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SYMPOSIUM ON NUTRIENT–HORMONE INTERACTIONS IN ANIMAL PRODUCTION

Hormones and metabolism: a background

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This paper is not intended to refer directly to animal production nor by its nature can it aim to be comprehensive or detailed. Rather it is intended to provide a bird's eye view of current thinking in relation to the subject of hormones and metabolism. In doing so it is hoped to provide some perspective in which to place the subsequent papers in this Symposium which involve detailed studies on a number of different aspects of chemical transmission in relation to metabolism.

Chemical transmission: endocrine, neural and paracrine

Endocrine. Although some of the ideas of endocrinology were being considered in the nineteenth century, the term 'hormone' did not gain currency until it was used by Starling in 1905 (Starling, 1905) following the discovery of the gut hormone secretin (Bayliss & Starling, 1902). Furthermore, as the ideas of a role for hormones in metabolism could not progress until the concepts of metabolic energy had been formalized, the subject of the endocrine control of metabolism is mainly a development of the twentieth century.

At a meeting in Newcastle it is perhaps apt to recall that one of the earliest scientific investigations in this field emanated from this Medical School. At the request of a committee appointed by the Clinical Society (1883), Sir Victor Horsley engaged in experimental work which established that myxoedema and cretinism were due to a loss of thyroid function. This led Horsley to suggest that grafting a portion of sheep thyroid gland would be a rational method of treatment (Horsley, 1890). It had not yet been established that the thyroid gland produced an internal secretion but Professor G. R. Murray, Consulting Physician to the Infirmary at Newcastle and later to be the Medical School's first Professor of Comparative Pathology, decided to try the therapeutic effects of extracts from thyroid glands of

freshly killed sheep (Murray, 1891*a*) on a 46-year-old female who had become slow in speech and action and who had lost the energy to go about her daily work (Murray, 1891*b*). Treatment, which was at first given by subcutaneous injection, was subsequently given orally once it had been established that this also was effective (Fox, 1892; Mackenzie, 1892). On treatment, the patient remained free from myxoedema and died of cardiac failure at the age of 74 (Murray, 1920). It is perhaps salutary to note that a lack of success with this treatment reported in 1892 (Clarke, 1892) turned out to be the result of the butcher supplying thymus instead of thyroid gland (Murray, 1920).

From such beginnings evolved the concept, as we now know it, of chemical substances transmitted from one part of the organism to another to produce excitation or inhibition of target tissue. It soon became evident that these chemicals are varied in nature and include substances as diverse as steroids, monoamines, glycoproteins and peptides.

Neural. Also in the latter half of the nineteenth century the idea was mooted that chemical transmission was involved in the synaptic cleft between nerve terminals and between nerves and their post-synaptic target tissue. Proof of the existence of this mechanism again was not forthcoming until the early years of the twentieth century (Dale, 1953). The nervous system was initially considered an entirely separate system of communication developed during the course of evolution to permit more rapid and evanescent excitation and inhibition. Perhaps partly for this reason and perhaps also because of the electrical features associated with conduction in nervous tissue and the earlier strong support for the notion of electrical transmission at synapses, the investigations of the nervous and endocrine systems have tended, until recently, to proceed on largely independent lines. Although there is some justification for considering the endocrine and nervous systems separately it is important not to overlook the similarities and the frequent functional interrelationships of these two systems. The early studies of gastrointestinal function, for example, were marked by a difference in emphasis between the Russian Pavlovian school of nervous control and the English Bayliss and Starling school of chemical control. This polarization of views was probably responsible, at least in part, for the delay in the recognition that the vagus nerve will stimulate the acid-secreting parietal cells of the stomach not only directly (Pevsner & Grossman, 1955) but also indirectly by causing the release of gastrin (Lanciault *et al.* 1973) and that the hormonal stimulation of adipose-cell lipase is reduced by denervation of adipose tissue (Havel, 1964).

Undoubtedly the activation of the brain is influenced by hormones (de Wied, 1969; Koranyi *et al.* 1977) and experiments with radiolabelled hormones suggest that there may be selective binding by hormones to specific neurones in the central nervous system (Sar & Stumpf, 1973). The control of food and fluid intake, which is integrated within the central nervous system, appears to involve not only neurotransmitters such as noradrenaline, acetylcholine, serotonin and dopamine (Bray *et al.* 1981) but also several peptide hormones (Smith & Gibbs, 1979; Fitzsimons *et al.* 1982). It is in this connection interesting that a number of

peptides, found in the gastrointestinal tract endocrine cells, have been identified in central and peripheral neurones (Dockray, 1977; Polak & Bloom, 1979; Dockray *et al.* 1981).

Paracrine. The chemical communications systems of the body are not limited to the endocrine and nervous systems. There is at least a third category of chemical communication that involves transmission within the extracellular spaces between cells located at various distances apart within an organ or tissue. The chemical messengers in this instance are sometimes referred to as local hormones or autocoids and the mechanism is referred to as paracrine.

Reference has already been made to the interrelation between the nervous and endocrine systems in relation to the control of gastric acid secretion. The sensitivity of the gastric parietal cells to chemical transmitters of both these systems is significantly affected by the presence of histamine which is believed to reach the parietal cells from the nearby mast cells. Drugs which block histamine H_2-receptors significantly inhibit the acid-secretory responses to nervous and hormonal stimulation (Parsons, 1977; Hirschowitz & Gibson, 1978). It seems probable that prostaglandins also are a significant component of the paracrine system, capable of modifying nervous and hormonal activity.

Any one chemical messenger is not necessarily specific to one communications system. Catecholamines, for example, may function as neurotransmitters at nerve terminals or as hormones released into the circulation from the adrenal medulla. In recent years evidence has accumulated which suggests that certain peptides may function as transmitters in relation to the nervous, endocrine and paracrine systems.

Gut hormones and other hormones

Just as in the past too rigid a conceptual demarcation has been made between the nervous and endocrine systems, so may this be true between the gut hormones and other hormones of the endocrine system. In this connection there has been a recent revival of an earlier concept that there is duodenal hormonal control of pancreatic endocrine function (Moore *et al.* 1906).

Following oral administration of glucose, insulin concentrations in the circulation are greater than after its intravenous administration despite greater circulating glucose concentrations after intravenous glucose (McIntyre, 1965). The name incretin has been suggested for this hormone (La Barre, 1932). In recent years it has been established that many of the hormones or putative hormones isolated from the gastrointestinal mucosa will cause insulin release but it is questionable whether a physiological role can be ascribed to any of these. At the present time the most favoured is gastric inhibitory peptide (GIP) which has been shown to have an insulinotropic effect only in the presence of a raised blood sugar (for this reason the acronym GIP is sometimes used also to mean Glucose-dependent, Insulinotropic Peptide). It has been suggested that the physiological purpose of such a hormone may be to help ensure that potentially

harmful quantities of insulin are not released in the presence of insufficient substrate concentration (Andersen *et al.* 1978).

Receptors

The receptor hypothesis is currently assigned a central role in chemical transmission and this too has resulted in a convergence of the interests of investigators of the neural, endocrine and paracrine systems. The first real evidence in support of the concept of receptive substances on the surface of cells was also presented in the latter part of the nineteenth century. Interestingly, this evidence was provided both by Langley, working on the nervous system, and by Ehrlich, working on circulating chemicals. Twentieth-century technology has, however, facilitated a remarkable increase in knowledge about cell receptors (Open University, 1977).

Chemical transmitters are thought to bind to receptors, typically protein in nature, and in consequence to elicit a response characteristic of the target cell. It is now evident that receptors are present not only in the cell membranes but also in the cytoplasm and the nucleus. The binding of transmitter to receptor is believed to produce some conformational change in the receptor which in turn induces cellular messengers to modulate cellular activity with the production of the response characteristic of the target cell. There has been some progress towards the isolation and characterization of receptors in chemical and physico-chemical terms and it seems that chemical transmitters become attached to receptors by virtue of their structural and electronic properties. There is considerable specificity in the transmitter–receptor interaction which allows discriminative selection by target cells from an array of potential stimuli. Receptor specificity is, however, relative and a number of chemicals with sufficiently similar structural features may influence one receptor. Steroid hormones appear to bind to cytoplasmic receptors, thyroid hormones to nuclear receptors and, in general, peptides and neurotransmitters bind to receptors in the plasma membrane.

Cytoplasmic receptors. The binding of steroid hormones to these receptors produces transmitter–receptor complexes that bind to nuclear chromatin (Chan & O'Malley, 1978). These complexes then modulate the production of messenger RNA and therefore of protein synthesis by the cell. In the chick oviduct, for example, the production of mRNA for ovalbumin is increased by exposure to oestrogen (Chan & O'Malley, 1978).

Nuclear receptors. The binding of thyroid hormone appears to be in the nucleus itself (Oppenheimer *et al.* 1976) and the production of mRNA for growth hormone, for example, appears to be under the control of thyroid hormones (Seo *et al.* 1977).

Cell plasma membrane receptors. The transmitter–receptor complex in this instance may influence an intracellular messenger, sometimes referred to as a second messenger to differentiate it from the primary interaction of transmitter and receptor. One such cellular messenger is the cyclic nucleotide adenosine-3,5′-monophosphate (cAMP) which is produced from ATP after the transmitter–receptor complex activates a membrane-bound enzyme, adenylate

cyclase, whose active site lies inside the cell (Sutherland & Rall, 1957). The interaction between the receptor complex and adenylate cyclase involves other intermediaries such as a guanine nucleotide regulatory protein, ionic magnesium, and ionic calcium possibly acting through the protein calmodulin (Berridge, 1975; Cheung, 1980). It is possible that transmitter–receptor interaction may involve alternative membrane enzymes such as guanylate cyclase as chemical messengers, but the evidence for this is not so strong as it is for adenylate cyclase (Goldberg & Maddox, 1977). Ca ions undoubtedly play an important role in regulating cellular function and, although cAMP and calmodulin are involved in a highly integrated regulatory network, it seems that ionized Ca may also initially act independently as a chemical messenger (Gardner & Jensen, 1981).

Target cell sensitivity

The sensitivity of a target cell to a circulating hormone may be modified in the presence of other chemical transmitters produced by the endocrine, paracrine or nervous systems. There are undoubtedly considerable species differences in this respect. In sheep at least the water-retaining action of vasopressin and the sodium-retaining action of aldosterone appear to be decreased in the presence of excess cortisol and their activity can be restored by the administration of prolactin (Horrobin, Manku & Robertshaw, 1973; Horrobin, Manku & Burstyn, 1973). In rats and guinea-pigs, secretin or vasoactive intestinal peptide (which increase intracellular cAMP) in the presence of cholecystokinin–pancreozymin (CCK–PZ) (which increases intracellular Ca^{2+}) produce greater hydrolase secretion from the pancreatic acinar cell than either hormone acting alone (Gardner & Jackson, 1977; Robberecht *et al.* 1977).

Modification of the effect of one transmitter by the presence of another may, on occasion, be explained by the activation of more than one receptor complex. The potentiation of pancreatic enzyme secretion that follows simultaneous stimulation by CCK–PZ and secretin in herbivores is thought to be the result of the combined effects of the stimulation of cAMP and Ca^{2+}. It is probable that the same explanation applies to the potentiation observed between the actions of vasoactive intestinal peptide, found in the autonomic nerves to the pancreas (Polak *et al.* 1978), and CCK–PZ. It seems that in these circumstances the cAMP and Ca^{2+} cellular messengers are each responsive to a number of different classes of receptor (Gardner & Jensen, 1981). It is possible that the existence of multiple receptors linked to different cellular messengers results in varying sensitivity of target cells to transmitter substances depending upon the permutations of receptor activation.

However, the kinetics of transmitter–receptor interaction are complex and differ for different transmitters on the same receptor so that this fact too may modify the responsiveness of target cells. Also, the sensitivity of a target cell to chemical transmitters is influenced by the number of receptors which may live for hours or days. The regulation of protein synthesis affects receptor numbers on target cells (Speir *et al.* 1982) and there may be up- or down-regulation of receptor numbers in response to local alterations in hormones and neurotransmitters (Baxter & Funder,

Table 1. *Some of the effects observed in man following the injection of gastrin*

Organ	Effect
Oesophagus	Increase in lower oesophageal pressure
Stomach	Strong stimulation of acid
	Strong stimulation of intrinsic factor
	Weak stimulation of pepsin
	Stimulation of motility
Pancreas	Strong stimulation of enzymes
	Weak stimulation of water and bicarbonate
Liver	Weak stimulation of water and bicarbonate
Gall bladder	Weak stimulation of contraction
Intestine	Stimulation of tone and motility
Body of stomach, Small intestine, Pancreas	Stimulation of tissue growth

1979; Catt *et al.* 1979). It is evident that many hormones are able to regulate their own receptors (Catt & Dufau, 1977) and excessive insulin release will, for example, cause a reduction in insulin receptors and a decreased sensitivity to insulin (Olefsky, 1976).

Many tropic hormones possess trophic effects capable of stimulation of growth and development in their target tissues (Johnson, 1980) (Table 1). It has been suggested that the effects of growth-promoting hormones in target cells may be conveniently considered in two groups. One group relates to rapid changes in the rate of cellular actions not directly responsible for the promotion of growth, and these involve alterations such as those in cAMP, Ca^{2+} and energy production. A second group relates to the slower mechanisms of stimulation of RNA and protein synthesis and probably involves yet another type of receptor (Tata, 1976). These effects are, however, no doubt interrelated so that the earlier changes facilitate the later biosynthetic events which in turn influence receptor numbers.

Hormones not only may regulate the numbers of their own receptors but may also alter the numbers of other types of receptor. It is known, for example, that injection of corticosterone during the stage of maturation will cause a premature increase of gastrin receptors in the gastric mucosa of newborn rats (Peitach *et al.* 1981).

Studies in man indicate that metabolic disturbances may arise from the presence of abnormal receptors (Griffin & Wilson, 1977) or from the development of receptor antibodies (Mehdi & Kriss, 1978; Harrison *et al.* 1979).

Investigative problems

Although the interaction between transmitters of the endocrine, paracrine and nervous systems is important for the control of metabolism, there is no doubt that it is one of the factors that complicates the analysis of physiological function. Another difficulty relates to the problems of interpreting the earlier literature in which there is the tendency to ascribe to hormones actions that have been observed during the study of impure extracts of endocrine glands. With the advent

of the present high standards of chemical purification and synthesis it seemed that it should be readily possible to characterize the physiological functions of pure hormones. It soon became evident, however, that many pure hormones have multiple actions; for example, some of the many actions which can be ascribed to gastrin are listed in Table 1 (Nillson, 1980). Another example is the tetradecapeptide somatostatin which was originally isolated from ovine hypothalamus (Brazeau *et al.* 1973) and at first was thought to be specifically concerned with the suppression of the release of growth hormone and then of thyroid-stimulating hormone. Subsequently, this peptide was found in larger quantities in the gastrointestinal tract (Arimura *et al.* 1975; Polak *et al.* 1975) and was shown to inhibit the release of many other hormones such as insulin, glucagon and gastrin in addition to having a direct inhibitory effect on acid secretion by the parietal cells of the stomach (Gomez Pan *et al.* 1975). There is also evidence that somatostatin is released from nerves (Uvnas-Wallenstein *et al.* 1977; Iversen *et al.* 1978) and that in turn it may inhibit the release of adrenergic and cholinergic transmitter substances (Guillemin, 1976; Cohen *et al.* 1978).

When hormones are found experimentally to have multiple actions the question must arise as to whether these can be accounted for by some unifying hypothesis or whether many of the effects must be regarded as pharmacological rather than physiological. The generalized inhibitory effect of somatostatin could, for example, be explained if the mechanism of releasing autonomic nervous transmitter substances and for releasing hormones is similar and so susceptible to inhibition by the same substance or, if somatostatin inhibits hormone release, by its inhibitory effects on neurotransmission (Cohen *et al.* 1978). On the other hand it is difficult to explain in this way the different actions ascribed to a hormone like gastrin.

It seemed that if the very small concentrations of hormones in the circulation could be measured it should at least be possible to differentiate physiological from pharmacological effects. During the past 20 years the development of chromatographic and immunoassay techniques has revolutionized the measurement of hormones in the circulation. However, leaving aside the possibility of discrepancies between assay values and the biological activity of a material (Blair *et al.* 1982) it soon became evident that, as in the case of gastrin (Gregory, 1974; Yalow, 1975), many of the peptide hormones circulate in a number of different molecular forms some of which may be the result of post-translational processing (Blair *et al.* 1977). These molecular variants may be present in widely differing concentrations and have different potencies and different half-lives in the circulation. In order, therefore, to understand physiological function it is necessary to measure and evaluate the significance of different circulating molecular forms of hormones. The study of circulating hormones is further complicated by the fact that, quite apart from diurnal changes in their concentration, some hormones like growth hormone (Hunter *et al.* 1968) and secretin may be released into the circulation intermittently under physiological circumstances (Schaffalitzky de Muckadell & Fahrenkrug, 1978). In addition, hormones in the circulation may be bound in an inactive form.

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