel disease commonly occurring in long-term diabetic patients [1, 2]. Light microscopic studies of diabetic coronary arteries have shown increased amounts of PAS-positive material in tunica media [3] and proliferation of myomedial cells in the smaller arterioles [4].

Cultures of arterial myomedial cells have been used for several years in model experiments to elucidate the nature of atherosclerosis and other kinds of human large vessel disease [5]. Originally this technique was employed in morphological studies, demonstrating, e.g., proliferation of myomedial cells in media containing hyperlipaemic or diabetic serum [6].

In earlier morphological studies in cell cultures we have shown that addition of small amounts of growth hormone to the incubation medium results in proliferation of arterial myomedial cells [7], and that addition of growth hormone antiserum suppresses the proliferative effect of diabetic serum [8].

In cell cultures as well as in the arterial wall, myomedial cells are embedded in a PAS-positive matrix of basement membrane material [9]. Recently we have described a technique for isolation of basement membrane (BM) material from cultured aortic myomedial cells [10].

The present study deals with the effect of growth hormone on basement membrane material isolated from cultures of myomedial cells. It was found that the

addition of small amounts of growth hormone led to accumulation of basement membrane material and a reduction in its content of sulphate.

Methods

Cells

Cultures of arterial myomedial cells were grown from thoracic aortas obtained from male rabbits weighing 1.5–2 kg. Explants of tunica media (13–15 pieces) were incubated in tissue culture flasks (264 ml, Nunc, Roskilde, Denmark) with 15 ml of a standard growth medium at 37°C and 5% CO_2 in air. The standard growth medium used in all the various experimental situations was composed of Eagle's basal medium, Hanks' salt solution, ascorbic acid (50 $\mu\text{g}/\text{ml}$) and 5% rabbit serum + 5% newborn calf serum (Gibco, UK). The medium was changed every second day and the primary non-trypsinized cultures were grown for about 5–6 weeks, at which time the "stationary growth phase" was reached [11].

Preparation of basement membrane-like material

The isolation procedure was carried out as reported earlier [10]. Briefly, the incubation medium was decanted and the cell layer washed three times with 0.15 mol/l NaCl followed by incubation for 15 min at room temperature with 1 mol/l NaCl. All solutions were buffered with 10 mmol/l Tris/HCl, pH 7.4 and the following proteinase inhibitors were added: 0.5 mmol/l N-ethylmaleimide, 0.5 mmol/l phenylmethanesulphonyl fluoride and 1 mmol/l benzamidine-HCl (Sigma, USA). Cells and extracellular matrix were scraped off the culture flask using a rubber policeman and sonicated three times for 30 s at a wave length of 16 μm with cooling in ice water between bursts. After evaluation by phase contrast microscopy to ensure satisfactory dis-

Fig. 1. Protocol for the accumulation studies of arterial basement membrane-like material. hGH = human growth hormone (1 ng/ml)

Fig. 2. Accumulation pattern of tritiated leucine labelled basement membrane-like material as a function of time. 1 bar = 1 SEM. $n = 5$ cultures

Fig. 3. Protocol for the cold chase studies of arterial basement membrane-like material. hGH = human growth hormone (1 ng/ml)

ruption of cells, BM-like material was sedimented from the sonicated cell suspension by centrifugation at 2100 g for 10 min at 4°C and washed three times with 1 mol/l NaCl, twice with 0.15 mol/l NaCl and finally in sterile distilled water. Between washes the BM-like material was sedimented by centrifugation as described above. Approximately 200–500 μ g BM-like material was isolated from each culture flask.

Accumulation studies

Cultures were preincubated for 24 h in the standard growth medium supplemented with 1 ng hGH/ml (Fig. 1). The standard growth medium was then discarded and substituted with medium including either tritiated amino acid mixture (0.5 μ Ci/ml, Amersham International, UK) or [$\text{Na}_2^{35}\text{SO}_4$] (5 μ Ci/ml, 20–40 Ci/mg, Amersham International, UK) in addition to 1 ng hGH/ml. However, the effects of hGH concentrations of 0.2 ng, 0.5 ng, 3 ng and 10 ng per ml medium were also analysed. After 24 h incubation BM-like material was isolated and its radioactivity as well as protein content was estimated. Protein was measured by a Lowry technique using bovine albumin as standard after solubilization in 1 N NaOH [12]. The experiments were performed twice with cultures from two aortas. In preliminary studies it was found that a 24-h incubation period was appropriate for evaluation of the accumulation of BM-like material (Fig. 2).

The size of the intracellular amino acid pool was evaluated using [^3H]leucine in 20 cultures, of which 10 received 1 ng hGH/ml. The incubation conditions were as described for the accumulation studies. The removed cell layer was solubilized in 0.1% Triton X-100 containing 260 mg non-radioactive leucine per liter. The cell layer was sonicated for 1 min. Total protein was estimated and trichloroacetic acid (10%) was added to precipitate protein. Radioactivity was measured

in the supernatant after centrifugation and expressed as cpm/mg total protein.

Human growth hormone (Nanormon, Nordic Insulin Laboratories, Gentofte, Denmark) has been found to be immunological equivalent to a Wilhelmi human growth hormone preparation (no. 1216s) [8].

Cold chase experiments

The effect of growth hormone on degradation of arterial BM-like material was evaluated in a series of cold chase studies (Fig. 3). The cultures were incubated in standard growth medium supplemented with either tritiated amino acid mixture (0.5 μ Ci/ml) or [$\text{Na}_2^{35}\text{SO}_4$] (5 μ Ci/ml) for 24 h. Then the cultures were rinsed in 5×10 ml growth medium and chased into standard growth medium without isotopes but now supplemented with 1 ng hGH/ml. The concentration of unlabelled amino acids and sulphate in the growth medium corresponds to the level in human plasma. BM-like material was isolated and its radioactivity and protein content measured after a 24-h chase period. This investigation was carried out twice with cultures from two aortas.

Separate experiments were carried out in order to evaluate the possible reincorporation of radioactive amino acids released into the medium. A number of 19 cultures was labelled with 0.1 μ Ci [^3H]leucine/ml for 24 h, then rinsed and chased for 24 h into a standard growth medium as described above. The chase medium was pooled, supplemented with glucose (5 mmol/l) and then added to a new series of 19 cultures of which 10 cultures received 1 ng hGH per ml. After a period of 24 h the BM-like material was isolated as described above.

Endocytosis

The effect of hGH on endocytosis rate of arterial myomedial cells was measured using horseradish peroxidase (HRP). For endocytotic measurements cultures were incubated with 1 mg HRP/ml standard growth medium for 4 h at 37°C [14]. Adherence of HRP to cell surfaces, matrix and culture flask was evaluated by keeping three cultures in each experiment at an incubation temperature of 4°C. Protein and HRP were determined in the supernatant from a cell homogenate centrifuged at 5000 g for 10 min at 4°C. HRP was measured by a odianisidine method [13]. The experiment was performed twice on cultures from two aortas.

Lysosomal enzymes

Lysosomal acid phosphatase (EC 3.1.2.2), cathepsin D (EC 3.4.4.23) and β -N-acetyl glucosaminidase (EC 3.2.1.30) were estimated in the supernatant of a cell homogenate obtained from arterial myomedial cell cultures grown for 24 h in standard growth medium supplemented with 1 ng hGH/ml. The supernatant was obtained by centrifugation at 4000 g for 15 min at 4°C after addition of Triton X-100 to a final concentration of 0.05% [14]. The analysis was carried out twice with cultures from two aortas.

Lysosomal acid phosphatase activity was determined using p-nitrophenyl phosphate as substrate (type 104, Sigma), and β -N-acetyl glucosaminidase by measuring the release of p-nitrophenol from p-nitrophenyl-N-acetyl- β -glucosaminide (Sigma) [14]. Finally cathepsin D was estimated with bovine haemoglobin as substrate (Sigma) and the production of TCA-soluble protein was assayed by a Lowry reaction [14].

SDS-PAGE

The protein pattern of isolated radiolabelled BM-like material was evaluated by exponential (5–15%) polyacrylamide gel electrophoresis in 0.1% SDS according to Laemmli [15]. The gels were processed for

Fig. 4. Effect of 24 h incubation of hGH (1 ng/ml) in standard growth medium on the accumulation of [^3H]amino acids into arterial basement membrane-like material isolated from the cell layer of cultured aortic myomedial cells. C = controls, hGH = human growth hormone

Fig. 5. A comparison of the effect of hGH (human growth hormone) concentrations at 3 ng and 10 ng per ml medium on accumulation of tritiated leucine labelled arterial basement membrane-like material after a 24-h incubation period

fluorography as described by Bonner and Laskey [16]. Densitometry was carried out using a laser-scanner (LKB, Bromma, Sweden).

Statistical methods

The results were based on at least two experiments each including 20–30 cultures from the same aorta allocated into control and experimental groups. Calculations were performed as described in detail previously [7]. Student's *t*-test or Wilcoxon's test for paired and non-paired comparison was used, and a *p*-value of less than 0.05 was considered as limit of significance (two-tailed test) [17].

Results

The effect of hGH at a final concentration of 1 ng/ml (standard growth medium) on arterial BM-like material was analyzed. It was demonstrated that accumulation of radioactive amino acids in BM-like material was significantly enhanced in cultures supplemented with hGH as compared to control cultures. The increase amounted to approximately 26% (Fig. 4, $2p < 0.005$). There were no statistically significant differences between the effect on accumulation of 0.2 ng and 0.5 ng growth hormone respectively, as compared to controls. But 3 ng and 10 ng hGH per ml both increased statistically significantly the accumulation of BM-like material ($2p < 0.01$). However, there was no difference between the effect of growth hormone concentrations at 3 ng and 10 ng per ml (Fig. 5).

Estimation of the intracellular free amino acid pool using [^3H]leucine revealed no difference between control and hGH treated cultures (505 ± 44 vs 533 ± 45) (cpm/mg total protein, $\bar{x} \pm \text{SEM}$, $n = 10$).

We observed no gross differences in the band pattern between BM-like material obtained from hGH-treated and non-treated cultures using SDS-PAGE as shown in the electrophoretogram (Fig. 6a). Densitometry of the gels is shown in Fig. 6b.

In a cold chase study we directed our analysis to the effect of hGH on degradation of BM-like material. We found a significantly larger amount of radioactive amino acids in BM-like material from myomedial cells incubated with hGH than in control cultures, indicating that hGH inhibits degradation of BM-like material. The difference amounts to about 16% (Fig. 7, $2p < 0.01$). Control values at the start of the cold chase period were 72.1 ± 4.58 (cpm/ μg BM-like material, $\bar{x} \pm \text{SEM}$, $n = 6$).

There was no difference in reincorporation of amino acid into BM-like material obtained from cultures treated without and with 1 ng hGH/ml (1370 ± 50 vs 1400 ± 120) (cpm/mg BM protein, $\bar{x} \pm \text{SEM}$, $n = 9$ and 10).

We found a reduced accumulation of glycosaminoglycans in BM-like material isolated from cultures added with hGH when [$^{35}\text{S}\text{O}_4^-$] was used as a marker for glycosaminoglycans. The difference was small (8%) but significant (Fig. 8, $2p < 0.01$). When the effect of hGH on the degradation of glycosaminoglycans from BM-like material was investigated, no difference could be demonstrated between experimental and control groups (68320 ± 2030 vs 71760 ± 3050) (cpm/mg BM-like material, $\bar{x} \pm \text{SEM}$, $n = 20$).

Endocytosis

At present it is unclear if BM-like material is removed by intracellular degradation. In the present experiments HRP was used as a tracer of fluid phase endocytosis. However, when the rate was compared between cul-

Fig. 6a. Protein pattern of arterial basement membrane-like material isolated from the cell layer of cultures of aortic myomedial cells and separated by polyacrylamide gel electrophoresis in SDS. The tritiated labelled proteins were visualized by fluorography. A = control, B = human growth hormone (1 ng/ml). **b** Laser scanogram of the labelled protein pattern shown in Figure 6a. A = control, B = human growth hormone (1 ng/ml)

Table 1. Activity of lysosomal enzymes from arterial myomedial cell cultures exposed to human growth hormone for 24 h. hGH = human growth hormone. The results are expressed as mean $\pm$ SEM, n = number of cultures

	Control	hGH
Acid-phosphatase $\mu\text{mol}/\text{mg protein per h}$	7.61 ± 0.24 ($n=19$)	7.80 ± 0.86 ($n=22$)
β -N-acetyl glucosaminidase $\mu\text{mol}/\text{mg protein per h}$	4.96 ± 0.21 ($n=21$)	5.23 ± 0.16 ($n=22$)
Cathepsin D $\mu\text{mol}/\text{mg protein per h}$	54.14 ± 2.17 ($n=21$)	52.35 ± 1.78 ($n=21$)

tures added with and without hGH (1 ng/ml), no difference was revealed (151.77 ± 17.96 nl/mg protein/h vs 145.88 ± 6.07 nl/mg protein/h [$\bar{x} \pm \text{SEM}$, $n=10$]).

Lysosomal enzymes

Although hGH does not seem to influence endocytosis the diminished degradation of BM-like material might be controlled by the activity of lysosomal enzymes (acid phosphatase, cathepsin D and β -N-acetyl glucosaminidase). However, we were not able to demonstrate any effect of hGH on these enzymes (Table 1).

Fig. 7. Effect of hGH (1 ng/ml) on the degradation of tritiated leucine labelled arterial basement membrane-like material obtained from the cell layer of cultured aortic myomedial cells. C = controls, hGH = human growth hormone

Fig. 8. Effect of hGH (1 ng/ml) on the accumulation of $[^{35}\text{S}]\text{SO}_4^{2-}$ in arterial basement membrane-like material isolated from the cell layer of cultured aortic myomedial cells. C = controls, hGH = human growth hormone

Discussion

Arterial myomedial cells are surrounded by abundant amounts of BM-like material in vivo as well as in vitro. Earlier we have shown that this structure can be isolated from the *cell layer* of cultured arterial myomedial cells. Previous analysis of isolated BM-like material has disclosed that 5–10% was collagen-like peptides with a molecular weight in the range of 105K–350K and was neither type I nor type III collagen [10]. The main part of BM-like material appeared to be glycoproteins comprising, among other components, fibronectin. Moreover, it was found that approximately 1% represented glycosaminoglycans, of which heparan sulphate was the main component [18, 19].

Previous studies have shown that cultures of aortic myomedial cells are very sensitive for growth hormone. The production of collagen type I and fibronectin into the medium was increased by growth hormone [11]. Moreover, it was demonstrated that growth hormone enhanced cell proliferation [7] and finally that anti-growth hormone serum abolished the growth effect of diabetic serum with increased growth hormone level [8].

In the present study non-diabetic serum with and without hGH was used. In our previous studies using serum from normal and diabetic subjects we found that diabetic serum increased the growth of arterial myomedial cells [6]. In these mentioned experiments we used a conventional technique in which serum was diluted ten to twenty times, e.g. a hGH concentration of 10 ng/ml in serum from diabetic subjects would give a final concentration of 0.5–1.0 ng/ml in the culture flask. Thus the concentration we are using now is similar to that of our earlier growth experiments with the serum of diabetic patients. Moreover our data showed that the maximal effect on accumulation is reached between 0.5 and 1 ng hGH, and no further increase is observed at higher concentrations. The same results were obtained investigating the growth effect of hGH [8].

The present study demonstrates that small amounts of hGH inhibit the removal, supposedly the degradation, of arterial BM-like material. It also appears from our data that this effect on degradation cannot be due to enhanced reincorporation of radioactive amino acids released into the incubation medium.

Previous studies have shown that $[\text{Na}_2^{35}\text{SO}_4]$ is a suitable marker for glycosaminoglycans in myomedial BM-like material. 96% of labelled material has been identified as specific types of glycosaminoglycans using a combination of HNO_2 and chondroitinase ABC treatment [19]. Our present results suggest that hGH may either decrease sulphatation or reduce the amount of glycosaminoglycans in arterial BM-like material. Although the differences are small, it should be kept in mind that hGH augments the incorporation of amino acids by 26%. Therefore a larger reduction in the $\text{SO}_4/\text{protein}$ ratio than the observed may occur. The physiological implication of this observation is not clear, but it has been shown that glycosaminoglycans can bind large amounts of lipoprotein [20].

We were not able to show any effect of hGH on the activity of lysosomal enzymes. However, precaution should be exercised in the interpretation of lysosomal enzymic activity since locally increased activity may well occur without being detected when measuring activity of a whole cell-homogenate. Therefore it is an open question whether hGH induces BM-degradation via lysosomes.

It is generally acknowledged that some growth hormone effects are mediated through somatomedins [21]. Clemmou et al. [22] have shown on cultures of fibroblasts that growth hormone may induce production of

somatomedin C into the culture medium. Of course, the same possibility may be considered in the present investigation, although we have not been able to demonstrate any effect of somatomedin A (being identical to somatomedin C) and B on growth of arterial myomedial cells in culture [23]. The results obtained in the present study make it more likely that there is a direct effect of hGH on arterial myomedial cells, since reduced incorporation of sulphate does not fit with a somatomedin effect, as most of the somatomedins primarily stimulate the uptake of sulphate. A direct effect of hGH has also been suggested from studies of growth of arterial myomedial cells [7, 8], of longitudinal bone growth in rats [24] and of cultured pancreatic islets [25].

It has been hypothesized that elevated growth hormone in diabetic patients may be one causal factor in the development of diabetic angiopathy [1]. In the present study we have demonstrated that small amounts of hGH leads to accumulation of BM-like material from arterial myomedial cells. It is noteworthy that several histopathological studies have found accumulation of a PAS-positive material in larger and smaller arteries from diabetic patients, and that the PAS-positive material exhibits negative reaction for glycosaminoglycans [3, 4, 26]. Recent biochemical studies indicated a reduced synthesis of $[\text{}^{35}\text{S}\text{O}_4^-]$ glycosaminoglycans aorta obtained from rats with experimental diabetes [27]. The same kind of data has come from studies of glomerular basement membrane in diabetic patients and in animals with experimental diabetes [27–29]. All these observations are reconcilable with the results obtained in the present study which shows an accumulation of arterial BM-like material with a reduced content of glycosaminoglycans.

Concerning the biological relevance of some of the differences observed in the present experiments (26%, 16% and 8%) it has to be kept in mind that the results are obtained in a culture system covering an experimental period of 24 h only, whereas cells *in vivo* are exposed to abnormal growth hormone or other putative components for decades before alterations can be discovered. Therefore minor metabolic and structural changes may be of great pathophysiological importance.

However, it is important to remember that our analysis on BM-like material is performed on cultures of isolated arterial myomedial cells from rabbit aorta. It is not possible to draw conclusions from the results presented here to the situation prevailing in the large *ves-*
sels of diabetic patients. Nevertheless our data, together with other evidence including, i.e., the demonstration of elevated plasma growth hormone in diabetic patients [30]; the inhibition of the development of diabetic retinopathy after hypophysectomy followed by hormonal substitution (without growth hormone) [31]; the surprisingly low incidence of vascular abnormalities in growth hormone deficient dwarfs with diabetes mellitus [32]; suggest that growth hormone may be involved in

the pathological processes leading to small vessel disease and perhaps also to abnormalities in the large vessels.

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References

- Lundbæk K, Christensen NJ, Jensen VA, Johansen K, Olsen TS, Hansen AaP, Ørskov H, Østerby R (1970) Diabetes, diabetic angiopathy and growth hormone. *Lancet* 2: 131-133
- Gerich JE (1984) Role of growth hormone in diabetes mellitus. *N Engl J Med* 310: 848-849
- Ledet T (1968) Histological and histochemical changes in the coronary arteries of old diabetic patients. *Diabetologia* 4: 268-272
- Ledet T (1976) Diabetic cardiopathy. Quantitative histological studies of the heart from young juvenile diabetics. *Acta Pathol Microbiol Scand Sect A* 84: 421-428
- Fischer-Dzoga K, Wissler RW (1976) Stimulation of proliferation in stationary primary cultures of monkey aortic smooth muscle cells. Part 2. Effect of varying concentrations of hyperlipemic serum and low density lipoproteins of varying dietary fat origins. *Atherosclerosis* 24: 515-525
- Ledet T, Fischer-Dzoga K, Wissler RW (1976) Growth of rabbit aortic smooth-muscle cells cultured in media containing diabetic and hyperlipemic serum. *Diabetes* 25: 207-215
- Ledet T (1976) Growth hormone stimulating the growth of arterial medial cells in vitro. Absence of effect of insulin. *Diabetes* 25: 1011-1117
- Ledet T (1977) Growth hormone antiserum suppresses the growth effect of diabetic serum. Studies on rabbit aortic medial cell cultures. *Diabetes* 26: 798-803
- Haust DM, Geer JC (1972) Smooth muscle cells in atherosclerosis. In: Monographs on Atherosclerosis. Pollak OJ, Simms HS, Kirk JE (eds) Karger, Basel, Vol 2, pp 1-138
- Heickendorff L, Ledet T (1983) Arterial basement membrane-like material. Isolated and characterized from rabbit aortic myomedial cells in culture. *Biochem J* 211: 397-404
- Ledet T, Vuust J (1980) Arterial procollagen Type I, Type III, and fibronectin. Effects of diabetic serum, glucose, insulin, ketones and growth hormone studied on rabbit aortic myomedial cell cultures. *Diabetes* 19: 964-970
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 242: 1918-1922
- Steinman RM, Cohn ZA (1972) The interaction of soluble horse-radish peroxidase with mouse peritoneal macrophages in vitro. *J Cell Biol* 55: 186-204
- Barret AJ (1972) Lysosomal enzymes. In: Dingle JF (ed) *Lysosomes*. North-Holland Publishing Company, Amsterdam, London, pp 46-135
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685
- Bonner WM, Laskey RA (1974) A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. *Eur J Biochem* 46: 83-88
- Snedecor GA, Cochran WG (1972) *Statistical methods*. The Iowa University Press Ames, Iowa, USA
- Heickendorff L, Ledet T (1983) The carbohydrate components of arterial basement membrane-like material. Studies on rabbit aortic myomedial cells in culture. *Biochem J* 211: 735-741
- Heickendorff L, Ledet T (1984) Glycosaminoglycans of arterial basement membrane-like material. Studies on rabbit myomedial cells in culture. *Biochim Biophys Acta* 798: 276-282
- Iverius P-H (1973) In: Porter R, Knight J (eds) *Atherogenesis. Initiating Factors*. Elsevier Amsterdam London New York, pp 185-196
- Chockinov RH, Daughaday WH (1976) Current concepts of somatomedin and other biologically related growth factors. *Diabetes* 25: 994-1007
- Clemmons DR, Underwood LE, van Wyk JJ (1981) Hormonal control of immunoreactive somatomedin production by cultured human fibroblasts. *J Clin Invest* 67: 10-19
- Ledet T (1981) Diabetic macroangiopathy and growth hormone. *Diabetes* 30: (Suppl 2) 14-17
- Isakson OGP, Jansson J-O, Gause IAM (1982) Growth hormone stimulates longitudinal bone growth directly. *Science* 216: 1237-1239
- Nielsen JH (1982) Effects of growth hormone, prolactin, and placental lactogen on insulin content and release, and deoxyribonucleic acid synthesis in cultured pancreatic islets. *Endocrinology* 110: 600-606
- Blumenthal HT, Alex M, Goldenberg S (1960) A study of lesions of the intramural coronary artery branches in diabetes mellitus. *Arch Pathol* 70: 13-28
- Brown DM, Klein DJ, Michael AF, Oegema TR (1982) [³⁵S]glycosaminoglycan and [³⁵S]glycopeptide metabolism by diabetic glomeruli and aorta. *Diabetes* 31: 418-425
- Parthasarathy N, Spiro RG (1982) Effect of diabetes on the glycosaminoglycan component of the human glomerular basement membrane. *Diabetes* 31: 738-741
- Cohen MP, Surma ML (1981) [³⁵S]sulfate incorporation into glomerular basement membrane glycosaminoglycans is decreased in experimental diabetes. *J Lab Clin Med* 98: 715-722
- Hansen AP (1972) Serum growth hormone patterns in juvenile diabetes. *Dan Med Bull* 19 [Suppl 1] 1-32
- Lundbæk K, Malmros R, Andersen HC, Rasmussen JH, Bruntse E, Madsen PH, Jensen VA (1969) Hypophysectomy for diabetic angiopathy: A controlled clinical trial. In: Goldberg MH, Fine SL (eds) *Symposium on the treatment of diabetic retinopathy*. Publ Health Serv Publ 1890, Washington, pp 291-311
- Merimee TJ (1978) A follow-up study of vascular disease in growth-hormone-deficient dwarfs with diabetes. *N Engl J Med* 298: 217-222

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