DDT Vol. 6, No. 20 October 2001

Insulin therapies – past, present and future

Omathanu Pillai and Ramesh Panchagnula

The discovery of insulin is one of the greatest milestones in medical history. This discovery revolutionized the use of peptides and proteins as therapeutic agents. For more than six decades, insulin from different animal sources was used, until the breakthrough in biotechnology made it possible to produce human insulin in sufficient amounts. The evolution of the biotechnological era gave rise to modified insulins to solve some of the bottlenecks in insulin therapy. Efforts are currently focused towards developing non-invasive insulin delivery systems, and there are several competing technologies in different stages of development. The next few years will see several novel approaches to mimic the endogenous release and kinetics of insulin, and also many improved analogues designed to achieve better control and effective treatment of diabetes.

Omathanu Pillai and *Ramesh Panchagnula
Department of Pharmaceutics
National Institute of Pharmaceutical Education and Research (NIPER)
Sector-67, Ph X
SAS Nagar
160 062 Punjab
India
*tel: +91 172 214 682/7
fax: +91 172 214 692
e-mail: panchagnula@yahoo.com

▼ The discovery of insulin in 1922 by Banting and Best (http://www.diabetes.ca/about_diabetes/banting) is one of the greatest discoveries in the history of medicine, and ushered in a new discipline of 'protein therapeutics'. The 1923 Nobel Prize in Physiology and Medicine (http://www.nobel.se/medicine/laureates) was awarded for this landmark discovery, and this was the first time it had been awarded to a Canadian. Further, insulin has been the subject of two more Nobel prizes in Chemistry: Sanger was recognized for elucidating the structure of insulin, and the prize was also given to Hodgkin for determining the three-dimensional structure using X-ray diffractometry (http://www.nobel.se/chemistry/laureates). Perhaps insulin is the only molecule known to have been the subject of three Nobel prizes in two different scientific disciplines.

After its discovery, crude extracts of the pancreas of cow, pig or sheep[1] were used for more than a decade to treat diabetes until Scott purified insulin in 1936 by crystallizing it in the presence of zinc ions. This also formed the basis for the development of insulin formulations with prolonged-action (Lente insulins), using zinc ions in later years[2]. However, a breakthrough in the purification of insulins came in the 1960s, when chromatographic techniques were used to separate the high molecular weight impurities found in insulin, and which were later identified as the root cause of the immunogenicity of insulin preparations[3]. As a result, highly purified single component- or monocomponent-insulins were introduced into the market[4].

Standardization of insulin was traditionally done by rabbit glucose or the mouse convulsion method, and in 1956 Berson and Yalow developed a radioimmunoassay (RIA) for the first time using insulin[5]. RIA is now a routine immunochemical method for the assay of hormones and drugs. Therefore, insulin has been a 'fancied protein' for scientists all over the world, and is thus one of the most extensively studied proteins (Table 1). However, from a patient's point of view, non-invasive insulin delivery remains a 'mirage'. Nevertheless, based on the rich experience gained from the past, coupled to current technological advances[6], there is a great deal of optimism prevailing within the scientific community with regard to the improved management and treatment of diabetes.

Beginning of the 'biotech era'

Eli Lilly (Indianapolis, IN, USA) was the first company to demonstrate the commercial feasibility of recombinant DNA technology, with the introduction of human insulin in 1982. This marked a paradigm shift in drug discovery and development, leading to the start of the 'biotech era', and it would not be an exaggeration to state that the genesis and development of protein therapeutics is synonymous with

1359-6446/01/$ – see front matter PII: S1359-6446(01)01962-6

the development of insulin[7]. There are now 50 approved biotech drugs and more than >350 in various phases of development (http://www.phrma.org), and the biotech industry is expected to remain the fastest growing industry in the healthcare sector in the next 5–10 years[8].

Modern advances in biotechnology mean that it is possible to produce 'designer insulins' of changed amino-acid sequences, thereby imparting new structural, physicochemical and biological properties[9]. So far, >300 insulin analogues[10] have been produced, and some of these analogues have reached the market or are in various stages of development (Table 2). These modified insulins have been designed for altering the duration of action and/or improving the stability of native insulins[11]. The 'biotech era', which started with recombinant insulin, has now reached a stage where the human genome stands completely deciphered, thus offering numerous possibilities in more than one branch of science[12].

Table 1. Major milestones of insulin

Scientist	Achievements*	Year	Ref.
Banting and Best[a]	Isolation of insulin from pancreas	1922	52
Abel	Insulin – first protein to be crystallized	1926	53
Scott	Zinc – insulin crystallization	1936	54
Hagedorn *et al.*	Protamine insulin	1936	55
Hallas-Moller *et al.*	Lente insulins	1951–52	56
Sanger[b]	Insulin primary structure elucidation (first protein)	1955	57
Berson and Yalow	Radioimmunoassay for insulin (first compound)	1956	5
Hodgkins[c]	Three-dimensional structure of insulin	1969	58
Goeddel *et al.*	Recombinant human insulin (first healthcare product)	1979–1982	59

[a]Nobel Prize for Medicine in 1923.
[b]Nobel Prize for Chemistry in 1958.
[c]Nobel Prize for Chemistry in 1964.
*Landmarks given within parentheses.

Non-invasive delivery of insulin

Despite the significant developments made in insulin therapy over the past 60 years, non-invasive insulin delivery remains an elusive goal. Insulin is the only molecule to have attracted so much attention from drug delivery scientists, as witnessed by >2,000 published articles and >100 granted patents on several aspects of insulin delivery. Therefore, it is not surprising that every possible route has been explored for insulin delivery[4]. Subcutaneous injections continue to be the only viable option for diabetics, despite

Table 2. New analogues of insulin

Insulin analogue	Properties	Status	Company	Ref.
Short-acting				
Insulin Lyspro[a]	Short-acting and rapidly absorbed	Introduced in 1996	Eli Lilly, Indianapolis, IN, USA	60
Insulin Aspart[b]	Short-acting and rapidly absorbed	Introduced in 1999	Novo Nordisk, Bagsvaerd, Denmark	61
Long acting				
Insulin glargine[c]	Once-a-day long-acting insulin	Introduced in 2001	Aventis Pharmaceuticals, Parsippany, NJ, USA	62
N (epsilon) – palmitoyl Lys (B29)	Long-acting, less variable and highly reproducible pharmacokinetic profile	Preclinical and early clinical trials	Eli Lilly, Indianapolis, IN, USA	63
Lys B-29, tetradecanoyl. *des* (B30)[d]	Long-acting, peakless action and less pharmacokinetic variability	Preclinical and early clinical trials	Novo Nordisk, Bagsvaerd, Denmark	64

[a]Insulin Lyspro: transposition of proline and lysine in positions 28 and 29 of B-chain.
[b]Insulin Aspart: Substitution of proline with charged aspartic acid at position 28 of B-chain.
[c]Insulin glargine: Glycine at position 21 of A-chain and arginine at positions 31 and 32 of B-chain.
[d]Non-esterified fatty-acid-coupled insulins can bind to albumin in the body and prolong the absorption of insulin.

Table 3. New technologies for insulin delivery

Technology	Company	Status	Source
Peroral delivery of insulin using amphiphilic polymer conjugates	Nobex Corporation[a]	Phase II	http://www.nobexcorp.com
Peroral delivery of insulin using novel carriers	Emisphere[b]	Phase II	http://www.emisphere.com
Macrulin™ – peroral delivery of encapsulated insulin	Provalis[c]	Phase I	http://www.cortecs.com
Orasome™ – liposome-based formulation to protect insulin from harsh GI tract conditions	Endorex[d]	Preclinical	http://www.endorex.com
Insulin delivery through oral cavity (Oralgen™/Oralin™) using RapidMist™ device	Generex Biotech[e]	Phase II/III	http://www.generex.com
Pulmonary delivery of insulin using PulmoSol powder technology	Inhale Therapeutics[f]	Phase III	http://www.inhale.com
Pulmonary delivery of insulin using microprocessor based AERx iDMS[q]	Aradigm Corporation[g]	Phase II	http://www.aradigm.com
Pulmonary delivery of insulin using battery powered Spiros System™	Dura Pharmaceuticals[h]	Phase II	http://www.durapharm.com
Pulmonary delivery of insulin using low density porous particles (AIR™ technology[r])	Alkermes[i]	Phase I	http://www.alkermes.com
Powdered insulin through skin using helium gas	PowderJect[j]	Preclinical	http://www.powderject.com
Iontophoretic delivery of insulin (E-Trans™)	Alza[k]	Preclinical	http://www.alza.com
MicroPor™ technology – creates tiny pores for milliseconds using thermal process	Altea Development[l]	Preclinical	http://www.alteatech.com
Biphasix™ microencapsulated system for transdermal delivery of insulin	Helix Biopharma[m]	Preclinical	http://www.helixbio.com
Transferosomes™ – liposome-based formulation for insulin delivery	Idea[n]	Preclinical	http://www.idea-ag.de
Basulin™ – long-acting insulin using nanoparticles	Flamel Technologies[o]	Phase I	http://www.flamel.com
Depofoam™ – sustained release multivesicular liposomal formulations of insulin	DepoTech[p]	Preclinical	http://www.depotech.com

The above companies are specialized drug delivery companies with platform technologies for the delivery of insulin.
[a]Research Triangle Park, NC, USA; [b]Tarrytown, NY, USA; [c]Deeside, UK; [d]Lake Forest, IL, USA; [e]Ontario, Canada; [f]San Carlos, CA, USA; [g]Hayward, CA, USA; [h]San Diego, CA, USA; [i]Cambridge, MA, USA; [j]Oxford, UK; [k]Mountain View, CA, USA; [l]Atlanta, GA, USA; [m]Ontario, Canada; [n]Munich, Germany; [o]Cedex, France; [p]San Diego, CA, USA; [q]AERx iDMS, this insulin diabetes management system is a proprietary device and dosage form combination for pulmonary delivery of human insulin solutions; [r]AIR, unique dry, porous-particle aerosol technology developed for both fast and slow-acting pulmonary insulin formulations.
Abbreviations: GI, gastrointestinal.

improvements in treatment regimens and hormone preparations. Surveys have repeatedly shown that the complexity of the syringe and vial system is a huge hindrance to compliance for diabetics, and the factors mainly influencing patient compliance (in decreasing order of importance) include perception of a regimen's complexity, difficulty, duration, safety and cost[13].

Several advances, in the form of newer, long-acting insulins, short-acting insulins, and innovations such as pen injectors and insulin pumps have, to some extent, alleviated the discomfort associated with insulin injections[14,15]. Meanwhile, there have been numerous attempts to deliver insulin non-invasively through other routes of administration, although these have met with limited success[16]. In this regard, oral delivery and use of the respiratory tract have been a major attraction because of the convenience and perceived advantages. Some of the potential insulin delivery technologies under current investigation are listed

in Table 3. Of these, the pulmonary route offers promise because of the large surface area (comparable to a tennis court!), rich blood-supply low proteolytic activity[17]. Several companies have products in the late-stages of clinical trials, and insulin aerosols can be expected to be in the market within the next few years[18].

An interesting approach is the delivery of insulin through the buccal mucosa, where the wastage of aerosol particles in the oral cavity has been exploited for insulin delivery. This was achieved by the careful control of particle size so as to retain the particles in the oral cavity, and the rich blood-supply of the buccal mucosa can result in rapid absorption and onset (http://www.generex.com). The quest for oral delivery of insulin has been underway since its discovery, and most of the strategies have been focused on surpassing one of the three barriers to intestinal absorption; namely the epithelial cell layer, proteolytic enzymes and chemical environment[19]. Delivery approaches that have been investigated for insulin include liposomes, nanocapsules, liquid emulsions, prodrugs, polymer–inhibitor conjugates and colon targeting, and these have met with little to moderate success[19–24]. Microspheres are potential carrier systems, and these can be custom-designed to provide both protease inhibitory and permeation enhancement characteristics[20]. A recent novel approach developed by Emisphere Technologies (Tarrytown, NY, USA) includes 'protenoid microspheres', which use modified amino acids or peptides as carriers for biologically active molecules, including insulin, for delivery through the intestine[25]. Although there are significant challenges, the potential therapeutic and commercial benefits in terms of patient convenience and the market potential for non-invasive insulin delivery remains high.

Physiological replacement of insulin

Simulating the physiological release and kinetics of insulin has been the Holy Grail of diabetes therapy[26]. Insulin in the body is secreted according to a circadian rhythm, with higher levels in the hepatic portal than in the peripheral circulation. By contrast, insulin injected into a subcutaneous depot seeps into the general circulation, exposing all tissues to an equal concentration of insulin for a prolonged time period, thus leading to the development of a nexus of metabolic and cardiovascular complications that might manifest as a part of the diabetic complications[27]. Although the introduction of large doses of insulin into the gastrointestinal (GI) tract is shown to result in the absorption of some intact insulin (from the upper intestinal tract) into the blood with a consequent reduction in blood-glucose levels, it could upset a physiological control system, in which locally produced insulin in the GI tract and insulin receptors plays an important role[28]. The deep rectal (colon) and intraperitoneal routes are the only routes that can empty insulin directly into the portal circulation, but issues of patient convenience and compliance prevent the routine delivery of insulin via these routes[16]. It is pertinent to note here that insulin is the first and only molecule for which the presence of first-pass effect, inherent to the rectal anatomy, has been exploited beneficially to elicit a pharmacological response[29].

One of the major difficulties with insulin delivery is the associated risk of severe hypoglycemia, especially in cases where stable and tighter glycemic control is the target. In 1970, the design of the first implantable pump for intravenous infusion[30] led to the concept of glycemia-controlled insulin infusion[31] (artificial pancreas). As a sequel to this, the first implantation of a pump in diabetic patients in 1979 led to expectations that a fully implantable closed-loop system would be available soon[32]. Unfortunately, glucose sensors are still under development and implantable insulin pumps are not widely used, although they have been tested in randomized trials for type 1 and type 2 diabetes[33].

Despite some technical problems, such as insulin precipitation in catheters, pumps and tubings, the long-term safety and efficacy of insulin pumps has been demonstrated in a large cohort of patients[34]. Closed-loop delivery systems contain glucose biosensors capable of measuring the blood-glucose level, and adjust the insulin delivery rate based on a computer algorithm. Insulin is released from the pump and is rapidly absorbed through the peritoneum, mimicking normal insulin secretion in the body, and this comes with a decreased risk of the side effects associated with intensive insulin therapy[35]. In contrast, in open-loop delivery systems the patient has to adjust the insulin delivery rate in the pump, according to the blood-glucose levels[36].

'Intelligent stimuli-responsive' delivery systems using hydrogels have been investigated that can release insulin in a pulsatile fashion in response to blood glucose levels[37]. One such approach is based on immobilized glucose oxidase in pH-sensitive polymers that can swell in response to glucose and release the entrapped insulin in a pulsatile fashion. Another approach is based on competitive binding of glycosylated insulin and glucose to a fixed number of binding sites in concanavalin, where insulin is displaced in response to glucose stimuli, thus functioning as a self-regulating insulin delivery system.

Iontophoresis coupled to reverse iontophoresis through the skin appears to be one of the potential areas for developing a truly closed-loop insulin delivery system[38]. A glucose-monitoring system using reverse iontophoresis,

which can extract glucose through the skin under the influence of electric current, has been approved by the US FDA and will soon be available for the continuous monitoring of blood glucose (http://www.glucowatch.com). However, several bottlenecks must be overcome if coupling reverse iontophoresis (for glucose monitoring) to iontophoretic insulin delivery is to become a reality, thereby setting a new standard for diabetes management[39].

Rapid advances in molecular biology hold some promise for realizing the long-term goal of physiological replacement of insulin[40]. Bioengineered islet cells encapsulated in various polymer-matrix systems have been successful in the laboratory[41]. A recently reported novel approach is the use of fusion proteins to cluster insulin within the endoplasmic reticulum, which can be cleaved to release insulin according to physiological need by oral administration of a small molecule[42]. The implications of these findings suggest that insulin can be made to secrete from any non-pancreatic cell, thereby avoiding the issues of tissue rejection and the short supply of pancreas for transplantation. Further, the possibility of producing expanded human pancreatic islet cells has led to a conceptually appealing approach of implanting islet cells with a regulatable insulin gene that can respond to a range of glucose concentrations similar to stimulation of normal beta-islet cells[43,44].

Closing thoughts

Based on the findings of Diabetes Control and Complications Trial (DCCT) research group*, it is now recognized that there is a need for tighter control of blood glucose to avoid the complications associated with diabetes, a major contributing factor to the mortality caused by the disease[45]. In this regard, it would be more realistic to combine two different routes of administration, such as rapid post-prandial insulin levels with pulmonary delivery and sustained basal levels with new long-acting insulins. Bearing in mind the current pace of developments in biotechnology, in future we should see many 'tailor-made insulins' for improving stability and reducing pharmacokinetic and pharmacodynamic variability, leading to selective action on the liver and thereby reducing the metabolic effects on peripheral tissues[46]. Further efforts are also being directed to produce insulins suited for different delivery approaches.

Newer strategies based on computer-aided drug design should lead to insulin 'mimetic' small molecules from both natural sources[47] and synthetic routes that can be administered in the form of an oral pill. When the tangible benefits of the Human Genome Project are realized, it will not be surprising to find 'personalized insulin genes' on the market[48]. Alternatively, there will be novel ways of administering insulin through the skin using iontophoresis, ultrasound waves and electroporation, with more emphasis on chronopharmacokinetics-directed insulin delivery, mimicking the pulsatile release from the pancreas[49]. Advances in microelectronics and material sciences will also give rise to more sophisticated next-generation insulin delivery systems, including 'insulin-on-a-chip' and 'microfabricated needles' for controlled or pulsatile insulin delivery in response to external- or feedback-stimuli[50,51]. Therefore, it is the belief of the authors that by 2020, marking 100 years of insulin discovery, a diabetic will have several options to deliver insulin according to his or her choice. Until that time, the 'Flame of Hope' at Banting Museum, Ontario, Canada continues to be a hope not only for the millions of diabetics, but also for scientists around the world.

Acknowledgements

The authors acknowledge the suggestions and comments of Anurag Sood and Vinod Nair (NIPER, India) in preparation of this manuscript. One of the authors, Omathanu Pillai (NIPER, India), is supported by a research fellowship from the Department of Science and Technology (DST), New Delhi, India.

References

1 Sumra, M. and Gupta, V.B. (1995) Insulin to human insulin. *Indian Drugs* 32, 356–361
2 Brange, J., ed. (1987) Insulin preparations. In *Galenics of Insulin. The Physicochemical and Pharmaceutical Aspects of Insulin and Insulin Preparations* (Brange, J. *et al*., eds), pp. 17–70, Springer Verlag
3 Home, P.D. (1982) The new insulins. Their characteristics and clinical indications. *Drugs* 24, 401–413
4 Trehan, A. and Agsar, A. (1998) Recent approaches in insulin delivery. *Drug Dev. Ind. Pharm*. 24, 589–597
5 Yalow, R.S. and Berson, S.A. (1960) Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* 37, 1157–1175
6 Pillai, O. *et al*. (2001) Drug delivery – an odyssey of 100 years. *Curr. Opin. Chem. Biol.* 5, 439–446
7 Drews, J. (2000) Drug discovery: a historical perspective. *Science* 287, 1961–1964
8 Drews, J. (2000) *Quo vadis*, biotech? – Part 1. *Drug Discov. Today* 5, 547–553
9 Brange, J. (1990) Monomeric insulins and their experimental and clinical implications. *Diabetes Care* 13, 923–954
10 White, J.R. *et al*. (1997) Insulin analogues: new agents for improving glycemic control. *Postgrad. Med.* 101, 58–70
11 Vajo, Z. and Duckworth, W.C. (2000) Genetically engineered insulin analogs. Diabetes in the new millennium. *J. Pharmacol. Exp. Ther.* 52, 1–9
12 Peltonene, L. and McKusick, V.A. (2001) Dissecting human disease in the postgenomic era. *Science* 291, 1224–1229

*The Diabetes Control and Complications Trial research group is supported under cooperative agreement and a research contract by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Heart, Lung and Blood Institute (NHLBI), the National Eye Institute (NEI), the National Center for Research Resources (NCRR) [all of which form part of the National Institutes of Health (Bethesda, MD, USA)], and various corporate sponsors[65].

13 Roller, K. (1999) New insulin delivery systems key to diabetes management, compliance. *Drug Store News* 3, CP8

14 Jeandidier, N. and Boivin, S. (1999) Current status and future prospects of parenteral insulin regimens, strategies and delivery systems for diabetes treatment. *Adv. Drug. Deliv. Rev.* 35, 179–198

15 Bohannon, N.J.V. (1999) Insulin delivery using pen devices. *Postgrad. Med.* 106, 57–68

16 Hoffman, A. and Ziv, E. (1997) Pharmacokinetic considerations of new insulin formulations and routes of administration. *Clin. Pharmacokinet.* 33, 285–301

17 Newhouse, M.T. (1999) Tennis anyone? The lungs as a new court for systemic therapy. *Can. Med. Assoc. J.* 161, 1287–1288

18 Patton, J.S. *et al.* (1999) Inhaled insulin. *Adv. Drug Deliv. Rev.* 35, 235–247

19 Sood, A. and Panchagnula, R. *Peroral* route: An opportunity for protein and peptide drug delivery. *Chem. Rev.* (in press)

20 Carino, G.P. and Mathiowitz, E. (1999) Oral insulin delivery. *Adv. Drug Deliv. Rev.* 35, 249–257

21 Iwanaga, K. *et al.* (1997) Oral delivery of insulin by using surface coating liposomes. Improvement of stability of insulin in GI tract. *Int. J. Pharm.* 157, 73–80

22 Dage, C. *et al.* (1988) New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carriers. *Diabetes* 37, 246–251

23 Marschutz, M.K. *et al.* (2000) Design and *in vivo* evaluation of an oral delivery system for insulin. *Pharm. Res.* 17, 1468–1474

24 Swaffran, M. *et al.* (1986) A new approach for the oral administration of insulin and other peptide drugs. *Science* 233, 1081–1083

25 Bay, L. *et al.* (2001) Modified amino acids for drug delivery. *US Patent* 6180140

26 Zinman, B. (1989) The physiological replacement of insulin – an elusive goal. *New Engl. J. Med.* 321, 363–370

27 Stout, R.W. (1992) Insulin and atherosclerosis. In *Diabetes and Atherosclerosis* (Stout, R.W., ed.), pp. 165–201, Kluwer Academic Publishers

28 Saffran, M. *et al.* (1997) Insulin and the gastrointestinal tract. *J. Control. Release* 46, 89–98

29 Ritschel, W.A. and Ritschel, G.B. (1984) Rectal administration of insulin. *Methods Find. Exp. Clin. Pharmacol.* 6, 513–529

30 Blackshear, P.J. *et al.* (1970) A permanently implantable self-recycling low flow constant rate multipurpose infusion pump of simple design. *Surg. Forum* 21, 136

31 Albisser, A.M. *et al.* (1974) Clinical control of diabetes by the artificial pancreas. *Diabetes* 23, 397–404

32 Blackshear, P.J. *et al.* (1979) Control of blood glucose in experimental diabetes by means of a totally implantable insulin infusion device. *Diabetes* 28, 634–639

33 Selam, J.L. (1999) Implantable insulin pumps. *Lancet* 354, 178–179

34 Broutin, H. *et al.* (1995) On behalf of the EVIDAC group. Feasibility of intraperitoneal insulin therapy with programmable implantable pumps in IDDM. *Diabetes Care* 18, 388–392

35 Duckworth, W.C. (1992) Why intraperitoneal delivery of insulin with implantable pumps in NIDDM? *Diabetes* 41, 657–661

36 Irsigler, K. *et al.* (1985) Controlled drug delivery in the treatment of diabetes mellitus. *Crit. Rev. Ther. Drug Carrier Syst.* 1, 189–271

37 Kost, J. and Langer, R. (1991) Responsive polymeric delivery systems. *Adv. Drug. Deliv. Rev.* 6, 19–50

38 Guy, R.H. (1995) A sweeter life for diabetics? *Nat. Med.* 1, 1132–1133

39 Panchagnula, R. *et al.* (2000) Iontophoresis revisited. *Curr. Opin. Chem. Biol.* 4, 468–473

40 Levine, F. and Leibowitz, G. (1999) Towards gene therapy of diabetes mellitus. *Mol. Med. Today* 5, 165–171

41 Bae, Y.H. *et al.* (1998) Extracellular matrix for a rechargeable cell delivery system. *J. Control. Release* 53, 249–258

42 Rivera, V.M. *et al.* (2000) Regulation of protein secretion through controlled aggregation in the endoplasmic reticulum. *Science* 287, 826–830

43 Efrat, S. (1998) Development of engineered pancreatic β-cell lines for cell therapy of diabetes. *Adv. Drug Deliv. Rev.* 33, 45–52

44 Yang, Y.W. and Kotin, R.M. (2000) Glucose responsive gene delivery in pancreatic islet cells via recombinant adeno-associated viral vectors. *Pharm. Res.* 17, 1056–1061

45 The Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New Engl. J. Med.* 329, 977–986

46 Brange, J. and Volund, A. (1999) Insulin analogs with improved pharmacokinetic profile. *Adv. Drug Deliv. Rev.* 35, 307–335

47 Zhang, B. *et al.* (2000) Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. *Science* 284, 974–977

48 Lee, H.C. *et al.* (2000) Remission in models of type 1 diabetes by gene therapy using a single-chain analogue. *Nature* 408, 483–488

49 Pillai, O. *et al.* Non-invasive delivery of peptides and proteins through skin. *Drugs Future* (in press)

50 Santini, J.T., Jr, *et al.* (2000) Microchips as controlled drug delivery devices. *Angew. Chem., Int. Ed. Engl.* 39, 2396–2407

51 Henry, S. *et al.* (1998) Microfabricated microneedles. A novel approach to transdermal drug delivery. *J. Pharm. Sci.* 87, 922–925

52 Banting, F.G. and Best, C.H. (1922) Pancreatic extracts. *J. Lab. Clin. Med.* 7, 464–472

53 Abel, J.J. (1926) Crystalline insulin. *Proc. Natl. Acad. Sci. U. S. A.* 12, 132–136

54 Scott, D.A. (1934) Crystalline insulin. *Biochem. J.* 28, 1592–1602

55 Hagedorn, H.C. *et al.* (1936) Protamine insulinate. *J. Am. Med. Assoc.* 106, 177–180

56 Hallas-Moller, K. *et al.* (1952) Crystalline and amorphous insulin-zinc compounds with prolonged action. *Science* 116, 394–399

57 Sanger, F. (1959) Chemistry of insulin. *Science* 129, 1340–1344

58 Adams, M.J. *et al.* (1969) Structure of rhombohedral 2 zinc insulin crystals. *Nature* 224, 491–495

59 Goddel, D.V. *et al.* (1979) Expression in *Escherichia coli* of chemically synthesized genes for human insulin. *Proc. Natl. Acad. Sci. U. S. A.* 76, 106–110

60 Burge, M.R. *et al.* (1998) Lispro insulins. Benefits and limitations. *Trends Endocrinol. Metab.* 9, 337–341

61 Lindholm, A. *et al.* (1999) Improved postprandial glycemic control with insulin aspart. type 1 diabetes. *Diabetes Care* 22, 801–805

62 Gillies, P.S. *et al.* (2000) Insulin glargine. *Drugs* 59, 253–260

63 Baker, J.E. *et al.* (1997) Acylation of human insulin with palmitic acid extends the time of action of human insulin in diabetic dogs. *Diabetes* 46, 637–642

64 Markussen, J. *et al.* (1996) Soluble fatty acid acylated insulins bind to albumin and show protracted action in pigs. *Diabetologia* 39, 281–288

65 The DCTT Research Group (1986) The Diabetes Control and Complications Trial (DCCT). Design and methodologic considerations for the feasibility phase. *Diabetes* 35, 530–545