RESEARCH AND DEVELOPMENT CHALLENGES IN EMERGING MEAT PROCESSING TECHNOLOGIES

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Abstract

Enzyme processing will continue to emerge as an important meat processing technology. The use of specific, tailored proteases, coupled with other hydrolases (lipases, carbohydrases) will be used to alter the structure and flavour precursors of meat to promote new kinesthetic and organoleptic changes in processed meat products. Enzyme based biosensors could also play a role in meat processing via on-line quality determinations.

Transgenic technology will have an impact on the meat quality of animals, meat yields and disease resistance. Both transgenic and enzyme technologies are set to play important roles in the future of meat processing.

1. Introduction

There are many ways of processing meat, ranging from the simple slicing in basic butchery, be it manual or automated, through to meat reforming technology based on the use of functional proteins or polysaccharides.

Indeed, as more knowledge is gained about specific native protein structure and its relationship to function, using genetic and protein engineering technologies, the potential for inducing specific functionality by the actual design of proteins cannot be too distant.

The impact of these technologies on meat and meat production in the 21st century could be profound. We already have transgenic animals. These animals carry artificially inserted genes within each nucleus of each cell. To be truly transgenic the inherited gene has to be a heritable entity. The potential of this technology cannot be ignored.

Traditional breeding techniques, augmen[ted] with artificial insemination techniques us[ing] elite germ plasm has had a significant eff[ect] on the commonly reared meat animals.

As new biotechnology comes of age its imp[act] on food animals will grow in many spheres.

Enzyme processing technology is anot[her] relatively new area which also has gr[eat] potential for meat processing. Indeed, p[ost] mortem autolytic changes in meat br[ing] about a conditioning process leading [to] textural changes and the generation [of] flavour precursor compounds which react [on] heating to generate the characteristic arom[as] and flavours of cooked meat.

Other techniques that are being investiga[ted] in meat processing are the use of high pre[s]sure and low frequency ultrasound. Some a[d]vances are being made with both processi[ng] and packaging, such as with a range [of] microwaveable (microwave cooking) produc[ts].

Current and emerging processing technolog[ies] do not exist in isolation. There are a num[ber] of factors which influence them. The cons[u]mer of the meat and meat containing produ[cts] has a pivotal role. There is currently [a] trend for the eating of leaner meats, part[ic]ularly beef and pork, and also an increas[ed] emphasis on the eating of poultry and [...]. The economic pressures on producers to r[ear] meat animals more cost effectively is a[lso] part of the equation, as is the ability [of] manufacturers to use more and more of t[he] carcase in expanding ranges of new m[eat] based products.

Legislation also plays a part in the over[all] picture and will have further impacts as n[ew] biotechnological processes are brought [to] bear, ranging from the genetic manipulati[on] of the animals themselves and their p[lant] based food, to modified microorganis[ms] implanted into rumens and gut, through [to] engineered enzymes used as processing aid[s].

2. Muscle Composition

Muscle, the basis of meat, contains a comp[lex] array of proteins, some fats, carbohydra[tes] and inorganic ions.

The proteins of muscle can be classified i[nto] discrete groups depending on their cell[ular] localisation. Those that constitute [...]

contractile apparatus are the myofibrillar proteins. The sarcoplasmic class of proteins include all the metabolic enzymes found in the muscle cell, the pigment myoglobin and the components of the muscle cell nucleus and the lysosomes. The connective tissue proteins constitute the third major class. These are focussed outside the muscle fibres and make up the extra cellular matrix. This gives support to the muscle in life.

The major myofibrillar protein is myosin, a large structural protein comprising of two large, heavy chain polypeptides and four smaller subunit light chains. The protein has a molecular weight of around 480,000 (Godfrey and Hassington, 1970). Actin is a globular protein associated with myosin, and has a molecular weight of 42,000 (Elzinga et al, 1973). A large amount of work has been performed on these proteins in connection with their contractile ability. However, we will be concerned with these proteins from the viewpoint of their functionality and their behaviour under various methods of meat processing.

Fats are a major chemical constituent in the carcase of a meat animal and can be around 18% - 30% of the carcase weight of cattle and from 12–20% of the live weight of the average pig. The term fat is usually taken to encompass all the lipid species, including triglycerides (the major species), phospholipids, sterols, sterol esters and other minor lipids such as certain essential fatty acids.

Lipids are found intermuscularly, intramuscularly, in adipose tissue, in the blood and in nervous tissue. The fatty acid composition of the fats varies depending on the animal species. There are also dietary effects on the triglyceride composition of meat animals.

A rigorous evaluation of fats is inappropriate in this paper, but the reader is directed to the review by Dugan, 1987.

There are a number of carbohydrate moeities associated with muscle. The major one is glycogen, essentially a polymer of glucose. It is found at levels of around 0.5% - 2% in mammalian skeletal muscle. Other carbohydrates are the glycosaminoglycans and proteoglycans associated with the collogenous extracellular matrix. Free glucose and the above carbohydrates will have a significant effect on the meat. The amount of glycogen present at slaughter, together with the rate and degree of postmortem hydrolysis affects the colour, texture, firmness, emulsifying capacity and water holding capacity of the meat.

The flavour of cooked meat is also influenced by Maillard reaction browning products produced by glucose–protein/amino acid interaction.

A knowledge of the status of muscle carbohydrates should be an indicator of the overall freshness of the meat and perhaps also may say something about the flavour that will be developed later.

3. Functionality and Processing

There are excellent reviews of the functionality of muscle proteins (Acton et al, 1983, Acton and Dick, 1984, Jones 1984, Regenstein 1984 and Zeigler and Acton, 1984) and this area will not be described here.

Processing of meats begins with choosing the basic lean meats, fatty tissues and other ingredients, grinding, cutting, salting, curing, acidifying, smoking, reforming, cooking, shaping and packaging. During these stages, biological, chemical and physical changes occur.

These basic processes are well known and are the basis of many established technologies (Whiting, 1988). However, there are certain novel areas of processing which should have an exciting future. Such processes are with enzymes, high pressures (Berry et al, 1986), and low frequency ultrasound (Vimini et al, 1983). Heating procedures such as Ohmic heating, microwaving and the various aseptic processing methods will not be considered.

Enzyme Processing

Enzyme processing of meats has a long history, although the directed use of specific enzymes is relatively modern. Originally, animals were hung in some cultures to improve the flavour and texture. This was due to tissue autolysis postmortem. Other cultures used plant enzymes, notably the bromelain in pineapples to tenderise meats, yet others used a partial hydrolysis and natural curing (Mongol horsemen placed meat under the saddles of their horses to be consumed days or weeks later).

Thus enzymes for meat process can come from two sources, the meat itself (endogenous hydrolytic enzymes) and external sources (exogenous hydrolytic enzymes).

Proteolytic Enzymes Within Meat

There are two broad classes of proteolytic enzymes found in skeletal muscle, exopeptidases and endopeptidases. Exopeptidases cleave proteins from the C or N termini and are further subdivided into amino peptidases, carboxypeptidases, and also dipeptidases and pipeptidylpeptidases depending on exactly where they cleave the peptide (Bird and Carter, l980; Barnett and McDonald, l980).

The endopeptidases cleave within the peptide itself, at some distance from the termini. Some may hydrolyse terminal peptide bonds too, but relatively slowly (Mihalyi, l972).
These enzymes are the serine proteases, thiol proteases, carboxy proteases and metalloproteases. Some of the characteristics of exo and endopeptidases are given in tables 1 and 2. These enzymes acting in concert within living muscle tissue, are responsible for the normal turnover of proteins, modification of proteins, remodelling of the muscle and 'housekeeping' duties. The normal functions of these proteases decline after slaughter.

The muscle is metabolically active after slaughter and anaerobic respiration generates lactic acid and a consequent decrease in muscle pH. This leads to microenvironment favourable to the activity of some proteases and certain of these release others.

It is these endogenous, now liberated proteases that are used to condition meat by means of a controlled autolysis. This is important, because at the cessation of anaerobic glycolysis, as the ATP level falls below a critical level, 10–20% of its initial value (Bendall, l979), the actin and myosin form a rigid complex via a bridging process. This is the processes called rigor mortis. If cooked in this condition, the meat is relatively tough and bland to the taste.

TABLE 1 - EXPOEPTIDASES OF SKELETAL MUSCLE

Enxopeptidase [1]	Mol wt $x10^{-3}$	pH Range	Cellular Distribution
Aminopeptidases			
Leucine amino peptidase	150	7.8–8.0	Cytosol
Neutral arylamidase	257	7.0	Cytosol
Acid arylamidase	105	6.0	Cytosol
Carboxypeptidases			
Cathepsin A	100	5.5	Lysosomal
Cathepsin B25.	52	5–6.0	Lysosomal
Dipeptidases			
Prolinase	300	8.0–8.8	Cytosol
Prolidase	108	7.5–8.2	Cytosol
Dipeptidytpeptidases			
Dipeptidylaminopeptidase I	200	5.0–6.0	Lysosomal
Dipeptidylaminopeptidase II	130	4.5–5.5	Lysosomal
Dipeptidylaminopeptidase III	80	7.0–8.5	Cytosol
Dipeptidylaminopeptidase IV	250	7.5	Microsomal

1 Adopted from Bird and Carter l980, Ashgar and Bhatti l987

TABLE 2 – ENDOPEPTIDASES OF SKELETAL MUSCLE

Endopeptidase [2]	Mol wt x15[3]	pH Range	Cellular Distribution
Serine proteases			
Myofibriller s. protease	25	8.3–9.0	Cytosol
Myosin cleaving protease	26	7.5–9.5	Cytosol
Group specific proteases	24	9–10.5	Cytosol
Cytosolic protease	25	9.5	Cytosol
Myofibrillar protease	31	9.5	Cytosol
Alkaline protease (ATP activated)	550	7.8	Cytosol
Thiol Proteases			
Cathepsin B1	25	4.0–6.5	Lysosomal
Cathepsin H	28	5.5–6.0	Lysosomal
Cathepsin L	24	3.0–6.5	Lysosomal
Aspartate proteases			
Cathepsin D	42	3.0–5.0	Lysosomal
Caltoepsin E	100	2.0–3.5	Lysosomal
Metalloproteases			
Collagenase	33	7.5	

2 Adapted from Barret and McDonald l980, Ashgar and Bhatti l987.

Enzyme Processing

In the industrial situation, it is not uncommon to find meats, meat mixes (comminuted meats, fat, offal), offals (lung, melts, spleen), runners (intestine), tripe (stomachs etc.), slurries etc. This is particularly true of the petfood industry which is dedicated to the upgrading of relatively low quality meats (in the general sense) and offals into a product which is both appealing to the consumer, who purchses and serves it, and to the consumer, a cat or dog most commonly.

The scope for enzyme processing in this industry is wide, and greater than for human foods. Indeed, for human meat based foods enzymes are used usually for tenderisation (either pre or post mortem) and thus really to induce a textural change. There must also be opportunties to use enzymes to induce positive organoleptic changes to meats which will result in new, tastier products or which will help to upgrade the final product in relation to the source materials.

Let us consider some applications of enzymes which could be beneficial in meat based or meat containing pet foods.

Enzymes and Palatability

Different meats have differing palatabilities. The way those meats are processed will also affect their palatability, as will other components of the product, such as acidity regulators, cations, other proteins, fats, carbohydrates and flavour systems.

Thus, for instance, dogs prefer pork to beef and beef to chicken when presented raw, and the same meats are preferred in the same order when boiled, but not so much when thermally processed in cans. The raw meats are preferred to the boiled meats, which suggests that flavours and taste promoting chemicals are changing and being volatilised during processing.

There is a similar palatability preference amongst offal meats, with liver being highly palatable, particularly to cats, tripe for dogs and chicken viscera to both.

Fresh chicken viscera is not as palatable as aged or autolysed chicken viscera. The viscera contains not only proteases found in muscle tissue (smooth muscle) but also digestive enzymes from the gut and associated organs (ie the pancreas). The enzymes present are not just proteases but lipases too.

These enzymes act on the viscera, and other added meats (if any) to alter the texture of hydrolysis. During this process peptides and some free amino acids are generated, together with free fatty acids from lipolysis of fats.

If these partially hydrolysed mixes are then heated with reducing sugars, Maillard reaction products are formed. The digests can be stabilised by post process acidification with phosphoric acid. If these digests are absorbed onto biscuits or cooker extruded kibbles, the palatability of the bland kibbles is dramatically enhanced. When added to canned products a similar situation would be expected.

The way forward with this basic technology is by the directed use of exogenous hydrolases. Papain has been used to treat meats and preslaughter animals. The enzyme is a cysteinyl protease and will degrade myofibrillar and connective tissue proteins. It has quite a high temperature optimum and considerable thermostability, with the greatest hydrolysis being obtained at around $50^{o}C$. Thus, although undenatured collagen is resistant to papain, at elevated temperatures certain domains of the collagen molecule denature and can be cleaved by papain. However, excessive use of papain causes the build up of bitter peptides which have a negative effect on palatability. The Maillard reaction can offset this for some pet products, but it does not abolish the effect entirely.

Bitterness effects and heterogenous hydrolysis are due to the nature of the proteases used together with the general poor control of the protolytic process. Bitterness can be offset by the use of quite newly available enzymes to the food industry, namely amino peptidases.

If the result of protein hydrolysis is th production of a high proportion of peptid with hydrophobic amino acid residues their N-termini, then bitterness will perceived. Small soluble peptides with high proportion of non-polar amino aci either at the centre of the peptide chain at the carboxy terminus can also contrib to the bitter flavour. Thus, if a prot component of meat or an added bulki protein contains a significant proportion these amino acids, proteolysis will gener give rise to a bitter tasting end produ (Adler-Nissen 1986).

Masking agents, process control and th careful exclusion of selected proteins ha been used in attempts to reduce bitterness.

The aminopeptidases for industrial use (alph aminoaclypeptide hydrolases) can be deriv form microbial sources (e.g. Streptococc lactis). They are active within the range $6^{o}C$–$40^{o}C$, although those from thermoph microorganisms have a considerably extend thermal range of activity.
The effect of aminopeptases is to remo single or dipeptides from the N-termin end of a protein or polypeptide. The cleavage of N-terminal hydrophobic amino aci from peptides generated by protein hydroly will lead to a debittering effect, without th production of an excess concentration free amino acids. Although the major us for such enzyme application is soy and da protein processing, these enzymes could used in digests produced for pet foods an human meat product applications, particula products that do not retain the macroscop texture of the meat from which they we made.

Sourcing of Processing Enzymes

Autolysis of meat products is the most co effective route of hydrolysis in terms enzymes, but can be a relatively sl process for some non-offal meats. Th reaction rate can be enhanced by addi enzymes, which are usually produced fermentation. With pet food enzyme processing, high levels of palatable pa hydrolysates have been produced usi mammalian enzymes such as pancreatic lipas which also has proteolytic effects.

Fungal microbial and plant proteases tended to give lower palatability products. It may be that the specificity of the enzyme with respect to its clearage pattern is important. The difference being the production of flavoursome (or even bland) peptides on one hand as opposed to bitter peptides on the other.

The use of recombinant DNA technology (rDNA) should enable the production of specific food processing enzymes in bulk, by cloning the relevent cDNA sequence into suitable producer organisms, such as certain filamentous fungi, Bacillus species or Actinomyces species. Indeed, optimum production may be via the 'natural' sequence or by the insertion of a synthetic gene with a codon usage more in keeping with that of the producer host. The advances being made in the protein engineering of enzymes with respect to structural and functional correlations, coupled with rDNA technology, should result in the biomolecular engineering of highly specific proteases which could be used as processing tools within the food industry.

Unfortunately these technical advances are not matched in pace and sometimes outlook, by the regulatory authorities. Non-one would want to use a process or substance that was not safe, particularly in the area of food and food processing technology. Obviously enzymes or other products produced by biotechnology have to be shown to be safe, non-toxic etc. The enzymes themselves are proteins and behave exactly as proteins on thermal processing. Certainly one way of ensuring safety is to limit the use to thermally processed foods - but there are foods which are 'live', where prolonged enzyme activity is essential, such as cheeses.

We should exploit all opportunities, assessed on their technological merits (activity, production process, purification etc.) and not allow novelty to become a vice.

Other Uses of Enzymes

Enzymes are used widely in an increasing range of Biosensors. A biosensor is a device comprising a biological sensing element (enzyme, DNA, antibody, whole cells, receptor organelles) a transduction system (electrochemical, optical, calorimetric, acoustical, mechanical) and a suitable readout mechanism which may be direct or indirect (Turner et al 1986). The biological specificity and selectivity offers the opportunity of the production of highly specific sensors. The advantages of such sensors are:

a) Specificity of analysis in complex mixtures, which eliminates extended sample preparation.

b) The potential for on-line analysis and consequent feed back control, achieved by interacting with computer technology.

c) Ease of use by untrained personnel.

d) Short analysis times.

e) Low cost potential.

An area where this approach could be used is meat freshness determination. In chilled fresh meat the surface microbial flora will consume free glucose from the tissue. This results in a glucose concentration gradient within the surface layers of the meat. In turn, this gradient reflects the level of contamination. A measurement of the glucose profile at different depths should enable a relative shelf life to be assigned to the meat.

The glucose biosensor contains the enzyme glucose oxidase. When catalysing the oxidation of glucose, hydrogen peroxide is generated. This can be split by catalase and the liberation of oxygen detected via an oxygen electrode. Alternatively a change in the election balance of the reaction can be detected using a mediator such as ferrocene and amperometric detection.

Other biosensors could detect aldehydes, for freshness determination of fats, histamines for fish, or hypoxanthine, a degradation product of ATP (again an indicator of freshness) or ATP levels themselves (using luciferase/luciferin and optical transduction).

These examples show that enzyme processing has many dimensions and has the potential to become an important process technology in its own right, applied directly to meat and meat products and also to meat wastes. In the UK there is an annual generation of over 90 tonnes of offal, rind fat, fat, fat and meat sludge, protein and fat and fish shells and bone waste. There is an opportunity for treating certain wastes and upgrading them. Enzymes can play a part in this too

(Fulbrook l983, Ashley, l983).

4. Transgenic Animal Technology – An Opportunity or a Problem for the Food Industry?

There are strong, emotive issues concerning genetic engineering which usually stem from ignorance on the part of the public, but which makes them easy prey to scaremongers and "anti technologists". There are also political issues concerned with even greater production, or overproduction of food in the West, even when there is a surplus.

Will the food industry receive 'bad press' if it becomes whole heartedly involved with this technology? It is easy to say that, with high levels of child mortality in the third world, this technology could have an important long term impact there, and deflect criticism in this way. However, the technology is here to stay. It should also be bourn in mind that for centuries man has genetically manipulated animals by selective breeding. This process can now be speeded up and made more specific by direct manipulation of DNA. However, the approaches should be well considered and address industry or producer needs, and not performed simply to demonstrate the technology itself.

5. Genetic Enhancement of Animal Species

There are a number of methods that have been used to successfully introduce foreign genes into animals (fig. 1). The best method would, of course, be to introduce foreign genes into sperm cells and thus rely on the natural egg penetrative process of the spermotozoon. At present, the highly coiled nature of the sperm DNA presents a barrier to the successful integration of foreign genes and then insertion into the egg. There are groups working on this technique and it could become a major pathway for the production of enhanced transgenic animals.

The DNA constructs, or transgenes, which are to be introduced into the fertilised egg have to be of the right design. They have to be complete genes, within the normal compelment of exons, introns and appropriate flanking sequences. Without all the correct pieces, high levels of expression (or indeed any expression at all) would not occur.

The selection of a suitable promotor sequence from a gene in the host animal is m important for the success of transgene particularly if tissue specific productio the gene product is desired, as it usually i

In theory, the metabolism of an animal c be regulated at several levels by transgen Once these genes have been successf integrated into the chromosome, then th are heritable elements and can be faithf transmitted to their offspring.

The introduction of somatotropin genes h been performed with cattle and pigs (a mice!), rainbow trout (Maclean and Renn l987) while salmon is another candida This is a relatively simple and straightforw approach. Other factors that could b manipulated include cell based recept (i.e. increasing their number) or the struct of the hormone to give it a longer circulat life time.

Although an increase in protein synthes rates would give advantages, perhaps t prevention of protein degradation mi also help to increase the rate of m production. This may be achieved by a sense RNA or antisense DNA which can used to selectively switch off specific genes.

Also, genetic manipulation doesn't alw have to be the option of choice. For i nce, vaccinating cattle with an antibody bovine somatotropin would cause the produ tion of an anti-antibody which itself wo act as a somatotropin. Thus an'overeffect' growth would be expected from a pro withthe correct somatotropin configurati but which is not samototropin technology.

There are a number of possible benef that may come from transgenesic (Goldsp l987).

a) Fecundity and fertility increase,

b) Sexing of sperm,

c) Growth factor control and musc quantity increase,

d) Muscle fibre type ratio manipulation,

e) Quality and quantity of lipids,

f) Collagen and meat toughne (manipulation),

g) Milk yields,

h) Milk, protein and lipid content,

i) Wool yield,

j) High quality wool increase (i.e. angora),

k) Disease resistance.

Work at present is at the comparatively early stages and much more research is required into the control of tissue growth (i.e. muscle). Ultimately elite strains of animals with enhanced characteristics will exist. The patent situation surrounding such animals is at present uncharacterised, but patents have been granted in the USA for a transgenic mouse. The prospect of royalty payments and licensing may be unusual for, and unwanted by, the farmer, but it would bring the 'transgenic farmer' into line with the rest of the biotechnology industry.

6. Conclusions

Of the number of emergent or novel processing technologies (or technologies that will affect meat processing) the main ones that will exert a profound effect on meat are enzyme processing technologies and transgenic animal technologies. Both technologies have considerable unexploited potential.

As they are used in more directed, controlled fashions then their use in the mid term to long term future (5-50 years) will increase dramatically. Biotechnology is the science of the 21st century, and it is here today, and here to stay.

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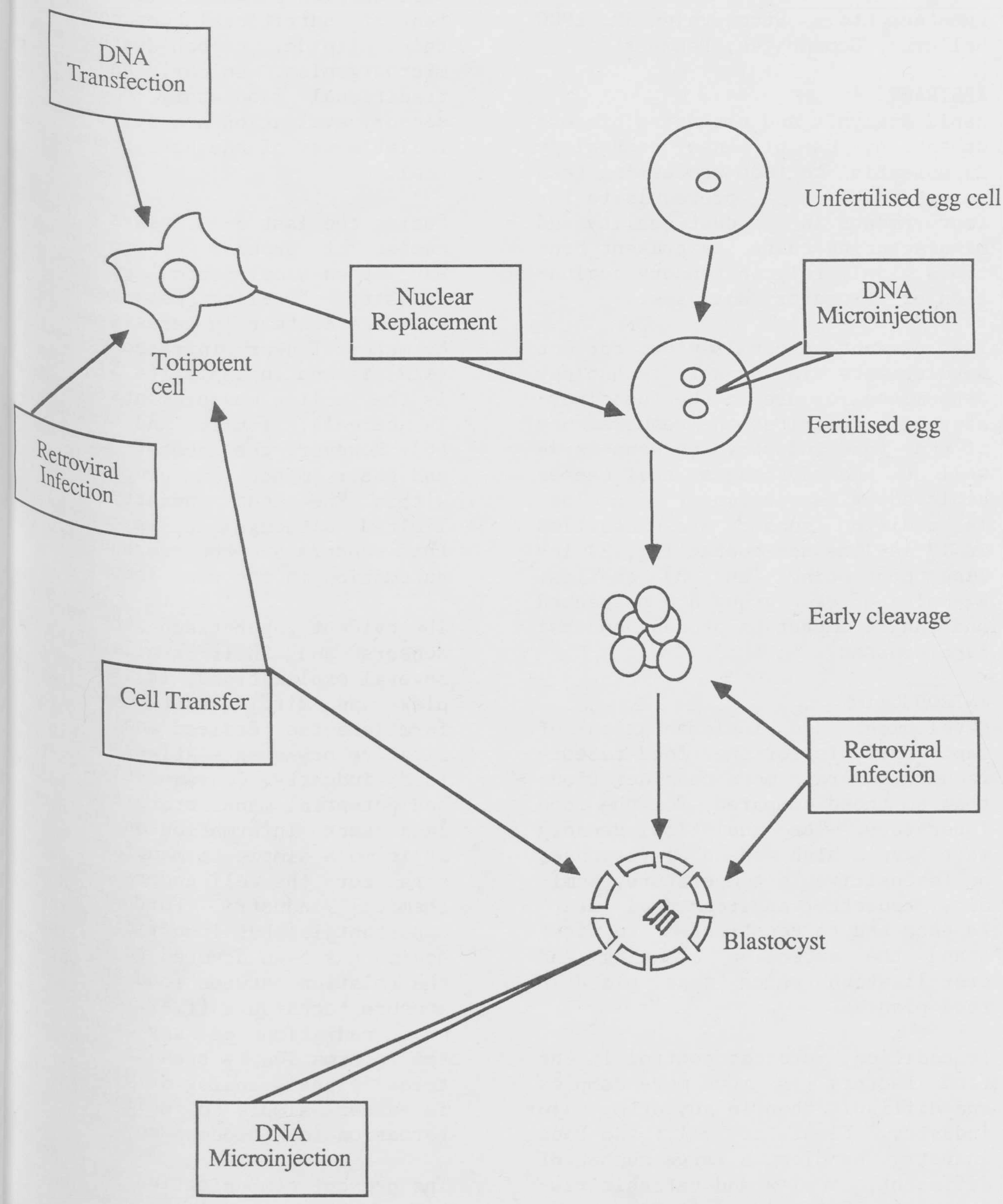
FIGURE 1. GENE TRANSFER METHODS



(After Goldspink, 1987)