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Human insulin

Until recently diabetic patients dependent on or requiring insulin have been treated by injections of insulin derived from beef or pork pancreases. The amino acid sequence of human insulin[1] differs from that of porcine insulin by just one residue[2] at a site which is not thought to be critically concerned in binding to the insulin receptor[3]: the C terminal amino acid of the B chain in the human insulin molecule is threonine, while it is alanine in the pig. The primary structure of beef insulin is slightly more discrepant, with three substitutions: alanine again at B30 but valine at position 10 on the A chain and alanine at A8 instead of isoleucine and threonine.[4] The chief arguments for introducing human insulin for routine treatment of diabetics rest not, then, on any expected large difference in biological action but on the hope of reduced immunogenicity and—with newer production methods—reduced cost and greater worldwide availability.

Small quantities of human sequence insulin can be extracted from cadaver pancreases[5 6] or the fluid used to maintain islet cells in culture, or it may be chemically synthesised.[7 8] These methods are not suitable for largescale industrial production, however, and the present clinical availability of human insulin stems from two recent and substantial achievements in biotechnology. Firstly, techniques were developed in the late 1970s for producing insulin of recombinant DNA origin, and this insulin was first tested in man in 1980 (probably the first "genetically engineered" protein to be administered to humans).[9] Shortly afterwards "semisynthetic" human insulin became available, a product manufactured from porcine pancreatic insulin by the trypsin catalysed removal of the B30 alanine and its replacement by threonine.[10] At present most recombinant DNA insulin is made by inserting synthetic genes for the A and B chain into plasmids at a promoter gene site—for example, β galactosidase or tryptophan synthetase—and then into bacteria (*Escherichia coli* K12).[11 12] After fermentation the chimeric gene product is cleaved, purified, and the chains combined to make active insulin.[13] Manufacture of human insulin from recombinant DNA proinsulin has been accomplished and may eventually be a major commercial source.

Formulations of highly purified human insulin are now marketed in Britain as short acting (soluble), lente, ultralente, and isophane types, and as premixed 50-50% and 30-70% combinations of soluble and isophane insulin. Recombinant DNA insulin is also known as "biosynthetic" and identified by the letters crb (chain, recombinant DNA, bacteria) and semisynthetic insulin as emp (enzyme modified porcine). In Britain about 6% of the amount of insulin currently sold is of human sequence; the proportion is considerably higher in some other countries (about 20% in West Germany).

Both the in vitro properties—for example, receptor binding and biological responses in isolated cells and tissues such as adipocytes, lymphocytes, fibroblasts—and the in vivo properties—for example, blood glucose lowering and plasma insulin pharmacokinetics—of intravenously administered biosynthetic and semisynthetic human insulin are virtually identical with the properties of purified pork insulin.[14-16] Differences in the counterregulatory hormone responses to induced hypoglycaemia have been reported in a few studies with small numbers of patients and sometimes with conflicting results,[17 18] but these need confirmation before they can be properly interpreted.

Subcutaneously injected human insulin is, however, absorbed slightly more quickly than porcine insulin; this has been shown for both short acting insulin[19 20] and for intermediate acting (particularly isophane) depot preparations.[21 22] In some studies human ultralente insulin had a similar duration of action to beef ultralente.[23] Possibly the reason for the accelerated absorption of human insulin is that it is more soluble; the B30 threonine has more hydrogen bonding capability than alanine, and comparative *x* ray diffraction studies of the tertiary structures of human and porcine insulin show differences only at the B30 region, where changes in the water structure are apparent.[24]

In several clinical trials comparing the control of blood glucose in diabetic patients treated by twice daily subcutaneous injections of animal or human short and intermediate acting insulin differences have been shown, with higher fasting blood glucose concentrations during human insulin treatment.[25-28] Presumably this is because of the shorter duration of action of the intermediate acting insulin injection given in the evening. Overall glycaemic control tends to be slightly worse, then, on transferring patients to human insulin from purified pork insulin, but adjustments in the ratio and amounts of insulins given throughout the day usually allow comparable control to be achieved after a short

while.[27] In the few patients who retain a raised blood glucose value before breakfast alternative strategies may be needed (whatever the species of insulin used) such as delaying the evening injection of intermediate acting insulin until bedtime,[29] using one[23] or two[30] daily injections of ultralente insulin as the basal insulin supply, or employing continuous subcutaneous insulin infusion.[31] These regimens are equally suitable approaches to obtaining near normoglycaemia in most diabetic patients, the choice depending, as always, on local skills and resources and on patient preferences and responses.

Clinical trials of human insulin have confirmed its safety and have also provided evidence that human insulin treatment can be less immunogenetic—that is, produces less circulating anti-insulin antibodies—than treatment with either beef or pork insulin. In predominantly adult patients not previously treated with insulin, after 12 months the proportion with detectable insulin antibodies was less after treatment with biosynthetic human insulin (44%) than with purified porcine insulin (60%).[32] In children with newly diagnosed type I diabetes insulin antibodies were lower in those treated for one year by semisynthetic human insulin than in those on porcine insulin.[33] The picture is less clear for established diabetics transferred from animal insulin to human insulin. Human insulin is definitely less immunogenic than beef insulin—as is pork insulin[34]—but several studies have indicated no detectable change in antibody concentrations on switching from pork to human insulin or vice versa.[26 27 34]

But what is the clinical importance of anti-insulin antibodies? They cause the lipoatrophy at the site of insulin injection[35] and the substantial insulin resistance formerly seen in some patients, but both events are rare now that purified pork insulin is in common use.[36] At least two patients have been reported with antibody mediated resistance to even purified pork insulin who were later managed successfully with human insulin (decrease in insulin requirement and antibody concentrations).[37 38] Local and systemic allergies to insulin are very rare—and they also occur with human insulin treatment.[39] Sometimes a patient cannot be successfully desensitised with porcine insulin whereas human insulin achieves a rapid effect.[40] One report of diabetics newly treated with insulin found that 19% developed insulin specific IgE antibodies on beef insulin, 17% on pork, but only 6% on human insulin.[40] Human preparations seem the sensible choices then, for patients who have developed allergies to insulin or those at risk of allergic reactions—patients intermittently treated with insulin, for example.

Interest has recently been revived in the possible contribution of insulin antibodies in modifying metabolic control in diabetics. Short term, in hospital conditions, insulin antibodies are known to prolong the intravenous half life of injected insulin[41] and to delay the appearance in the circulations of a subcutaneously administered insulin bolus.[42] Patients with moderate concentrations of insulin antibodies also show delay in recovery from induced hypoglycaemia[43 44]—but on the other hand they lose control less quickly after insulin withdrawal and may be relatively protected from ketoacidosis.[45 46] Yet, despite these hints, usually neither the amounts nor the binding characteristics of anti-insulin antibodies can be linked with the degree of diabetic control in individual patients in ordinary conditions of life.[47] The several other postulated adverse effects of insulin antibodies (crossing the placenta in pregnant diabetics and causing or contributing to neonatal hypoglycaemia and macrosomia; association with microangiopathy) have not been conclusively proved.[39 48] Nevertheless recent studies do provide further evidence that the development of insulin antibodies in diabetic children is associated with a shortened "honeymoon" remission period, higher insulin dosage, and impaired endogenous insulin secretion (as measured by C peptide responses). Indeed insulin antibodies proved to have the strongest relationship among various factors thought to influence residual β cell function (HLA status, sex, age at onset).[49] And in the context of insulin immunogenicity an additional interesting suggestion is that anti-insulin antibodies may cross react with nerve growth factor and perhaps contribute to the development of diabetic autonomic neuropathy.[50]

In Britain biosynthetic human insulin is of comparable price to purified pork insulin. On both medical and economic grounds, therefore, human insulin seems a justifiable first choice in newly diagnosed diabetics and those needing short term or intermittent treatment, such as gestational diabetics and type II diabetic patients during surgery. There is currently no good reason for transferring established diabetics from pork to human insulin—except perhaps those who have developed insulin allergy or those at risk or with a history of allergy.

Without doubt the advent of new, biotechnological processes for insulin production should be welcomed for their potential value. Not only may novel analogues of insulin be synthesised, with perhaps usefully altered biological activity (including proinsulin); but—theoretically at least—biosynthetic insulin may lead to much lower costs and increased supplies unlimited by the availability of animal pancreases. Whether the latter consideration will ever limit the provision of insulin remains arguable—and many other factors cause hindrances to insulin treatment in developing countries, including difficulties in distribution, storage, and syringe and needle supplies. Nevertheless, the hope that one day large scale production will be possible of cheap and pure human insulin must be relevant to diabetic patients everywhere.

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Endotracheal intubation: friend or foe

Endotracheal intubation is an essential part of modern anaesthetic practice, providing, as it does, both safety and convenience. Yet the doctor who attempts intubation and passes the tube into the oesophagus will—if he does not recognise the mistake—most likely kill the patient.

Obstetric anaesthesia is the only branch of the specialty in which we have accurate mortality data, and the most common cause of such deaths is "failed intubation." Possibly obstetric patients present greater problems for intubation than non-pregnant patients, but nevertheless some such preventable deaths must be occurring in non-obstetric practice.

Another worrying category of deaths is those associated with the resuscitation of unanaesthetised patients with cardiovascular collapse. When an anaesthetist attends a cardiac arrest he usually performs endotracheal intubation (though not as the initial attempt to fill the lungs with air or oxygen); properly placed, the tube is of the greatest value in maintaining artificial respiration and safeguarding the airway. The result has been that intubation has come to be seen as an essential part of cardiopulmonary resuscitation even when no anaesthetist is present. While some doctors who have not received anaesthetic training can pass tubes correctly, nevertheless the skill is one that requires fairly continuous practice. Even more important is the ability to diagnose incorrect placement quickly. There are very few absolute signs that the tube is in the trachea; in most cases reliance is placed on good visualisation of the larynx during intubation, the "feel" of the lungs during bag compression, the presence of reasonable breath sounds, and appropriate thoracic movement. The restarting of spontaneous respiration with the appearance of bag movement is reassuring. If the apparatus is available the detection of carbon dioxide in the expired air is diagnostic. The presence of bronchospasm or pneumothorax can confuse the picture—but while these possibilities should be borne in mind their presence should be accepted only with good evidence. Difficulty in ventilating is more likely to be because the tube has been placed in the oesophagus than because of bronchospasm. The detection of breath sounds is notoriously misleading, as these may be mistaken for the sound of the air passing along the oesophagus.

The most convincing evidence of the position of the tube is the patient's response to attempted ventilation. If a blue patient goes pink the tube is unlikely to be anywhere but in the trachea. If the pink patient goes blue it probably is not. Both these possibilities require some cardiovascular adequacy. The patient with a palpable pulse whose alveoli contain a reasonable amount of oxygen will not remain blue in normal circumstances. The paradoxical danger of preoxygenation in this context is important, as it may be several minutes before cyanosis appears, by which time the anaesthetist may not suspect a misplaced tube. This is not a reason to avoid preoxygenation, but the fact that the patient has remained pink for some minutes does not exclude oesophageal intubation.

Patients do not die from a "failure to intubate." They die either from failure to stop trying to intubate or from undiagnosed oesophageal intubation. Ventilating with a mask and oral airway appears to be a dying art—even among anaesthetists—but it is easier to teach than endotracheal intubation. Those not trained in anaesthesia are best advised to use mouth to mouth breathing only.

One major advantage of an endotracheal tube is the protection it affords against aspiration of gastric contents into the lungs. If aspiration seems a possibility and endotracheal intubation has failed, or has not been attempted, an oeso-