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Immunological Approaches for Manipulation of Animal Growth, Body Composition and Fecundity

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Introduction

The endocrine system plays a central role in the regulation of reproduction, growth and body composition, and a number of approaches have been adopted to utilize hormonal control of these processes, or indeed to ablate such controls in situations which were detrimental to certain animal production systems. For example, in the beef industry producers receive less for heifer calves than steers because their rate of body weight gain is 10% less and food conversion efficiency is reduced to a similar extent. Perhaps more importantly, heifers also run the risk of unwanted pregnancy. Surgical removal of the ovaries or the use of expensive prostaglandins to induce abortion are attempts to control the problem, whereas in males surgical castration has been used traditionally to minimize aggressive behaviour. When maximal fecundity is required (e.g. for lamb production) systems have often been limited by low fertility rates. This has been attributed to oestrogen levels in clover pasture.

Probably the best known and most widely used method of influencing animal production systems has been the use of anabolic steroids as growth promotants. Their abuse in man, notably in athletes, as well as incidents such as their appearance in Italian baby food has created understandable concern over their use in animals used for meat production. This fear led ultimately to a ban on their use in the EEC, despite the fact that a scientific committee

Abbreviations: ACTH, adrenocorticotrophic hormone; bGH, bovine growth hormone; CFA, Complete Freund's adjuvant; CRF, corticotrophin releasing factor; Fab, fragment of antibody; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotrophin releasing hormone; GRF, growth hormone releasing factor; hCG, human chorionic gonadotrophin; hGH, human growth hormone; IGF-I, insulin-like growth factor I; LH, luteinizing hormone; MV10, quillabasaponin; PMSG, pregnant mare's serum gonadotrophin; RIA, radioimmunoassay; TGF, transforming growth factor; TRF, thyrotrophin releasing factor; TSH, thyrotrophin/thyroid stimulating hormone; VIP, vasoactive intestinal protein.

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reported favourably on their safety if used correctly (Lamming *et al.*, 1987). Indeed there is more concern, now that they are banned, that they are being used illegally and in an inappropriate and less well-monitored fashion. It is also interesting to note that these compounds are still permitted and used widely in the USA.

The growth of both consumer and animal welfare groups meant that pressure intensified to develop alternatives to the use of surgical procedures such as castration, as well as the use of anabolic steroids. Immunological approaches involving vaccination procedures would appear to be more acceptable and, in terms of regulation of fertility, this has been investigated quite extensively and with considerable promise. In terms of growth and lactation, however, early attempts to develop alternatives involved novel hormonal as well as molecular biological techniques.

Two major alternative hormonal approaches have been proposed to replace steroid hormones, namely β-adrenergic compounds and growth hormone. The naturally occurring β-adrenergic hormones, adrenaline and noradrenaline, promote lipolysis in white adipose tissue, and a number of synthetic analogues have been developed which have been assessed for their ability to prevent obesity in humans. In fact, some of the most potent of these were effective because of their thermogenic (i.e. energy-wasting heat production) effects (Rothwell and Stock, 1986). Such energy-wasting would be inappropriate for animal production systems so it was surprising that two such compounds, cimaterol and ractopamine, had a marked ability not only to reduce adipose tissue mass but also to stimulate lean tissue growth in a number of species, including cattle and sheep (reviewed by Hanrahan *et al.*, 1986). The anabolic effects appeared to be due to direct effects on muscle protein synthesis and degradation (Bergen *et al.*, 1987; Eadara *et al.*, 1987; Maltin *et al.*, 1987). Several limitations to the use of these compounds exist: since they are orally active any residues are potentially hazardous to those consuming the meat; they appear to require extremely short withdrawal periods (time from stopping treatment to time of slaughter) with much of the effect being lost within 7 days. Perhaps of greatest concern has been the adverse effects of these compounds on meat quality and preservation properties (Warris and Kestin, 1988). These problems make it unlikely that such compounds will ever be licenced for use in meat animals.

Far greater prospects of success are evident for the use of pituitary growth hormone. It has been known for several decades that growth hormone can improve protein : fat ratios and feed conversion efficiencies in cattle (Brumby, 1959), sheep (Muir *et al.*, 1983) and pigs (Machlin, 1972; Chung, Etherton and Wiggins, 1985; Etherton *et al.*, 1987). The effects of growth hormone in pigs are particularly interesting since both anabolic steroids and β-adrenergic agonists are of limited efficacy. Growth hormone also produces dramatic increases in milk yield in dairy cows (typically 10–30%) (Bauman *et al.*, 1985). The ability to capitalize on these effects was prevented until quite recently because the only source of this peptide (almost 200 amino acids long) was by purification from pituitary glands. The advent of recombinant DNA technology has dramatically changed this situation and has meant that growth

hormone is being promoted very actively by a number of companies as a potential product for enhancing the efficiency of both meat and milk production. Ironically the description of anabolic steroids as 'growth hormones' has led to undue criticism of pituitary growth hormone which, since it is a polypeptide, is inactive by mouth. Indeed non-primate growth hormones in general are inactive even if injected into humans, a fact which severely limited the growth prospects of growth hormone-deficient children, since the only material of use to them was human growth hormone obtained from cadavers. Despite the fact that growth hormone appears to be safe and effective, successful licensing in the EEC is still by no means certain and, because of the necessity to inject, a suitable delivery system remains to be devised, although several promising approaches using delay vehicles have been described (Rijpkema *et al.*, 1987; Knight *et al.*, 1988).

An even more dramatic approach to animal growth regulation has developed from the ability to incorporate foreign genes into the germ line using molecular biological approaches. Palmiter *et al.* (1982) were first to describe gigantism in mice with extra copies of growth hormone genes. Experiments in sheep and pigs have followed, with some unwanted side-effects, including arthritic joints and reduced fertility, although Pursel *et al.* (1988) have described transgenic pigs overexpressing growth hormone, without any such ill-effects. Interestingly, gigantism has not been a feature of any of the studies in large animals, the benefits typically being an improved carcass composition, with increased protein and decreased fat content. There are, however, other subtle changes in body composition, most notably hepatomegaly (Shea, Hammer and Brinster, 1987). The major current challenge would appear to be regulation of gene expression in a site-specific fashion, for example linking the prolactin promoter to the growth hormone gene to enable prolactin secretagogues to be assessed for their ability to induce growth hormone secretion (Polge *et al.*, 1989). Such approaches clearly have the potential to make a dramatic impact on animal breeding systems but the degree of resistance to the use of molecular biological approaches of this nature has yet to be assessed.

The growing pressure to prevent the use of surgical castration, as well as the use of hormones and molecular biological approaches to increase the efficiency of animal production systems has meant that increasing efforts have been devoted to finding alternative, non-invasive, non-hormonal techniques to replace them. The remainder of this review will be devoted to a variety of approaches including immunoneutralization, immunoenhancement, cytotoxic antibodies and antibodies as mimics of hormones, with particular reference to the potential benefits of these techniques in the areas of reproduction, growth and lactation.

Immunocastration

Both male and female fertility is dependent upon the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secreted from the

pituitary under the stimulatory influence of gonadotrophin releasing hormone (GnRH) produced in the hypothalamus.

The ability to reduce fertility and prevent pregnancy using immunization against GnRH has been successfully demonstrated in rats (Fraser *et al.*, 1974), rabbits (Arimura *et al.*, 1973), dogs (Schanbacher *et al.*, 1983), monkeys (Chappel *et al.*, 1980), boars (Awoniyi *et al.*, 1988), heifers (Johnson *et al.*, 1988), sheep (Schanbacher, 1982) and mares (Garza *et al.*, 1988). In many of these studies the major problem has been uniformity of response. In males active immunization against GnRH typically results in high titres of antibodies to GnRH, reduced plasma testosterone levels and decreased testicular size. In bulls Robertson *et al.* (1982) described reduced libido, semen production and more docile behaviour, although only 50% of the animals developed high titres of antibodies. Clearly this approach has the potential to modulate reproductive activity but various problems still exist. Many of these studies have used Complete Freund's adjuvant (CFA) to induce antibody production, an approach which is untenable in production systems because of its ulcerative properties. In a study in heifers Johnson *et al.* (1988) compared the use of CFA and adjuvant containing quillabasaponin (MV103). CFA was superior in inducing antibodies to GnRH and LH than MV103 but not to human chorionic gonadotrophin (hCG, cross-reactive with LH). MV103 was still able to depress fertility when used with GnRH or LH despite the inferior antibody responses and, curiously, despite the fact that CFA and MV103 induced similar antibody responses to hCG, they were only effective in depressing fertility in MV103-treated animals. These results serve to emphasize the point that simply assessing antibody titres is not necessarily a good indicator of the biological outcome. Clearly, the ability of antibodies to immunoneutralize hormones may depend on the particular epitopes (antigenic sites) on the hormone to which the antibodies bind. If these epitopes are not involved in the binding of the hormone to its receptor, then the hormone may still be effective even with antibody bound to it.

Typically, GnRH has been conjugated to a carrier protein to enhance its immunogenicity and the importance of the conjugation technique is also apparent, since different methods have produced equivalent antibody titres but with differing capacities to neutralize hormonal activity. In this respect an azo-GnRH tetanus toxoid conjugate has been shown to produce antibodies with excellent specificity for GnRH (Singh, 1987).

The potential use of this approach has even been proposed for clinical purposes in the treatment of androgen-dependent carcinoma of the prostate, since anti-GnRH vaccines induce marked atrophy of the prostate (Jayanshankar *et al.*, 1989).

Boar taint

Although immunocastration of males reduces the risks of aggressive behaviour, it serves another important function in boars, that of reducing so called 'boar taint'. Meat from boars frequently emits a strongly unpleasant urine-like odour during heating, making it unacceptable to consumers. In the

United States such carcasses are condemned, and any exhibiting slight odour may be used only in comminuted sausage products. This odour is exhibited in about two-thirds of sexually mature animals and so is a major problem. Although immunocastration is a solution, this results in a reduced production of lean meat (about 10%) with a 10–15% reduction of feed conversion efficiency. The ability to keep boar pigs intact while selectively eliminating boar taint would have dramatic effects on the pork industry. A group of C_{19}-Δ^{16}-steroids have been proposed as agents responsible for the taint (Patterson, 1968; Thompson and Pearson, 1977). Synthesized in the Leydig cells of the testis, they are secreted into blood and transported to adipose tissue where they are stored. They are without androgenic activity, and function instead as pheromones, being released via the salivary glands in the form of $5\alpha_1$-androst-16-en-3α-ol (Gower, 1972). A number of studies have attempted to immunize against and thereby neutralize accumulation of 5α-androst-16-en-3-one (Shenoy, Daniel and Box, 1982; Williamson *et al.*, 1985) without any convincing effects (though concentrations of the steroid in adipose tissue were reduced by 30–70%). Brooks *et al.* (1986) reasoned that, since all of the steroids suspected of inducing boar taint were synthesized in the same metabolic pathway, auto-immunization against the initial C_{19}-Δ^{16}-steroids in the pathway (5,16-androstadien-3β-ol and 4,16-androstadient-3-one) should prevent the production of boar taint. Their studies did reveal significant reductions in both the adipose concentrations of C_{19}-Δ^{16}-steroids and boar odour intensity, although the results were somewhat variable between individuals. Encouragingly, however, and unlike immunocastration, no effects of immunization were evident on weight gain, efficiency of feed utilization, average backfat thickness or percent carcass muscle. Clearly this type of approach deserves further consideration.

Increasing fertility by immunization against steroids

Ovulation rates are largely dependent upon the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Their secretion is, in turn, inhibited by a negative feedback loop from the ovary, involving steroid hormones such as testosterone and oestradiol.

Early attempts to increase follicular development and ovulation rate have involved the use of pregnant mare's serum gonadotrophin (PMSG) which has both FSH- and LH-like properties. The responses, like those in similar trials in humans, are difficult to control and inconsistent, so that even when a seemingly appropriate dose is used there exists the risk of excessively large litters and ultimately complete infertility. In sheep the response to PMSG is further complicated since it has been shown to vary according to breed of animal and with season.

Recently a different strategy has been adopted in which animals are immunized against various gonadal steroids. In theory neutralization of these hormones should release the pituitary from the feedback mechanisms which inhibit FSH and LH release. Increased FSH and LH should then increase the number of follicles developing to mature ova and thereby increase fecundity.

Active immunization against androstenedione has been shown to improve the reproductive performances of ewes of many breeds with a wide range of natural lambing rates (Cox *et al.*, 1982; Geldard, Scaramuzzi and Wilkins, 1984). This work led to the introduction of a commercial product 'Fecundin'. Increases of 7–45% in lambing rates were achieved and were independent of whether the breeds were naturally prolific or not. The initial studies were carried out in Australian and New Zealand breeds of sheep but have been confirmed in several British breeds using both passive and active immunization (Land *et al.*, 1982; Rhind *et al.*, 1986). In passive immunization studies using antisera to testosterone it has become evident that there is an optimal level of antibody required to increase ovulation rate (Rhind *et al.*, 1987) which represents a moderate rather than a very high titre antibody response. This clearly represents a problem in active immunization schedules since antibody responses are notoriously difficult to regulate. In addition, body condition at the time of mating has a considerable effect on the response to passive immunization against testosterone (Rhind *et al.*, 1985). This increased both mean ovulation rates and mean lambing rates of Blackface ewes at low and moderate levels of body condition. Ovulation rate was also increased in ewes in a high body condition but this did not result in an increased number of lambs. This suggests that, when in good body condition, ovulation rate is not limiting. The number of embryos able to be sustained must therefore be the limiting factor and for such animals this immunization approach is probably inappropriate.

In at least two of these studies (van Look *et al.*, 1978; Rhind *et al.*, 1985) there was a trend towards higher proportions of female lambs in treated ewes. Although the hypothesis is that there is increased loss of male embryos, to date no evidence to support or explain this phenomenon exists. Several studies have also reported increased embryo mortality (Boland *et al.*, 1986; Croker *et al.*, 1987; Rhind *et al.*, 1987).

Active immunization against oestradiol has been examined in rams and shown to stimulate a number of reproductive parameters (Mon-et-Kuntz *et al.*, 1988). Oestradiol-immunized rams exhibited increased plasma concentrations of LH, FSH and testosterone as well as increased testis weights, Leydig cell numbers, spermatocyte and spermatid numbers.

Attempts have been made to reproduce these effects in cattle. Yearling heifers were actively immunized with conjugates of testosterone-3-carboxymethyloxime-ovalbumin (Price, Morris and Webb, 1987). All treated animals had measurable antibody responses and all became anoestrous and displayed ovarian cysts. Twenty-five weeks after boosting, three animals resumed cyclicity with several double ovulations and one quadruple. The remaining anoestrous animals had increased LH and decreased FSH concentrations and remained anoestrous for 11 months. Attempts to resume cyclicity with GnRH and prostaglandin $F_2\alpha$ injections succeeded in only one animal. In a similar study involving immunization of prepubertal heifers with either androstenedione or oestradiol, androstenedione-treated animals demonstrated consistently higher progesterone concentrations (indicative of ovulation) up to 14 months of age and exhibited greater numbers of larger follicles

within their ovaries. Although these results in cattle have not been as clear-cut as in sheep, they show sufficient promise to merit further study.

Although active immunization against androstenedione has been proposed as an alternative to the use of PMSG, it has actually been used in conjunction with it to induce out-of-season mating (Robinson and Scaramuzzi, 1986).

Immunomanipulation of growth

Growth hormone (GH), as its name implies, plays a major role in growth. Several dwarf rat and mouse models display extremely low serum GH levels and hypophysectomized rats, whose growth is completely retarded, can be induced to grow with injections of GH. It is not only this effect of GH which has proved to be of interest in animal growth studies but also its ability to induce the so-called 'repartitioning' of energy intake into protein deposition and away from fat storage. GH is both lipolytic and anti-lipogenic (Vernon and Flint, 1989) and also appears to enhance the ability of adipocytes to respond to more potent lipolytic agents, such as noradrenaline, possibly by increasing the number of β-adrenergic receptors on adipose tissue (Watt *et al.*, 1989). As already mentioned, one possible approach is to treat with growth hormone itself but a suitable form of implant has yet to be devised. An alternative strategy would be to stimulate endogenous GH secretion.

The release of GH from the pituitary is regulated by two major control mechanisms. Growth hormone releasing factor (GRF) stimulates GH release whereas somatostatin serves to inhibit GH release. Spencer, Garssen and Hart (1983a, b) hypothesized that immunoneutralization of somatostatin should lead to increased GH concentrations in serum and improved growth rates and/or carcass composition. This was in fact the case, with a staggering 76% increase in body weight gain of St Kilda sheep. Although similar findings have been demonstrated by other groups in sheep (Bass *et al.*, 1987) and in cattle (Lawrence *et al.*, 1986), others have failed to find an effect (Fitzsimons and Hanrahan, 1984; Galbraith *et al.*, 1985; Trout and Schanbacher, 1990) and there has even been one report of inhibition of growth (Varner, Davis and Reeves, 1980).

Passive immunization with antisera to somatostatin clearly results in increased circulating concentrations of GH in rats (Arimura, Smith and Schally, 1976; Terry and Martin, 1981). In studies involving active immunization against somatostatin, GH levels were increased in sheep (Varner, Davis and Reeves, 1980; Spencer, Garssen and Hart, 1983a, b), pigs (Dubreuil *et al.*, 1989) and cattle (Peticlerc *et al.*, 1988) whereas they were unchanged in other studies of sheep (Laarveld, Chaplin and Kerr, 1986) and cattle (Lawrence *et al.*, 1986).

One of the consequences of treatment with GH is an increase in circulating concentrations of insulin-like growth factor I (IGF-I) which, according to the somatomedin hypothesis (Daughaday, 1981) acts as a mediator of the anabolic (but not lipolytic) role of GH. Thus in animals actively immunized against somatostatin, an increase in plasma IGF-I concentrations would be anticipated. Such an increase has been found in two studies (Spencer,

Garssen and Hart, 1983a; Bass *et al.*, 1987) but not in another (Trout and Schanbacher, 1990). In the studies of Trout and Schanbacher (1990) immunization against GRF was also included. This induced a pronounced decrease in serum IGF-I concentrations, suggesting effective neutralization of GRF and thereby GH, but produced only a very small (6%) decrease in growth rate, questioning the importance of IGF-I as an endocrine mediator of growth. Perhaps the most convincing evidence that any growth effect induced by immunization against somatostatin is not simply mediated by increased GH concentrations is the fact that in none of the studies conducted so far has there been any change in the protein : fat ratios within the carcass. This is in direct contrast to the effects of GH treatment of sheep, cattle and pigs where GH consistently increases this ratio.

Perhaps the hypothesis was too simplistic since somatostatin not only regulates GH secretion but also that of thyrotrophin (TSH) (Reichlin, 1983), adrenocorticotrophin (ACTH) (Reisine, 1985), glucagon and insulin (Bozikov, 1980). Somatostatin is also found in abundance within the gastrointestinal tract and has been shown to influence the secretion of motilin. Fadlalla, Spencer and Lister (1985) demonstrated that passive immunization against somatostatin significantly reduced transit time of a marker in food, which might explain the increased efficiency of food utilization described by Spencer, Garssen and Hart (1983b).

Biological activity of growth hormone fragments

In attempts to resolve the problem of limited quantities of human GH for the treatment of pituitary dwarfism, a number of research teams began to examine the possibility of producing bioactive fragments of the GH molecule. GH is a polypeptide hormone with a complex tertiary structure and it appears to show a number of different biological actions: it stimulates growth, it is lipolytic and diabetogenic (i.e. antagonistic to the actions of insulin). These different properties have led to speculation that the different biological activities might reside in different parts of the molecule. Controlled enzymatic digestion of GH with chymotryptic, peptic and tryptic activity led Yamasaki, Kikutani and Sonenberg (1970) to the isolation of a small fragment of bovine GH that retained certain properties of the intact molecule and, somewhat surprisingly, was metabolically active in man. This was surprising since the intact bovine GH molecule is inactive in man. The fragment, incorporating residues 96–133, caused increased body weight gain and tibial width in hypophysectomized rats, both classical GH responses. One current concern regarding this possibility is that bovine GH, present in milk from cows treated with it to increase milk yield, could be digested in humans to produce a biologically active peptide. This seems unlikely since the transfer of GH into milk is low and no other group has reported similar findings. Such findings did, however, encourage a number of research teams to follow along similar lines, including synthesis of selected sequences of hGH. Many of these studies indicated that dissociation of the various biological actions of GH could be achieved in this manner. For example, several reports suggested that

diabetogenic activity resided in residues 44–77 and 170–191 (Wallis, 1982), that the paradoxical acute insulin-like effects of GH were evident in residues 1–13 and that the growth-promoting effects of GH might be located in the region of 80–140 (Retegui *et al.*, 1982b).

Despite these apparently encouraging results suggesting that peptide fragments might be produced exhibiting specific effects of GH, all of these peptides show drastically reduced biological activity and, as such, this approach seems unlikely to yield significant bioactive compounds in the foreseeable future.

This lack of biological activity has been reinforced by the extremely low affinity of synthetic peptides, for example corresponding to residues 19–128, 73–128 or 98–128, in radioimmunoassays for hGH (Retegui *et al.*, 1982b), emphasizing that the tertiary structure of the molecule may well be an essential requirement for full potency.

The production of monoclonal antibodies to GH have also been used to explore the interactions of GH with its receptor(s). Although the early studies of Retegui *et al.* (1982a) suggested that the same epitope on the GH molecule was responsible for binding to all GH receptors, more recent studies have provided conflicting results. The studies of Barnard *et al.* (1985) and Thomas *et al.* (1987) suggest that when monoclonal antibodies are bound to the GH molecule its ability to bind to GH receptors is restricted in some tissues but not in others.

After the cloning and expression of the GH receptor (Leung *et al.*, 1987), Smith, Linzer and Talamantes (1988) described two mRNA species in mouse liver and adipose tissue which hybridized with a GH receptor cDNA probe. The shorter of the two was considered to be the GH binding protein in serum, with the larger representing the receptor itself. More recently, Barnard, Rowlinson and Waters (1990) have demonstrated that although a number of monoclonal antibodies to the GH receptor reacted equally well with GH receptors from rabbit liver and adipose tissue, one of them reacted very poorly with adipose tissue but well with liver. When the receptors were removed from the membrane by detergent solubilization, however, no such discrepancy was apparent. This led the authors to propose that the ligand-binding domain for GH is identical but that tissue-specific membrane components may serve to mask certain epitopes on the receptor not intimately involved in the binding interaction. This may serve to explain the observations of Thomas *et al.* (1987), since a large molecular weight antibody bound to growth hormone would clearly have considerable opportunity to be sterically hindered by adjacent proteins, possibly even unrelated to the receptor, which might be present in some tissues but not others. Thus tissue specificity of binding could be induced even though there was but a single form of receptor.

Enhancing the actions of GH using monoclonal antibodies

Over 50 years ago the ability of antisera to increase rather than inhibit the activity of hormones was demonstrated for gonadotrophins which, when

injected for prolonged periods, induced antibody formation which augmented their effectiveness (Thompson, 1937; Rowlands, 1939). Ferguson (1954) demonstrated a similar phenomenon in terms of the effect of growth hormone on wool growth. Numerous explanations have been advanced to explain this phenomenon, one of the earliest of which was the concept of 'slow-release' of hormone or the protective effect of antibodies on proteolytic degradation of the hormone. Direct evidence supporting this proposal include studies such as those of Clark *et al.* (1985) which demonstrate that pulsatile delivery of growth hormone is much more effective in stimulating animal growth than an equivalent dose given as a single bolus. More active analogues of hormones such as D-ala$_2$-GRF appear to be more effective because of reduced degradation by proteases.

The studies of Shechter *et al.* (1979a) showed that enhancement of insulin activity by anti-insulin antibodies required bivalent antibodies, and they proposed that this might involve cross-linking and aggregation of receptors; a phenomenon known to occur for a number of receptors when they bind their specific ligand (Shechter *et al.*, 1979b). However, this mechanism of potentiation has been ruled out for growth hormone since univalent (Fab') fragments of antibody will also enhance growth hormone effects (Aston *et al.*, 1986, 1987).

Goodfriend, Webster and McGuire (1970) demonstrated the general applicability of this phenomenon by showing antibody-enhancing effects for angiotensin, bradykinin and melanocyte-stimulating hormone. Although they were unable to shed any light on the mechanism(s) involved, they proposed yet another hypothesis which is gaining increasing support; that of hormone 'targetting' to specific receptors by restricting access to other populations of receptors.

The production of antibodies to growth hormone in children given human growth hormone has been reported (Roth *et al.*, 1964). The advent of monoclonal antibody technology has allowed this phenomenon to be investigated in much greater detail and particular emphasis has centred on growth hormone action. With the aid of a panel of monoclonal antibodies, Aston and co-workers mapped a number of epitopes on the growth hormone molecule which exhibited enhancing properties (*see* Aston and Ivanyi, 1983, 1985; Aston, Cowden and Ada, 1989). Attempts to define these epitopes using short linear peptides derived from the GH sequence, however, proved unsuccessful, suggesting that the epitopes were conformational rather than linear sequences. These conformational epitopes arise from the close proximity of discontinuous amino-acid sequences often from distal regions of the linear peptide sequence.

A variety of strategies exist to allow prediction of such epitopes, including hydrophilicity (Hopp and Woods, 1981; Parker, Guo and Hodges, 1986) secondary and tertiary structure analysis (Nicoll, Mayer and Russell, 1986; Abdel-Meguid *et al.*, 1987; Blundell *et al.*, 1987; Cohen and Kuntz, 1987; Krchnak, Mach and Maly, 1987), and segmental or atomic mobility (Tainer *et al.*, 1984; Westhoff *et al.*, 1984). Geysen, Meloen and Barteling (1984) developed an impressive technique which allows the simultaneous synthesis

of hundreds of peptides, and were able to map an enhancing monoclonal antibody to the loop linking helices 3 and 4 of the bovine GH molecule.

One of the major limitations to achieving a better understanding of the enhancing properties of such antibodies is the inability to use *in vitro* techniques to assess some of the potential modes of action, such as antibody clearance or targetting of hormone to specific receptors. Even when parallel effects of antibodies have been achieved *in vitro* and *in vivo* they have, on occasion, been shown to be due to distinct mechanisms.

The elegant studies of Thomas *et al.* (1987) demonstrated that certain monoclonal antibodies could inhibit binding of human growth hormone to certain GH receptors but not to others and, intriguingly, that some of those antibodies inhibited growth hormone binding in livers from lean but not obese mice. Such findings clearly indicate that there may be distinct GH receptors which recognize distinct epitopes on the GH molecule and that the proportions of these receptors may change with physiological state.

The difficulties of elucidating the mechanism of this action are highlighted by the studies of Aston *et al.* (1986) which showed two monoclonal antibodies to human growth hormone to be inhibitory *in vitro* and enhancing *in vivo*, with no correlation of enhancement with receptor binding properties in several *in vitro* systems. Whatever the mechanism of enhancement, it does appear to be a true stimulation of GH-dependent mechanisms since circulating insulin-like growth factor I levels are increased considerably by such approaches (Wallis *et al.*, 1987). In the study of Wallis and colleagues, insulin-like growth factor I levels and growth rate showed a strong positive correlation, supporting an endocrine role for IGF-I in growth.

Although GH precomplexed to monoclonal antibodies induces effective enhancement (up to tenfold) of the biological activity of GH, for example when given to Snell Dwarf mice (Aston *et al.*, 1986), this approach is of limited practical value. The real potential lies in the ability of such enhancing antibodies to complex with endogenous GH. In the early studies of Aston and co-workers (using mice) this possibility could not be tested because the monoclonal antibodies to human growth hormone did not cross-react with the endogenous mouse growth hormone. One of their antibodies did, however, cross-react with marmoset GH and when this was administered alone (i.e. not complexed to GH) to young marmosets it produced a 30% increase in body weight gain over a 40-day period, presumably by complexing with endogenous GH (Holder *et al.*, 1985). This study also revealed a limitation of this particular approach involving passive immunization since the heterologous nature of the antibody administered induced an anti-immunoglobulin response which prevented subsequent enhancement.

Recently these studies have been extended to cattle where effects have been demonstrated on both growing and lactating animals. When bovine GH was given to lactating dairy cows it prevented the decline in milk yield that normally occurs. When the same bovine GH was pre-complexed to monoclonal antibodies it actually stimulated milk yield and serum IGF-I concentrations over and above those of GH alone (Pell *et al.*, 1989). Disappointingly, however, milk yield was not enhanced in animals given the monoclonal

antibody alone, although an appropriate control group (e.g. one receiving irrelevant mouse immunoglobulins) was not included, making it difficult to interpret any effect precisely.

As mentioned earlier, growth hormone exhibits a so-called 'diabetogenic effect' demonstrated most clearly by its ability to antagonize the effects of insulin on blood glucose concentrations. When insulin was given to sheep as an intravenous bolus it induced a hypoglycaemic state, with blood glucose decreased by approximately 2 mmol l^{-1}. When GH was given concurrently this decrease in blood glucose was reduced to around 1 mmol l^{-1} and when GH was given pre-complexed to monoclonal antibody the decline in blood glucose was prevented completely. There was also a significant effect of monoclonal antibody to GH when administered alone, reducing the decline in blood glucose to about 1·2 mmol l^{-1}, indicating that the antibody was capable of complexing with and enhancing the effectiveness of endogenous GH (Pell *et al.*, 1989).

Probably the most promising practical approach for antibody enhancement is the prospect of identifying the epitope responsible for enhancement, synthesizing it as a synthetic peptide and immunizing with such a peptide to produce a polyclonal antibody response of restricted specificity. Such antibodies would be capable of complexing with, and enhancing the effects of, endogenous hormone. Both bovine and human growth hormones appear to possess at least four non-overlapping antigenic determinants (Aston *et al.*, 1987). Attempts to define the precise binding site of individual monoclonal antibodies have proved difficult since they fail to cross-react with synthetic peptides derived from the growth hormone sequence. The alternative to this has been to raise antisera to synthetic peptides and examine their ability to bind to the native hormone. A variety of such anti-peptide antisera have been shown to react with native GH in solid-phase radioimmunoassay (RIA) in which GH is coated onto plastic. However, when examined in liquid-phase RIA, many of the antisera failed to react (Bomford and Aston, 1990). This higher reactivity in solid versus liquid phase is a common observation and may be due to several causes, including the greater sensitivity of solid-phase systems to detect low-affinity antibodies and the partial denaturation of the hormone when adsorbed onto plastic resulting in the exposure of linear sequences (corresponding to the synthetic peptide) (Walter, 1986). Despite these limitations, Bomford and Aston (1990) were able to identify a peptide, corresponding to the amino acids 35–53 of bGH, which produced antibodies cross-reactive with the intact hormone in liquid RIA and which also induced enhanced bioactivity of bGH when complexed with it. This region was interesting from several points of view. First, it was predicted as a region of a major turn likely to be an antigenic site, a proposal subsequently confirmed by X-ray crystallography of porcine GH (Abdel-Meguid *et al.*, 1987). It also overlaps the region of hGH (residues 32–46) which is deleted in the natural 20 kDa variant. This variant has different receptor-binding properties to the 22 kDa form of hGH (Smal *et al.*, 1987). Finally, and perhaps most interestingly, it has been shown that cleavage of GH at this site results in enhanced biological activity (Singh *et al.*, 1974; Lewis *et al.*, 1975) and that binding of

GH to liver receptors results in a proteolytic cleavage of GH in this region with a resultant increase in affinity for its receptor (Schepper *et al.*, 1984). These findings make it tempting to speculate that monoclonal antibody binding to these 'enhancing' regions of GH may be mimicking a natural proteolytic event which occurs during hormone binding.

The ability to produce enhancing antibodies by the use of synthetic linear peptide sequences is extremely encouraging, but ultimately the production of peptides mimicking epitopes involving the discontinuous amino-acid residues could yield even more potent agents for enhancement of a wide variety of hormonal effects.

Finally, an intriguing series of observations has been made which has demonstrated enhancement using a different approach. Instead of pre-complexing human growth hormone with antibodies, it was conjugated to ovalbumin or bovine serum albumin by using cross-linking reagents, including glutaraldehyde. When such conjugates were tested *in vivo* they significantly increased ^{35}S uptake into costal cartilage over and above that caused by growth hormone alone (Holder and Aston, 1989). In addition, when growth hormone was conjugated to itself, similar enhancement was obtained. These results suggest that the phenomenon of enhancement may not be limited to particular antigenic sites but may be an effect of increased molecular size. The determination of the mechanism of enhancement should provide exciting new insights into the potential for manipulation of hormone activity.

Antibodies as mimics of hormones

Jerne (1974) proposed the network hypothesis of regulation of the immune system. In this system any new antibody (Ab_1) produced to a novel antigen would itself be novel (and hence 'foreign') to the animal producing it, so that antibodies would also be produced to it (Ab_2). Since Ab_2 was novel it would evoke Ab_3, etc. Evidence for such networks does exist and they are currently considered to be important from the point of view of regulation of the immune response. Of greater relevance to the discussion of hormone mimicry is the production of a subset of antibodies which lead to the production of a so-called 'internal image' of the original antigen. Since Ab_1 is referred to as the idiotypic response, these internal images (Ab_2) are referred to as anti-idiotypes. Producing this anti-idiotype depends upon the fact that the antibody (Ab_1) to (say) GH will bind to the hormone in a situation loosely analogous to a glove around a hand. This Ab_1 is then used to produce a second antibody (an anti-idiotype) which also binds to the idiotype in analogous fashion to the hormone and hence may be a structural mimic of the hormone (or a part of it). Such anti-idiotypes have been produced for a number of hormones, including acetylcholine (Wasserman *et al.*, 1982), insulin (Sege and Peterson, 1978), β-adrenergic compounds (Schreiber *et al.*, 1980), TSH (Farid *et al.*, 1982) and growth hormone (Gardner *et al.*, 1990). In many cases these antibodies were also capable of inducing the biological response normally attributable to the respective hormone. Anti-idiotypes to β-adrenergic compounds and growth hormone have obvious application to

animal production systems as non-hormonal alternatives. Anti-idiotypes to growth hormone have been shown to bind to growth hormone receptors in sheep and rat liver and rat adipocytes but did not disrupt prolactin or insulin binding to the liver, indicating a high degree of hormonal specificity. When given to hypophysectomized rats for 3 days they also induced increased body weight gain (Gardner *et al.*, 1990). These results were produced using polyclonal antisera involving a mixture of antibodies probably mimicking a number of epitopes on the GH molecule. The real strength of this approach must lie, however, in the production of monoclonal anti-idiotypic antibodies which should mimic single epitopes on the GH molecule.

Recent studies by Elbashir *et al.* (1990) have demonstrated a panel of monoclonal anti-idiotypic antibodies to human growth hormone which show divergent effects in different receptor-binding systems. Several of these antibodies inhibited growth hormone binding to rat liver, enhanced binding to the human growth hormone serum-binding protein while having no effect on growth hormone binding to rabbit liver. This is possibly the most direct evidence to date to suggest that different epitopes of growth hormone are involved in binding to different receptor populations.

Such antibodies will allow a number of questions to be addressed: how many epitopes are involved in binding to GH receptors; do mimics of different epitopes bind to specific receptor populations in different tissues? Alternatively more complex questions may be asked about whether different populations of GH receptors exist within the same tissue and whether different epitopes induce different metabolic functions. This last point is a parallel approach to the concept of bioactive fragments but has a distinct advantage over such fragments. These antibody mimics essentially resemble the shape (rather than the primary amino-acid sequence) of the epitope on GH which they mimic. They are thus held in the appropriate conformation, which is unlikely to be the case for short synthetic or cleaved peptides. In addition, these antibodies may, quite conceivably, be mimics of epitopes which involve amino-acid residues held in close proximity to one another but derived from different parts of the polypeptide chain. The three-dimensional structure of porcine GH was recently elucidated by using X-ray diffraction (Abdel-Meguid *et al.*, 1987) and it seems likely that non-adjacent residues may well be involved in binding to the GH receptor. The primary amino-acid sequence of the GH receptor has also been deduced recently using recombinant DNA techniques (Leung *et al.*, 1987). Its three-dimensional structure and proposed sites of interaction with GH are eagerly awaited.

Another distinct advantage of this approach relies upon the very fact that it is an immunological technique and thus lends itself to a vaccination approach. The procedure would involve, first, identifying a monoclonal anti-idiotype which mimics GH. It should then be possible to identify the original idiotype (Ab_1) which induced its formation, also in monoclonal form. This monoclonal Ab_1 could then be used to immunize animals in order to induce an endogenous production of Ab_2, the growth hormone image. Since this is an amplification process, small amounts of Ab_1 can be used to induce large amounts of Ab_2. In addition, the half-life of such responses is of the order of

weeks compared to the half-life of injected GH (20–30 min). This approach does, however, have certain limitations since, to date, no mechanisms exist for regulating either the size or the duration of the response. Growth hormone is, however, a notable exception among hormones since excessive amounts in the blood of young, growing animals do not appear to produce problems for animal health. But note that in man, acromegaly is the dramatic result of excessive GH secretion in the fully grown adult. Finally, although this approach can be used as a possible alternative to many hormones, it is unlikely to be appropriate for mimicking steroid hormone actions where receptors are present in the cytoplasm rather than the plasma membrane.

The practical demonstration of anti-idiotypic antibodies as mimics of hormones has led to the proposal that in several disease states, including certain forms of insulin resistance and hyperactive thyroid syndromes, where antibodies to the insulin receptor and the TSH receptor are evident, these antibodies may be anti-idiotypes induced by an initial auto-immune response, not to the receptor itself but to the ligand that binds to it (Weetman and McGregor, 1984).

Cytotoxic antibodies to adipose tissue

A phenomenal amount of research has been devoted to the understanding of tumour biology, where cancerous cells appear able to escape from normal growth regulatory mechanisms and continue to multiply in an uncontrolled fashion. Clinical research has evaluated a number of different approaches including surgical removal of tumours, radio- and chemotherapy. Surgical removal is clearly only possible when the tumour is of an appropriate size and, in particular, when secondary tumours remote from the site of origin have not already arisen. Radiotherapy and chemotherapy, on the other hand, can attempt to deal with the problem on a whole-body basis (although radiotherapy can also be used in localized fashion). However, these alternative approaches not only kill tumour cells but lead to normal tissue destruction as well, and are often accompanied by quite severe side-effects.

These limitations have led to a different strategy involving the use of antibodies to kill tumour cells—the so-called magic bullet approach. Many descriptions exist of the ability of monoclonal antibodies to kill tumours against which they have been raised both *in vitro* and *in vivo*, although *in vivo* experiments typically involve simultaneous administration of the tumour and antibodies to experimental mice. In attempts to deal with established tumours, and in particular solid tumours *in vivo*, the results have been much less encouraging. Attempts to increase the effectiveness of such antibodies have included linking both radioisotopes and plant toxins to the antibodies although again, particularly in the case of the latter, non-specific toxic effects have been a major problem alongside inability to target most of the antibodies to the site of the tumour (typically around 5% of injected antibodies localize at the site of the tumour).

Even when such problems are resolved at least two other problems remain, one is the problem of tumour heterogeneity or 'antigen shedding' which

allows certain cells, no longer reactive with the antibodies, to escape the cytotoxic destruction. The second is simply the fact that, since the technique does not attempt to resolve the problem of growth regulation, even if 99·9% of the cancerous cells are destroyed the remaining 0·1% may regrow to form yet another tumour.

The growth properties of adipose tissue, however, are about as far removed from those of tumour cells as one might imagine. Although considerable cell division takes place in the neonate, with proliferation of numerous pre-adipocytes and their development into mature adipocytes, this rate of cell division slows dramatically around the time of puberty in many species. The mature adipocyte is a unique cell in that it stores a huge droplet of triacylglycerol within its core so that the cytoplasm of the cell is stretched thinly around it and the nucleus actually causes a pronounced protrusion on the surface of the cell. Purely from morphological reasoning, it has been suggested that mature adipocytes are incapable of undergoing mitosis. Recently, however, it has been proposed that mature adipocytes can release their triacylglycerol content, dedifferentiate, mitose and redifferentiate into mature adipocytes (Sugihara *et al.*, 1987). It is not within the scope of this review to consider this possibility in detail but even if it is argued that such events do occur, it is universally agreed that it is probably, quantitatively speaking, of limited importance. With such slow rates of cell division it could be argued that any cytotoxic event leading to loss of adipocytes should result in relatively long-term reductions in adipose tissue mass. Indeed, a number of studies involving surgical removal of adipose tissue have suggested that the capacity to regenerate the lost tissue is extremely limited and in some cases no regeneration occurred over periods of several months (Faust, Johnson and Hirsch, 1977; Bailey and Anderson, 1980).

In an attempt to reduce body fat in rats, antibodies have been raised in sheep against plasma membranes purified from isolated rat adipocytes. Such antibodies were capable of causing lysis of rat adipocytes maintained in *in vitro* tissue cultures. When the experiments were conducted in the absence of a source of serum complement (part of the effector mechanisms involved in antibody-mediated killing of cells) the antibodies actually stimulated both the transport of glucose into adipocytes and its subsequent incorporation into lipid (Flint *et al.*, 1986). Both of these phenomena are classical responses to insulin, suggesting that the antibodies may have been acting through insulin receptors. However, this was shown not to be the case, since, when the insulin receptors were removed from adipocytes (using mild trypsinization), the response to insulin was lost whereas that of the antibodies remained. The mechanism of this effect is currently unclear but a number of other agents known to perturb membranes in general, including Zn^{2+} and H_2O_2 also mimic the effects of insulin in a receptor-independent fashion.

When these antibodies to adipocytes were injected into young growing rats they produced a number of effects, some of which were quite unexpected. Several days after injecting rats for four consecutive days with antibodies to adipocytes, destruction of adipocytes was apparent along with a marked cellular infiltration of the tissue by lymphocytes and polymorphonuclear

nucleocytes (Futter and Flint, 1987). Such cellular responses indicate an alternative form of cell destruction, that of antibody-dependent cell-mediated cytotoxicity. The relative importance of this, as opposed to a complement-mediated mechanism, has yet to be determined.

After the initial 4 days of treatment the rats were left completely untreated. After 1 week the major fat depot affected, the parametrial depot, of the female rat was reduced by 50% in weight, and this could be accounted for by a 50% reduction in the number of cells in the depot. By 8 weeks the weight of this depot was reduced by 75% with no evidence of a recovery of lost adipocytes. The reduction in weight was even greater since those adipocytes which were not lysed by antibodies were apparently incapable of storing triacylglycerol as efficiently as those in control rats. At 8 weeks post-treatment total body fat was reduced by 30% (Futter and Flint, 1987).

The antiserum used in these studies was raised against a number of fat depots in the rat, including internal and subcutaneous depots, and reacted strongly against all of them. Interestingly, however, site-specific effects of the antibodies could be induced simply by altering the route of administration, with subcutaneous injections affecting subcutaneous depots more dramatically than internal and vice versa. This does not, of course, preclude the possibility of producing site-specific antibodies.

Preliminary experiments also revealed that treatment could be limited to a single day with effects as dramatic as those produced by 4 days' treatment. They also revealed a potential limitation of this approach in that antibody treatment could not be repeated after a period of more than 7 days, since the administration of the xenogeneic antiserum (i.e. sheep antibodies into rats) led to the production of rat anti-sheep antibodies which neutralized the anti-adipocyte antibodies when they were administered subsequently.

However, longer-term studies were very encouraging, suggesting that effects of treatment could last for at least 6 months (Panton *et al.*, 1990). Unexpectedly, rather than becoming lighter than the control rats, treated animals actually became heavier. For a period of about 4 weeks, the rats ate 20% more than controls and gained 40% more body weight leading to an increase in food conversion efficiency (weight gained per unit of food eaten) of around 15%. Thus, treatment led to increased body weight 7 weeks after injection but a reduction in the amount of fat in several major depots. After 2 months, food intake and rate of body weight gain returned to normal, although the difference in body weight was maintained until the end of the experiment at 6 months. The major fat depot, the parametrial pad, was still reduced in size at this time although other depots showed small (compensatory?) increases in weight. Surprisingly, however, the decrease in dissectable fat was exactly matched by an increase in fat that was not dissectable, presumably present in microscopic depots and interspersed within other tissues. The total increase in body weight of the treated animals was thus explained purely by increased protein and its attendant water content.

A number of hypotheses have been advanced to explain the regulation of body fat mass, including regulation by adipocyte number, size or total fat mass. The results of this experiment provided no evidence for regulation of

either adipocyte size or number but clearly did suggest that total body fat content might be monitored. How such regulation occurs is unclear, but it has been suggested that adipocytes may secrete a substance that regulates food intake in a negative manner; the more adipose tissue, the more substance produced, the greater the depression of food intake. No evidence exists to support such a hypothesis, although adipose tissue was recently recognized to secrete a novel protein, adipsin, the function of which is currently unclear (Rosen *et al.*, 1989).

Long-term effects of antibodies to adipose tissue have also been described using local subcutaneous injections of antibodies into pigs (Futter and Flint, 1990) and reductions in fat deposition in sheep treated in similar fashion have also been reported (Moloney and Allen, 1988; Moloney, 1990).

Inevitably such successes in animal models begs the question 'What about humans?' In theory, antibodies to human adipocytes would function in identical manner, but at least two major problems exist. First, since the objective would be to treat obese humans, with extremely large amounts of adipose tissue triacylglycerol to remove, it would be extremely important to know that the systems involved in lipid clearance would be able to cope and not lead to triacylglycerol deposition within the arteries. Secondly, since effects in humans would need to last much longer than 6 months, retreatment might be essential. In order to conceive of such a possibility, human monoclonal antibodies would seem to be essential in order to prevent the anti-immunoglobulin response elicited by xenogeneic serum injections. It would thus seem unlikely that this approach would be useful in clinical situations. It is perhaps worthy of mention that this approach does, however, differ from almost all other approaches to regulate obesity in humans which generally involve either reduction of appetite or 'energy wasting' techniques. For example, increased heat production using thermogenic drugs and malabsorption approaches such as inhibiting intestinal lipase activity to reduce fat absorption. These approaches would clearly be of no value in animal production systems where increasing appetite and increased efficiency of food utilization are of paramount importance.

Immunological ablation of specific endocrine cells

Whereas a number of the attempts to modulate various functions of the endocrine system described thus far have used antibodies to neutralize hormones within the bloodstream, the possibility of using antibodies to destroy endocrine tissues is also a distinct possibility. Surgical or chemical ablation techniques have been used to exemplify the particular role of certain endocrine organs as well as to control clinical syndromes such as hyperactivity of the pituitary and thyroid gland. Using antibodies to destroy endocrine cells could follow the approach of tumour immunology, by attempting to identify specific antigens on the surface of the cell which can be used to target antibodies to them in a specific fashion. Since many cells share common antigens, such as histocompatibility antigens, this can be a difficult and time-consuming approach. An apparently simpler technique has been devel-

oped which benefits from the fortuitous observation that cells which secrete particular hormones also appear to express those same hormones on the cell surface. This finding has been exploited to immunopurify, for example, somatotrophs (the growth hormone secreting cells of the pituitary gland) using an antiserum to growth hormone (Venetikou *et al.*, 1989). When an antiserum to rat growth hormone was given *in vivo* it produced a long-term reduction in growth and body weight gain of rats. When such rats received growth hormone releasing factor their response, in terms of growth hormone secretion, was extremely poor (Flint and Gardner, 1989; Gardner and Flint, 1990). Pituitary glands from these animals had reduced contents of growth hormone, indicative of pituitary cell destruction.

A novel and interesting variation on this theme has been demonstrated by Schwartz *et al.* (1987). They reasoned that since corticotrophin (ACTH) producing cells in the pituitary respond to corticotrophin releasing factor (CRF) by means of CRF receptors on the cell surface, these receptors might be suitable vectors for cytotoxic drugs. They therefore developed a conjugate comprising CRF linked to the plant toxin, gelonin, and showed it to be capable of destruction of pituitary ACTH-producing cells, based on the inability of pituitary cell cultures to produce ACTH in response to a subsequent challenge with CRF. Similar results have been obtained using toxins conjugated to human placental lactogen (Chang and Neville, 1977) and human chorionic gonadotrophin (Oeltmann and Heath, 1979). A subsequent study by Schwartz and Vale (1988), however, revealed one of two potential limitations of this approach. When using the CRF–gelonin conjugate described above, they showed that ACTH responses to CRF were severely reduced whereas the ACTH response to vasopressin was unaltered. This suggests that a population of ACTH-producing cells exists which do not possess CRF receptors. Thus total ablation of particular hormones is dependent on the presence of receptors for the hormone toxin on all hormone-producing cells. A second limitation is that the receptor being used for targetting of the toxin should only be expressed on the cells producing the specific hormone to be neutralized. This is clearly not the case for thyrotrophin releasing hormone (TRF) receptors. Although TRF was initially discovered because of its stimulatory effects on thyrotrophin (TSH) production from the pituitary gland, it also has potent stimulatory effects on prolactin release from pituitary lactotroph cells. This would suggest that TRF–toxin conjugates would reduce prolactin secretion and this has, in fact, been shown to be the case (Reichlin, Bacha and Murphy, 1983).

It seems likely, therefore, that techniques involving immunodetection of the target hormone to be ablated, on the surface of the cells producing it, will be a much more specific manner of regulating particular endocrine events.

Active immunization against hormones has generally been deemed preferable over passive techniques, at least in part due to the fact that passively administered antibodies are generally produced in a different species to those in which they are used. These antibodies are recognized as 'foreign' and are themselves typically immunoneutralized, often within one week of administration (as was the case for sheep anti-adipocyte antibodies given to rats). In a

recent series of experiments (Madon, Panton and Flint, 1991), however, the potential of passive immunization was clearly demonstrated, if the anti-immunoglobulin response is prevented. A group of young male rats were treated with sheep anti-rat growth hormone antibodies, and growth rate in all animals was severely retarded for about 3 days. After this time the majority of the animals started to grow again at the same rate as control rats. A number of the animals did not grow, however, and body weight gain was virtually zero for approximately 3 weeks before they, too, resumed normal growth. Blood samples revealed that in the group that started to grow after 3 days the sheep anti-(rat growth hormone) antibodies which had been injected were virtually completely cleared by 7 days and these rats had a large rat-(anti-sheep immunoglobulin) response, indicating the expected neutralization of the passively administered antibodies. In the group in which growth was retarded for 3 weeks the sheep anti-(rat growth hormone) antibodies remained in the circulation in large amounts and these rats showed a very weak anti-(sheep immunoglobulin) response. Thus, in animals unable to clear heterologous antibodies, there exists the potential for long-term effects of passive immunization. Why a small group of animals did not produce the typical anti-immunoglobulin response is unclear, but a wealth of data supports the concept of a large genetic component in immune responsiveness and these individuals may have all had a particular genetic make-up. There are, however, other ways of inducing a state of refractoriness, or tolerance, to non-self antigens, including neonatal administration of large doses of the antigen in a 'tolerogenic' (aggregate-free) form or the use of immunosuppressive drugs such as cyclophosphamide.

This approach, using antibodies to neutralize hormones within the blood, has advantages over immunotoxins in that the effects are clearly reversible when antibody is slowly cleared, and also offers the advantage over active immunization techniques of a much more uniform response that can be carefully titrated, compared with the often variable responses to active immunization.

Modulation of the actions of hormone-binding proteins

Many steroid hormones, because of their poor solubility in aqueous media, are carried in the bloodstream bound to carrier proteins. Many peptide hormones, on the other hand, have been considered to circulate in an unbound form. Recent findings, however, suggest that two hormones intimately involved in growth processes, growth hormone and insulin-like growth factor I, are also bound to carrier proteins within serum. The vast majority of IGF-I in serum is bound to a binding protein with apparent molecular weight of 150 kDa. In addition, several smaller molecular weight binding proteins exist, and one in particular appears to be secreted by various cell types and inhibits IGF-I activity. Baumann *et al.* (1986) first described a binding protein for human growth hormone within serum and Leung *et al.* (1987) demonstrated that the extracellular portion of the growth hormone receptor appeared to be identical to the growth hormone binding protein in serum, a

proposal supported by studies of the human growth hormone receptor and binding protein involving the use of a panel of monoclonal antibodies (Barnard, Quirk and Waters, 1989). The function of this protein remains uncertain, although it appears to increase the half-life of injected growth hormone and may thus serve some buffering capacity.

Antibodies raised against such binding proteins might actually block their ability to bind their respective hormones and thereby leave more free (biologically active) hormone. In support of this notion Szabo *et al.* (1988) have described a truncated form of IGF-I lacking the three N-terminal amino acids. This form of IGF-I does not bind to the 35 kDa (inhibitory) binding protein and possesses a tenfold greater biological activity *in vitro*. Whether such enhanced effectiveness is evident *in vivo* remains to be determined. Other inhibitors of IGF-I activity have also been described in, for example, certain clinical situations including uraemia, diabetes and malnutrition (Phillips *et al.*, 1984; Taylor *et al.*, 1987). The physiological significance of these inhibitors in non-pathological conditions is unknown but clearly immunological intervention could be a useful approach to interrupt their influence on IGF-I action.

Negative regulators of growth

Although binding proteins for hormones may be considered as negative regulators of growth because of their ability to interfere with hormone action, a growing number of hormones themselves appear to exert negative regulatory influences on growth. Clearly, any such factor shown to be relevant to specific growth processes could be the target for immunosuppression, resulting in the removal of a braking influence on growth.

The concept of endogenous inhibitors of growth has been hampered by the inability to purify specific compounds which would reproduce some of the inhibitory activities demonstrated in conditioned culture media from various cell types. However, in recent times several inhibitors have been purified and have increased the credibility of studies in this field. Transforming growth factor-β (TGF-β) was first described in conditioned medium from Moloney murine sarcoma virus-transformed mouse cells (De Larco and Todaro, 1978). It has been purified from various tissues, including bone, platelets and human placenta. TGF-β inhibits terminal differentiation of rat myoblasts, chicken skeletal muscle cultures (Massagué *et al.*, 1986) and mouse myoblasts (Olson *et al.*, 1986). TGF-β also inhibits developmental processes of adipogenesis *in vitro* (Ignotz and Massagué, 1985). In the case of both myoblasts and pre-adipocytes, TGF-β is without effect when added to cells after commitment to differentiation and did not affect cell proliferation. In mouse calvaria TGF-β induced bone resorption *in vitro* (Tashijian *et al.*, 1985) and may therefore also be a local regulator of bone growth. Since skeletal fibre number is determined early in gestation (Campion *et al.*, 1981) and a significant portion of adipocytes are also established in the fetus (Desnoyers *et al.*, 1980) the action of TGF-β may be most prominent in the fetus. This

may represent something of a technical barrier if inhibition of TGF-β action were desired.

TGF-β is only one of a number of growth regulators secreted by platelets, although none of the others is particularly well characterized (reviewed by Campion and Jones, 1988). Likewise, there have been numerous studies on liver-derived inhibitors, which include some of the somatomedin inhibitors previously mentioned, although again they have been relatively poorly characterized to date (*see* Iype and McMahon, 1984).

This is a rapidly expanding field and although immunological intervention may still seem premature, it offers considerable prospects for the future.

Lactation

Bovine growth hormone, when injected into dairy cows, can increase milk yield typically by up to 30%. The mechanism of action of growth hormone in mediating these effects is by no means clear, since to date no evidence exists to demonstrate specific growth hormone receptors in the mammary gland. This suggests that the effect is indirect, and two possible hypotheses have been proposed. Mobilization of adipose tissue is a well-known phenomenon during the early stages of lactation when food intake does not increase to match the increased energy output in milk; this is the so-called 'repartitioning' effect of growth hormone. Growth hormone is known to be anti-lipogenic and lipolytic in adipose tissue and also enhances the responsiveness of adipose tissue to β-adrenergic compounds (reviewed by Vernon and Flint, 1989); this may increase milk yield by 'partitioning' nutrients from adipose tissue to the mammary gland. The possibility that growth hormone stimulates milk yield by increasing the production of IGF-I (the somatomedin hypothesis of Daughaday, 1981) is supported by reports that IGF-I can increase mammary growth (Baumrucker, 1986; McGrath and Collier, 1988; Shamay *et al.*, 1988) and stimulate milk production when infused into the mammary gland (Prosser *et al.*, 1988).

Clearly, many of the proposed approaches for enhancing growth hormone action or GH-like activity in meat-producing animals will be relevant to the stimulation of milk production. In particular, the enhancing of monoclonal antibodies to GH has been shown to increase the effectiveness of GH in stimulating milk yield (Pell *et al.*, 1989b).

Spencer and Garssen (1985) reported that immunization against somatostatin led to increased milk production during lactation in goats, although Deligeorgis *et al.* (1988) failed to find any alteration in milk production of sheep immunized in similar fashion. Once again the potential usefulness of this approach appears unclear.

TGF-β appears to have a role in mammary growth since its administration during development of the murine mammary gland inhibited mammary growth and morphogenesis (Silberstein and Daniels, 1987). A mammary growth inhibitor of around 13 kDa has also been partially purified from mammary cells (Bohmer *et al.*, 1985) and shown to inhibit Ehrlich ascites mammary carcinoma cells, whilst a local inhibitory factor has been described

in goat's milk which may act as an autocrine regulator of milk production, although it is currently difficult to envisage how such an intracellular protein could be the target for antibodies (Wilde and Peaker, 1990).

Interactions of the endocrine and immune systems

The recognition of the usefulness of the immune system as a tool to modulate endocrine functions has led to a growing interest by endocrinologists in the more fundamental aspects of immunology in order to refine particular approaches. This interdisciplinary exchange has, however, by no means been one-way, as immunologists recognize the important regulatory role of the endocrine system on immune functions. Until recently most interactions were attributed to glucocorticoid hormones but it is now recognized that a wide range of peptide hormones also modulate immune responses. ACTH has been shown to suppress immunoglobulin synthesis and stimulate B-cell proliferation (Alvarez-Mon, Kehrl and Fauci, 1985) whereas thyroid stimulating hormone (TSH) enhances immunoglobulin synthesis (Blalock *et al.*, 1985). Growth hormone can enhance production of cytotoxic T-cells (Snow, 1985) whereas human chorionic gonadotrophin (hCG) can suppress this response (Alanen and Lassila, 1982) as well as suppressing natural killer cell activity and mixed lymphocyte reactions (Ricketts and Jones, 1985), possibly by the generation of suppressor T-cells (Fuchs *et al.*, 1982). These effects of hCG may be an important part of the suppression of immune responses which is an essential component of successful pregnancy. Siiteri *et al.* (1982) have proposed that this effect of hCG may be an indirect one mediated via progesterone.

The fact that both growth hormone and somatostatin, which suppresses histamine release and T-cell proliferation (Payan and Goetzl, 1985), have immunomodulatory functions means that in attempting to regulate growth via manipulation of these hormones subtle changes in immune responsiveness may also be initiated, which clearly need to be given appropriate consideration.

Not only do classical hormones influence immune function but classical immune products, such as interleukins and interferons, act as hormones to modulate secretion of pituitary and hypothalamic hormones. For example, both interleukins-1 and -2 stimulate ACTH release (Brown, Smith and Blalock, 1987) whereas interleukin-1 stimulates GH release (McCann *et al.*, 1989). An additional degree of complication is added to these immune–endocrine interactions by the discovery that numerous cells of the immune system actually secrete hormones similar, or indeed identical, to those of pituitary and/or hypothalamic origin. Lymphocytes have been shown to produce ACTH (Blalock and Smith, 1980), GH and prolactin (Hiestand *et al.*, 1986), whereas leucocytes and mast cells have been shown to produce vasoactive intestinal peptide (VIP) and somatostatin (Lygren *et al.*, 1984). Production of both TSH and hCG by T-cells has also been reported (Harbour-McMenamin, Smith and Blalock, 1984).

In the case of ACTH, the molecule produced by lymphocytes appears to

have sequence identity with pituitary ACTH, whereas in the case of GH evidence for higher molecular weight forms in lymphocytes compared with pituitary GH exists. Similarly, leucocyte-derived somatostatin appears similar but not identical to the hypothalamic peptide (reviewed by Blalock, 1989).

Regulation of ACTH release from leucocytes could be stimulated by corticotrophin releasing factor (CRF) and inhibited by dexamethasone; B-cell derived GH release could be stimulated by GRF and inhibited by somatostatin; and leucocyte TSH release could be enhanced by thyrotrophin releasing factor (TRF). Thus these immune cells appear to respond to hypothalamic regulatory factors in a fashion broadly similar to that of their pituitary counterparts (Blalock, 1989).

This rapidly expanding field must inevitably require some of the rather simplistic hypotheses concerning regulation of the endocrine system by immunization to be scrutinized much more carefully so that their wider-ranging effects can be taken into account.

Summary

The ability to manipulate certain physiological processes by using the immune system, so as to regulate endocrine secretions and/or actions is clearly possible. The dramatic effects of immunocastration and the ability to increase ovulation rates in sheep are probably the best examples. Other approaches along similar lines have produced equivocal results, the effects of immunization against somatostatin being the most notable case.

Although anti-idiotypic antibody approaches to producing hormone mimics have also been shown to be attainable and, indeed, possibly involved in certain auto-immune dysfunctions of the endocrine system, to date no successful applications of this approach have been demonstrated in commercial livestock.

The ability to enhance hormone action using antibodies is an extremely promising area. Its prospects probably hinge on the ability to synthesize suitable short peptides which will mimic epitopes on the hormone and so permit the development of active immunization techniques to produce polyclonal antibodies of restricted and enhancing specificity. It seems less likely that administration of hormones pre-complexed to monoclonal antibodies has any potential as a practical approach to manipulating animal productivity.

All of these approaches involving active immunization suffer the same limitations: the highly variable response of individual animals and the general inability to regulate the duration of the response; a need to find suitable adjuvants to replace the almost universally used and commercially unacceptable Freund's adjuvant; and the problem of trying to generate what, in most cases, is an auto-immune response.

A second group of approaches consists of attempts to use antibodies in their classical role, that is by targetting antigens or cells for destruction by the immune system. These include, for example, antibodies directed against adipose tissue or cytotoxic antibodies to specific hormones aimed at destruc-

tion of the hormone-secreting cells. Since these are passive immunization techniques, the antibodies can be assessed carefully *in vitro* and administered in appropriate doses. However, success in these applications is largely dependent on the inability of damaged tissues to regenerate, since re-treatment is generally precluded because of the anti-immunoglobulin response induced in treated animals. Toleragenic forms of such antibodies or the use of appropriate immunosuppressants may ultimately remove this limitation.

Perhaps the greatest current limitation to the use of all of these techniques in animal production systems, however, is public resistance to the use of such techniques. The decision to ban the use of anabolic steroids in meat-producing animals contradicted the scientific evidence on safety and, with the current bad publicity that bovine growth hormone is receiving, any technique involving manipulation of the endocrine system will probably be viewed with considerable scepticism.

From a scientific standpoint it is clear that the interactions of the endocrine and immune system are likely to provide an additional tier of complexity to our attempts to achieve specific physiological changes.

Where such approaches stand in relation to the potentially powerful approach of transgenic animal techniques is difficult to forecast, since transgenics themselves have created considerable controversy both from an ethical standpoint as well as one of patentable property.

Irrespective of these current limitations, and notwithstanding their applied objectives, there is no doubt that these techniques will help to increase our fundamental understanding of the molecular basis of hormone action, auto-immunity and immune–endocrine interactions.

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