0031-6997/79/3003-0247$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 30, No. 3
Printed in U.S.A.

Peptide Hormones and Their Analogues: Distribution, Clearance from the Circulation, and Inactivation in Vivo

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I. Scope and Introduction

THIS review concerns processes that are responsible for removing biologically active peptide hormones from the circulation, which thus terminate the signal that the hormone carried to its target cells. Mechanisms of clearance and inactivation are of interest physiologically because they are the means of terminating the hormonal message, therapeutically because they regulate concentrations of exogenous hormones and hence the duration and magnitude of their actions, and pharmacologically because they give information that may lead to the development of analogues with improved properties. In what follows, studies of the metabolism of endogenous hormones, of exogenous hormones at supraphysiological concentrations, and of peptide hormone analogues all will be considered.

Investigation of the metabolism of peptide hormones is complicated by a number of factors. The available assays suffer from limitations. Radioimmunoassays are usually not totally specific for the intact hormones and bioasays suffer from the same disadvantage; moreover, many of the degradation products, active or inactive, may escape detection. Use of radioactive tracer calls for careful preparation of the labelled hormone and extensive application of chromatographic techniques to separate the intact peptide and to characterize fragments. These methodological problems are the more serious because peptides are complex molecules capable of giving rise to many products. There are also difficulties in the selection of appropriate experimental animal models. Peptides do not readily cross cell membranes and the use of models in vitro, where cellular compartmentalization may be lost, is of limited interest unless the results can be related to observations in vivo. Experimentation is complicated in vivo by the rapidity and complexity of the processes involved, which can make it hard to decide, for instance, whether uptake into a tissue has occurred before degradation and, when degradation is extensive, where the primary sites of cleavage are.

Despite these difficulties a number of mechanisms of clearance and inactivation of peptides have been identified and the aim of this review is to assess the areas in which progress has been made and also to indicate some of the fundamental questions that remain unanswered.

A. Overview of Processes Known to Be Involved in Peptide Metabolism

Once in the circulation, peptides are subject to processes of distribution into tissues, which can be passive or involve active uptake, and also to processes of degradation.

Distribution into tissues appears in some cases to be a reversible phenomenon for which the mechanism has not been elucidated. However, in addition to reversible distribution, many peptide hormones are taken up by the kidneys by a process that is probably irreversible since it involves glomerular filtration followed by pinocytotic uptake by proximal tubule cells.

Degradation or inactivation of peptide hormones is usually considered to be carried out by peptidases. Although these enzymes are ubiquitous, the specificities of the enzymes at different sites in the body vary considerably. The degradation of a

peptide will therefore depend on which sites in the body it reaches. Thus, unless degradation occurs in the circulation or at the luminal surfaces of the vascular wall, the inactivation of a peptide hormone is linked to its distribution and transport. The assumption that degradation processes are proteolytic may be an oversimplification and one should observe caution in the interpretation of data from experiments that did not allow other mechanisms of inactivation to be observed.

B. Methods for the Assay, Characterization, and Identification of Peptides

Because peptide hormones are complex molecules normally present in small amounts, it is difficult to acquire absolutely specific information about their concentrations or identity. The following methods are most commonly applied.

1. Bioassay. Bioassays are useful for obtaining information about the concentrations of biological activity in samples. However, they do not give information about inactive products and may fail to indicate conversion of a peptide hormone to active metabolites. Bioassays can be very sensitive but sensitive, accurate, and practicable assays are not available for every peptide hormone.

2. Radioimmunoassay. Radioimmunoassays are usually sensitive and reproducible but, like bioassays, they lack specificity. Data obtained by radioimmunoassay can be seriously misleading since biologically inactive fragments may crossreact and be estimated as immunoreactive peptide hormone. Radioimmunoassays, therefore, even with careful characterization and validation, require cautious interpretation.

3. Isotopically labelled hormones. The use of labelled peptides has the advantage that quantitative assay is relatively simple and no confusion with endogenous peptides can arise. There are difficulties, however, in the preparation and choice of suitable labelled molecules and also in establishing whether the radioactivity in a sample represents the intact peptide or products of degradation. Ideally one should use a labelled molecule, essentially identical in physicochemical and biological properties to unlabelled peptide. This can be achieved by the use of ^{14}C or ^{3}H labelling, but such products are difficult to prepare. Labelling with radioactive iodine provides material of high specific activity but iodine-labelled peptides are chemically distinct peptide analogues and in addition, unless great care is taken, the peptide is likely to become chemically damaged during iodination.

4. Chromatography. All of the above assay methods can be rendered more specific and informative by combination with a chromatographic step. When isotopically labelled molecules are used it is essential to include some kind of separation procedure if any useful data are to be obtained, since otherwise one might be measuring end products of degradation.

5. Identification. Criteria for the identification of a peptide hormone or its metabolites depend on the circumstances of the study. For a natural hormone it is often taken to be sufficient to establish identical biological activity or immuno-crossreactivity and chromatographic behaviour. More rigorous identification can be carried out by isolation and amino acid analysis or sequencing. In practice this can require extensive purification of quantities of between 10 μg and 1 mg of peptide. An alternative procedure for identification is radiosequencing, which avoids the need for purification and can be carried out with small quantities of peptide. Radiosequencing, however, requires the preparation of peptides labelled in specific parts of the molecule and must be coupled to chromatographic procedures if complex mixtures of labelled products are present. The principle of the method is the determination of the number of sequential steps, each involving the removal of one N-terminal amino acid, that are required to release label from a known position in the peptide.

C. Biological Experimental Methods

In addition to difficulties in the assay of peptide hormones, studies of peptide hormone metabolism are complicated because it is rarely possible to observe separately simple processes of transport and degradation.

1. Blood and plasma in vitro. Provided care is taken to ensure that no activation of peptidases of the clotting mechanism occurs during collection, it may be assumed that studies of stability in blood or plasma will give reliable information. The experiments are simple, and mechanisms of inactivation can be elucidated. However, for many peptide hormones degradation in the blood is only a minor process in vivo; degradation in tissues is more important.

2. Tissue homogenates or slices. Although experiments in vitro may be easy to carry out, the results must be interpreted with caution. With homogenates the peptide hormone is exposed to enzymes that may never reach it in vivo and with tissue slices irrelevant enzymes may be released from cut surfaces or the peptide may have restricted access to capillary endothelial surfaces.

3. Isolated perfused organs and tissues. Studies with perfused systems can yield information about the capacity of a tissue to clear peptide hormones. When appropriate measurements are carried out they can indicate whether the peptide is accumulated or degraded in transit and give information on mechanisms of degradation. Results of perfusion studies cannot be applied quantitatively to metabolism in vivo. Furthermore, care should be taken to ensure that the perfused tissue is not damaged in such a way as to release peptidases into the perfusion medium.

4. Arteriovenous differences in vivo. This method can given quantitative information about the relative importance of various tissues in clearing a peptide hormone from the circulation. Clearance can result from tissue uptake, which may or may not be associated with degradation, or from degradation in transit through the capillary bed. Resolution of these possibilities can be accomplished by infusing the labelled peptide and by sampling tissues.

5. Blood or plasma kinetics in vivo. Although knowledge of the pharmacokinetics of peptides is important for an understanding of their biological actions, the interpretation of pharmacokinetic observations in terms of mechanisms of clearance is complex. Plateau blood concentrations reached after a period of constant infusion at a known rate give information on total body clearance under conditions where distribution has achieved an equilibrium or distribution and metabolism have reached a steady state. By studying total clearance at different infusion rates it is possible to establish whether clearance mechanisms depend on blood concentration. Another method of studying peptide hormone kinetics is to follow the decline of blood levels after intravenous injection or cessation of infusion or secretion. After a bolus injection it is usual to delay the first measurements for 2 to 3 min (to allow mixing in the circulation) and then to determine the blood concentrations at short intervals for the next 20 to 30 min. During this time the concentration may fall by several orders of magnitude and at the end may approach the limit of detection. Normally, the decline is approximately exponential and is therefore simply characterized by calculating a half-life. Although the half-life determined in this time interval is usually quoted as the half-life in a given species, for some hormones more careful investigation has revealed an early phase of more rapid decline and a late phase of slower decline. These findings and their interpretation in terms of distribution phenomena will be discussed later in greater detail.

6. Distribution studies in vivo. Investigation of the distribution and nature of radioactive peptide and its degradation products in plasma and tissues at various times after injection will give realistic information about the fate of the molecule in vivo. However, the interpretation of the

results in terms of simple mechanisms can be very difficult. The presence of a peptide fragment in a tissue does not indicate where or when it was formed. One must not conclude that only those tissues that accumulate label are involved in metabolism. For example, there are peptides that are extensively degraded in transit through the lungs. However, with a peptide for which it can be shown that fragments do not circulate over the period under consideration it is possible to interpret tissue uptake and degradation patterns in terms of specific roles played by the individual tissues.

7. Conclusions on methods of investigation. The most appropriate methods depend very much on the processes that are important for each individual peptide and generally it is necessary to compare the results of several experimental approaches to obtain insight into the mechanisms involved in the metabolism of a peptide hormone. There is also a danger that a given experimental approach may focus attention on certain specific aspects of the metabolism without revealing processes of major importance for the overall handling of the molecule.

II. Individual Hormones or Classes of Hormones

The sections on individual hormones or classes of hormones are organized as far as possible according to a set pattern in which topics are considered in the following order: introductory remarks about the properties of the hormone; stability in blood or plasma in vitro; plasma half-lives and decay curves; factors that affect plasma clearance such as tissue distribution, metabolism, and plasma protein binding; the role of specific organs as defined by tracer studies, measurements of arteriovenous difference, isolated organ studies, effect of exclusion of organs from the circulation on survival of the peptide hormone; mechanisms of enzymatic cleavage where data are available that are likely to be relevant to metabolism in vivo; and other information of interest, e.g., the behaviour of peptide analogues. Amino acid sequences of low molecular weight peptides are shown in Figure 1; for primary sequences of the larger hormones see Dayhoff (64). Since a number of reviews have been written on specific classes of peptide hormones and the total number of publications on peptide metabolism is very large, no attempt has been made to provide an exhaustive compilation of the literature.

A. Opiate Receptor Agonists

Two classes of potent opiate receptor agonists, the enkephalins and the endorphins, have recently been isolated from brain (134) and the anterior pituitary (37, 168, 169). One of the enkephalins and all the endorphins are homologous with sequences of the anterior pituitary peptide β-lipotrophin (β-LPH); residues 61–65, 61–76, and 61–91 correspond respectively to met-enkephalin, α-, and β- endorphin. It is likely that the endorphins are formed from β-LPH or a common precursor of β-LPH and corticotrophin (ACTH) (177), but the presence in the brain of a second enkephalin, leu-enkephalin, which is not homologous with β-LPH, makes the origin of enkephalins less certain.

Met- and leu-enkephalin are unstable in blood in vitro. The half-life is about two min and tyrosine is cleaved from the amino-terminus (119), which results in inactivation.

On incubation with brain, α- and β-endorphins have been reported to be more resistant to aminopeptidase attack than met-enkephalin (178) and it is likely that they are also more stable in plasma. It is probable that the half-lives of all these peptides are shorter in vivo than in vitro, but nothing is known about the clearance of the peptides or the importance of different tissues for inactivation.

Studies with the peptide opiate receptor agonists have, however, provided information on an interesting aspect of distribution: passage across the blood-brain barrier. Enkephalins and endorphins are active analgesics when administered intraventricularly (245), and β-endorphin is also active

OPIATE RECEPTOR AGONISTS

Met-enkephalin (porcine) TyrGlyGlyPheMet
Leu-enkephalin (porcine) TyrGlyGlyPheLeu
β-Endorphin (ovine) TyrGlyGlyPheMetThrSerGluLysSerGlnThrProLeuValThrLeuPheLysAsnAlaIleIleLysAsnAlaHisLysLysGlyGln

VASOACTIVE PEPTIDES

Bradykinin (bovine) ArgProProGlyPheSerProPheArg
Angiotensin II (bovine) AspArgValTyrValHisProPhe

NEUROHYPOPHYSIAL HORMONES

Oxytocin (multiple) CysTyrIleGlnAsnCysProLeuGlyNH_2

Arginine vasopressin or Antidiuretic hormone (ADH) (multiple) CysTyrPheGlnAsnCysProArgGlyNH_2

HYPOTHALAMIC HORMONES

Thyrotrophin-releasing hormone or Thyroliberin (TRH) (porcine and ovine) pGluHisProNH_2
Melanocyte-stimulating hormone-release-inhibiting hormone or Melanostatin (MRIH) (bovine) ProLeuGlyNH_2
Luteinizing hormone/Follicle stimulating hormone-releasing hormone or Gonadoliberin (LHRH) (porcine) pGluHisTrpSerTyrGlyLeuArgProGlyNH_2
Growth hormone-release-inhibiting hormone or Somatostatin (GHRIH) (ovine) AlaGlyCysLysAsnPhePheTrpLysThrPheThrSerCys

GASTROINTESTINAL HORMONES

Gastrin (human) pGluGlyProTrpLeuGluGluGluGluGluAlaTyrGlyTrpMetAspPheNH_2
Secretin (porcine) HisSerAspGlyThrPheThrSerGluLeuSerArgLeuArgAspSerAlaArgLeuGlnArgLeuLeuGlnGlyLeuValNH_2
Cholecystokinin (CCK) (porcine) LysAlaProSerGlyArgValSerMetIleLysAsnLeuGlnSerLeuAspProSerHisArgIleSerAspArgAspTyrMetGlyTrpMetAspPhe

ANTERIOR PITUITARY PEPTIDE HORMONES

α-Melanocyte-stimulating hormone or Melanotrophin (multiple) AcSerTyrSerMetGluHisPheArgTrpGlyLysProValNH_2
Adrenocorticotrophin or Corticotrophin (ACTH) (human) SerTyrSerMetGluHisPheArgTrpGlyLysProValGlyLysLysArgArgProValLysValTyrProAsnGlyAlaGluAspGluSerAlaGluAla-PheProLeuGluPhe

CALCIUM REGULATING HORMONES

Calcitonin (human) CysGlyAsnLeuSerThrCysMetLeuGlyThrTyrThrGlnAspPheAsnLysPheHisThrPheProGlnThrAlaIleGlyValGlyAlaProNH_2

Parathyroid hormone (PTH) (bovine) AlaValSerGluIleGlnPheMetHisAsnLeuGlyLysHisLeuSerSerMetGluArgValGluTrpLeuArgLysLysLeuGlnAspValHisAsnPhe^{34}....Gln^{84}

GLUCOSE REGULATING HORMONES

Glucagon (human) HisSerGlnGlyThrPheThrSerAspTyrSerLysTyrLeuAspSerArgArgAlaGlnAspPheValGlnTrpLeuMetAsnThr

GlyIleValGluGlnCysCysThrSerIleCysSerLeuTyrGlnLeuGluAsnTyrCysAsn

Insulin (human) PheValAsnGlnHisLeuCysGlySerHisLeuValGluAlaLeuTyrLeuValCysGlyGluArgGlyPhePheTyrThrProLysThr

when administered intravenously (292). Since β-endorphin is 31 residues long it is apparent that peptides of moderate size can cross the blood-brain barrier. The natural enkephalins are almost inactive when administered intravenously (42) but this does not necessarily indicate inability to cross the blood-brain barrier since enkephalins are more unstable than β-endorphin in blood and in brain preparations. In fact a stabilized analogue [D-Ala^2, MePhe^4, Met (O)^5-ol] enkephalin has been reported to have considerable analgesic activity after parenteral and even after oral administration (245).

Investigation of the stability of opiate peptides in brain homogenates and slices shows that amino-terminal cleavage can occur (178) and result in loss of activity. Analogues containing D-Ala^2 are more active (245). However, there is no direct information to indicate the mechanisms of inactivation that actually occur when peptide enters the brain from the blood stream or when endogenous peptides are released. Transmitter substances are often confined to restricted and specialized environments and it seems possible that the mechanisms of inactivation differ from those observed in brain preparations in vitro.

B. Vasoactive Peptides

Bradykinin and angiotensin are vasoactive peptides of nonendocrine origin. They are released rapidly from plasma proteins in response to various stimuli and as such are dissimilar to glandular hormones. Their metabolism is very rapid. The release and metabolic fate of bradykinin and of angiotensins I and II have been reviewed (300).

1. Bradykinin. The pharmacology of bradykinin and the other kinins, including their metabolic fate, has been extensively reviewed by Erdös (84, 85). Bradykinin, a nonapeptide, is the most studied of a family of peptides that are released by the action of proteolytic enzymes, the kallikreins, on circulating proteins, the kininogens. In addition to potent vasoactive properties, pharmacological effects of these peptides include increased capillary permeability, production of pain, and contraction of visceral smooth muscle. The potent effects of bradykinin make it undesirable that it persist other than at its site of production.

Bradykinin is rapidly inactivated in plasma by a carboxypeptidase (kininase I or carboxypeptidase N) that removes the carboxyl-terminal arginine residue (86) and by kininase II. Although the half-life in plasma in vitro is short [17 sec in the cat (91); 20 sec in man (252)], bradykinin is even more rapidly inactivated during passage through most tissue beds.

In the cat, 37% to 72% of the bradykinin administered was inactivated in a single passage through hindquarters, liver, and kidney but the pulmonary circulation was most efficient, inactivating 78% (91). Degradation of ^{14}C-labelled bradykinin has been studied in perfused lung (249). Radioactivity emerged in the form of small fragments indicative of extensive cleavage of Arg^1-Pro^2, Pro^3-Gly^4, Gly^4-Phe^5, Ser^6-Pro^7, and Pro^7-Phe^8 in a single passage. This degradation was probably caused in part by kininase II, which is present in rat lung and removes the N-terminal dipeptide, Phe^8-Arg^9 (318). This enzyme appears to be identical to angiotensin converting enzyme (276), a peptidase situated in the vascular endothelium of lung that facilitates extensive degradation of peptides in transit (251). Angiotensin-converting enzyme is also found in many other tissues (44) and is probably responsible for at least part of the rapid clearance of bradykinin observed in many tissue beds.

2. Angiotensins I and II. ANGIOTENSIN I. The pharmacology of angiotensin and its analogues has been reviewed by Regoli et al. (238). Angiotensin I, a decapeptide, is released from a plasma α_2-globulin, angiotensinogen, by the proteolytic enzyme renin. Interest in angiotensin I has mainly been concerned with its conversion, by removal of the carboxyl-terminal dipeptide His^9-Leu^{10}, to the biologically more potent angiotensin II, which has pressor properties and stimulates aldosterone secretion by the adrenal cortex.

Ng and Vane (213) showed that the major

site of conversion was the lung; kidney and hindquarters also inactivated large amounts of the hormone in a single passage. Ryan et al. (250) found that conversion by the lung of [^{14}C]-labelled angiotensin I takes place in transit without retardation of radioactivity. Converting enzyme is present in plasma membranes prepared from rat lung and has been immunochemically localized in the caveolae lining the capillary endothelia (251). Converting enzyme has been isolated and purified. The isolated enzyme appears to have a broad specificity for substrates; it cleaves carboxyl-terminal dipeptides provided there is a C-terminal carboxyl group and the bond to be cleaved does not involve an imino group (82). In the intact lung, however, the activity of the enzyme appears to be restricted to bradykinin inactivation and angiotensin conversion. Converting enzyme has been found in many other tissues (44) and indirect evidence suggests that substantial local conversion may take place in a number of extrapulmonary tissue beds (73–75, 154). It is likely that as a result of further metabolism only small amounts of angiotensin I will emerge from the tissues, as was found by Oparil et al. (216). Converting enzyme is also present in blood but in small amounts (213).

ANGIOTENSIN II. Hodge et al. (126) showed by bioassay that angiotensin II is inactivated by dog blood in vitro with a half-life of 100 to 200 sec. Angiotensin II is inactivated in plasma by an aminopeptidase (237). However, the blood itself plays little part in the inactivation of angiotensin II in vivo since biological activity is removed rapidly from the blood stream by the tissues (30, 31, 126), and the kidney, liver, and hindquarters but not the lung substantially inactivate angiotensin II in a single passage in vivo. The half-life of angiotensin II is of the order of one circulation time, which in the case of the dog is approximately 15 sec. The importance of the kidney, liver, and hindquarters in the degradation of angiotensin has been confirmed in experiments with isolated perfused organ preparations (17, 159, 160). In a single passage through the dog kidney 75% of [^{14}C-Ile^{5}]-angiotensin II is degraded but the label is not retarded (214). A major product was free isoleucine and in the same investigation replacement of L-Asp^{1} by D-Asp^{1} reduced the extent of metabolism of ^{131}I-labelled angiotensin II. This latter observation suggests that aminopeptidase attack is an important initial step in degradation.

Recently it has become apparent that removal of aminoterminal Asp from angiotensin II does not inactivate it but converts it to angiotensin III, a peptide with enhanced steroidogenic but reduced pressor properties (33, 45, 51). It has been suggested that angiotensin III may be an important mediator of aldosterone production in vivo (106). It has been reported to be a major component of angiotensin activity in rat and human blood (267, 268).

Summary. Bradykinin and angiotensin are extremely potent vasoactive agents. They are inactivated in blood but their inactivation or conversion in many tissues is even more rapid. The enzyme responsible for converting angiotensin I to angiotensin II is well characterized and appears also to be important for the inactivation of bradykinin in the lung and other tissues. Many of the major mechanisms for the inactivation of bradykinin and angiotensin in tissues, however, remain poorly defined.

C. Neurohypophysial Hormones

The neurohypophysial hormones, oxytocin and vasopressin, are stored in the posterior pituitary in association with the high molecular weight proteins, the neurophysins. Like bradykinin and angiotensin, these hormones have been the subject of intensive research since their isolation and structural elucidation in 1953. Although structurally related, they have different biological functions.

Arginine-vasopressin and its analogue [desamino-Cys^{1}, D-Arg^{8}]-vasopressin (DDAVP) are used in the treatment of diabetes insipidus. They have a direct antidiuretic effect on the kidney. Oxytocin has

weak vasopressor activity but is primarily concerned with stimulating contraction of the uterus during parturition and promoting milk ejection during suckling. Its main use clinically is to induce labour. The secretion and the metabolism of these two nonapeptides have been extensively reviewed (101, 123, 158). The structure of oxytocin is common to all species so far studied but two vasopressins have been isolated, Lys^8-vasopressin and Arg^8-vasopressin.

DECAY OF BLOOD PEPTIDE LEVELS IN VITRO AND IN VIVO. Both oxytocin and vasopressin are stable in normal plasma in vitro (1). In pregnant women "oxytocinase" activity appears in the plasma and inactivates oxytocin and vasopressin by cleavage of the pentapeptide ring between the cysteine and tyrosine residues (293). The low efficiency of this inactivation system makes it of doubtful physiological importance.

The half-life of oxytocin after intravenous injection is short in vivo. In the rat and man values of 1.7 min and 2.7 min, respectively, have been reported (103–105). The half-life of Arg^8-vasopressin is also short in vivo, but the value obtained has an unusual dependency on the dose administered. The half-life in the rat ranges from 0.85 min for a large dose (102) to 3.4 min for a low dose (61). Vasopressin, but not oxytocin, binds to a small extent to human plasma proteins (88).

ROLE OF TISSUES IN DECAY OF PEPTIDE LEVELS. The renal and hepatic circulations are the most important in the metabolism of both oxytocin and vasopressin since exclusion of the kidney and liver from the circulation of the rat prolongs the effective life of both hormones (102, 103). It has been suggested that during lactation the rat mammary gland may also be important in the inactivation of oxytocin after intravenous injection. Ginsburg and Smith (103) found that oxytocin bioactivity in the plasma continued to decrease despite the exclusion of the kidney and liver from the circulation. Neither vasopressin nor oxytocin are inactivated by the pulmonary circulation in single passage experiments in the rat (29).

After the intravenous injection of oxytocin, tritiated in the leucine or tyrosine residues, into the rat, radioactivity was concentrated mainly in the kidney and liver with smaller amounts found in skeletal muscle (11, 274). Walter and Shlank (314) found that when oxytocin, labelled with [^{14}C] in the glycine residue, was injected into the rat, the radioactivity was excreted in the urine in the form of glycinamide or the intact peptide. Similarly, Walter and Bowman (313) found that, when [^{14}C]-labelled oxytocin or vasopressin was added to the perfusate in an isolated perfused rat kidney preparation, radioactivity found in the urine was mainly in the form of glycinamide. Labelled glycinamide did not appear in the perfusate and radioactivity did not accumulate in the kidney. This shows that degradation occurs after glomerular filtration and that the products are not reabsorbed. With an isolated perfused rabbit kidney, Levi et al. (167) found that clearance was greatest when the concentration of arginine-vasopressin was small. However, Little et al. (170) found the converse to be true in the perfused rat liver. Since the liver is an important site of clearance, the latter observation may explain why the half-life of vasopressin is shorter in the rat at high doses.

IN VITRO STUDIES. Enzymic inactivation of arginine-vasopressin and oxytocin in rat kidney homogenates has been studied with the hormones labelled at Gly^9 (314). The results suggest that cleavage of Pro^7-Arg^8 is of major importance in the degradation of vasopressin whereas cleavage of Leu^8-Gly^9-NH_2 occurs most rapidly when oxytocin is degraded.

ANALOGUES OF THE NEUROHYPOPHYSIAL HORMONES. Many analogues of both oxytocin and vasopressin have been synthesized mainly in order to characterize the appropriate receptors [see Schwartz (260) for review]. Several analogues of the neurohypophysial hormones that are resistant to tissue peptidases in vitro have been synthesized (18). These peptidases are not nec-

essarily relevant in vivo but one in vitro-resistant analogue of arginine-vasopressin has turned out to be very successful clinically. This analogue, [desamino-Cys[1], D-Arg[8]]-vasopressin (DDAVP), was designed to be resistant to both aminopeptidase and trypsin-like activity (321). DDAVP is a remarkable drug in that it produces a prolonged antidiuretic effect on the kidneys of patients suffering from diabetes insipidus. This is a reflection of its metabolic stability and also, apparently, of increased receptor affinity (10, 80, 224).

The modifications in this analogue that have led to greater stability suggest that inactivation by aminopeptidases and by enzymes of the type proposed by Walter and Shlank (314) must occur in vivo at tissue sites that equilibrate with the circulation. In addition to being longer acting, DDAVP is almost completely free of pressor activity (302). Thus this analogue embodies a combination of metabolic stability and selective and enhanced biological activity.

Summary. Despite their stability in plasma in vitro both oxytocin and vasopressin have short half-lives in vivo. This is brought about by rapid inactivation by the renal and hepatic circulations. Little is known about the mechanisms of inactivation. In the lactating rat the mammary gland may also play a substantial role in lowering blood oxytocin levels. A study of the inactivation of arginine-vasopressin by tissue peptidases has led to the synthesis of a clinically useful analogue, [desamino-Cys[1], D-Arg[8]]-vasopressin, that is both metabolically stable and characterized by selective and enhanced biological activity.

D. Hypothalamic Hormones

The existence of hypothalamic hormones that stimulate or inhibit the release of anterior pituitary hormones has been known for many years. These low molecular weight peptides are secreted into blood vessels that pass from the hypothalamus to the anterior pituitary (121). It is only recently that some have been isolated and sequenced (93) and analogues synthesized (242). Since these hormones act locally for the most part, systemic studies may not be of physiological importance but are nevertheless relevant from experimental and therapeutic points of view.

1. Thyrotrophin-releasing hormone (TRH) or thyroliberin. TRH, a tripeptide that stimulates the release of the hormones thyrotrophin and prolactin, is used to test both thyroid and pituitary-hypothalamic function. Porcine and ovine TRH are identical. TRH is protected against classical exopeptidase attack by having a pyroglutamyl residue at its amino-terminus and an amidated carboxyl-terminus. Nevertheless, TRH is unstable in rat plasma in vitro (232), with a half-life of about 3 min. Inactivation is caused by the removal of the amide group from the carboxyl-terminus (207). The half-life of TRH in vivo in the rat after intravenous injection was estimated with tritiated or [^{14}C]-carbon labelled peptides to be 2.2 min (78, 233) or 6.5 min (253). The decay of total radioactivity in the blood was measured, and the fall in biologically active TRH is probably faster than these values suggest. The plasma half-life in man has been found to be 5.0 min by bioassay (166) and 5.3 min by radioimmunoassay (20).

Dupont et al. (78) found that [^{3}H]-TRH rapidly left the circulation and the deamidated form rapidly appeared. In several studies large amounts of apparently intact TRH (20, 166, 253), immunoreactive TRH (83), and deamidated TRH (78, 233, 253) were found in the urine.

Despite protection against carboxypeptidases, TRH is unstable in plasma in vitro and has a short half-life in vivo in the rat and man. A major route of inactivation in vitro and in vivo is deamidation but it is not clear whether this occurs entirely in the plasma or whether tissue beds are involved.

2. Melanocyte-stimulating hormone-release-inhibiting hormone (MRIH) or melanostatin. A tripeptide that inhibits the release of MSH from the frog pituitary has been isolated from porcine hypothalami. It is identical in structure to the carboxyl-

terminus of oxytocin. As in TRH and LHRH, the carboxyl-terminus of MRIH is amidated.

Nair et al. (206) found, through the use of radiolabelled peptide and a bioassay, that MRIH is inactivated by human plasma in vitro; the main mechanism of inactivation was removal of the proline residue from the amino-terminus. Some deamidation of the carboxyl-terminus was also found. The rate of inactivation was not studied.

The half-life of disappearance of radioactivity after the intravenous injection of tritiated and [^{14}C]-labelled MRIH is approximately 9 min in the rat (231) and 1.9 min in man (230). A slow component of decay with a half-life of 15.2 min was observed in man but this may reflect the clearance of radiolabelled peptide fragments and not intact peptide. The distribution of tritiated MRIH in the mouse and rat has been studied by direct measurement of tissue radioactivity and by whole body autoradiography (79, 231). After intravenous injection, radioactivity was concentrated in the pineal, pituitary, kidney, liver, and fat. Considerable amounts of intact MRIH are excreted in the urine by man after intravenous injection (230) but the rat excretes very little intact peptide (231).

At present it is not clear what processes are responsible for the short half-life of MRIH in vivo.

3. Luteinizing hormone/follicle-stimulating hormone-releasing hormone or luliberin (LHRH or folliberin (FSHRH)). LHRH is a decapeptide that stimulates the release of both luteinizing hormone and follicle-stimulating hormone from the anterior pituitary. Like TRH this hormone is apparently protected against exopeptidase degradation by the pyroglutamyl residue at its amino-terminus and the amidated carboxyl-terminus.

DECAY OF BLOOD LHRH CONCENTRATIONS IN VITRO AND IN VIVO. LHRH has been shown by bioassay to be only slowly degraded by rat, guinea-pig, chicken, dog, sheep, and human plasma (256) and to be almost completely stable in human plasma and whole blood (306) upon incubation in vitro. Since LHRH has a short half-life of a few minutes in vivo (table 1), it follows that the blood itself plays little part in reducing circulating levels of this peptide after injection. Table 1 lists the half-lives in vivo of synthetic LHRH in the rat, dog, pig, and man after bolus intravenous injection. Initially, the blood peptide levels fall very rapidly and, in man, in the first 2 min, about 80% of the dose leaves the circulation (139). The half-life values appear to be shorter for man than for the other species and indicate that in man LHRH is very rapidly inactivated. Recently, Pimstone et al. (219) found that by radioimmunoassay LHRH has a

TABLE 1

Estimates of in vivo half-lives of LHRH in the rat, dog, pig, and man

Species of Animal	Method of Assay	Half-lives (min)		Reference
		Initial	Final	
Rat	^{125}Iodine-labelled hormone	5–10	150–600	202
Rat	Tritium-labelled hormone	6.8		234
Dog	Bioassay	5.0		257
Pig	Bioassay	12.0		257
Man	Tritium-labelled hormone	2–4	30–60	202
Man	Tritium-labelled hormone	4.0	57.0	229
Man	Radioimmunoassay	3.6	19.3	148
Man	Radioimmunoassay	5.3	27.4	139
Man	Radioimmunoassay	3.6		8
Man	Radioimmunoassay	2.7	~30*	306
Man	Bioassay	2.9		306
Man	Bioassay	2.0	10.0	269

* Authors' estimate.

somewhat longer half-life of 5.5 to 9 min in man after intravenous infusion. Bolus injection may yield shorter values because of rapid distribution outside the vascular compartment. Observation of the decay of LHRH by use of isotopically labelled peptide or radioimmunoassay may produce misleading half-life values because neither method distinguishes between biologically active hormone and inactive fragments. In man the initial plasma half-life value for LHRH is, in general, lower by bioassay than by the other methods. This is also noticeable when decay rates at later times are considered. As with some other peptides, notably ACTH and calcitonin, a second much slower component of decay follows the initial rapid drop in blood hormone levels. Half-life values for this secondary decline in blood levels in man vary from 30 to 60 min when tritium-labelled hormone or radioimmunoassay is used, but by bioassay the value is only 10 min. Virkkunen et al. (306) concluded that the longer half-life by radioimmunoassay was mainly because of the slower elimination from the circulation of immunologically active, biologically inactive LHRH fragments. The second phase of decline in blood bioactive hormone levels could be caused by peptide binding to plasma protein or to peripheral tissues. The latter mechanism is more likely because, according to Virkkunen et al. (306), LHRH is not bound to plasma protein.

ROLE OF TISSUES IN DECAY OF BLOOD LHRH CONCENTRATIONS. Studies of the distribution of [^{3}H]-LHRH after intravenous injection into the rat have shown that the kidney and to a lesser extent the liver concentrate most of the radioactivity (234). Very little intact LHRH was found to be excreted in the urine of rats (bioassay) or man (radioimmunoassay), which confirms the ability of the kidney to accumulate or degrade the peptide (139, 234). Redding et al. (229) studied the nature of the radioactivity in human urine after the injection of LHRH labelled with tritium in the amino-terminal pyroglutamyl residue. They concluded that radioactivity was mainly excreted in the form of pyroglutamic acid and the dipeptide pyroglutamyl-histidine, which suggests that LHRH is probably inactivated, among other mechanisms, by the cleavage of these components from the amino-terminus. Because the label was present only at the amino-terminus these findings could indicate only part of the breakdown pattern.

IN VITRO STUDIES. Sandow et al. (255) have shown by bioassay that LHRH is rapidly inactivated by dog and pig kidney and liver homogenates. Muscle had little inactivating capacity. Hudson et al. (133) employed radiosequencing to show that LHRH labelled with [^{3}H] in trytrophan and [^{14}C] in proline is cleaved mainly at Tyr^5-Gly^6 in rat liver homogenates. Rat hypothalamic and brain homogenates inactivate LHRH in vitro (109, 150, 180). However, peptidases in these organs cannot play a role in limiting blood LHRH concentrations after intravenous injection since only a small fraction of the circulation passes through these tissues. In addition there is no evidence that endogenous hormones released from the hypothalamus are inactivated before they reach their target tissue, the pituitary.

SUMMARY. LHRH, despite structural characteristics that protect it against exopeptidases, has a short half-life in vivo, especially in man, but little is known about the processes responsible for this.

4. Growth hormone-release-inhibiting hormone (GHRIH) or somatostatin. Somatostatin, a tetradecapeptide first isolated and identified as hypothalamic growth hormone-release-inhibiting factor, was subsequently found also to inhibit release of glucagon, insulin, and gastrin. Since somatostatin-like immunoreactivity has been detected in many extrahypothalamic sites including pancreas and stomach (9), it seems likely that somatostatin is a locally secreted inhibitory factor and as such might be expected to be inactivated rapidly. Recent evidence suggests that somatostatin may also be released as a circulating hormone that plays a role in glucose homeostasis by

regulating glucose absorption from the intestine (294).

Cyclic somatostatin is rapidly converted to [des-Ala[1]]-somatostatin in heparinized rat plasma and whole blood in vitro [T½, 0.5–1 min (195)]. This is the only product formed after 2 min, and since it is probably biologically active (297) it is unlikely that rapid inactivation of somatostatin occurs in the blood. Brazeau et al. (38) estimated that the half-life of circulating somatostatin in vivo was certainly less than 10 min in the rat and McMartin and Purdon (195) found that 1 min after injection only about 5% of the dose was present in the circulation as somatostatin; 20% was present as [des-Ala[1]]somatostatin.

The linear or reduced form of somatostatin is fully active in vivo (297). In plasma in vitro the reduced form apparently ring-closes since it rapidly converts to a product that resembles cyclic somatostatin (195). After intravenous injection linear somatostatin is totally cleared from the circulation in 1 min but presumably sufficient cyclized somatostatin reaches the appropriate tissue receptors before clearance is complete.

Extracts of rat brain tissue degrade somatostatin very slowly in vitro (179–181). [D-Trp[8]]-somatostatin is slightly more resistant to brain peptidases. This analogue has been reported to be 8 times more potent than somatostatin itself both in vivo and in vitro (243). It is not clear whether this is because of increased metabolic stability in the circulation, more efficient activation of the appropriate receptor through increased binding, resistance to peptidase attack, or a combination of these factors.

Like the other hypothalamic hormones, somatostatin has a short half-life in vivo. [des-Ala[1]]-somatostatin is rapidly formed from the parent molecule in the rat in vivo and in rat blood in vitro. It seems likely that endogenous somatostatin acts locally as the intact molecule but that the biologically active circulating form is [des-Ala[1]]-somatostatin. The biological properties of exogenous somatostatin observed in vivo may well reflect the activity of the [des-Ala[1]]-form, which might differ in some respects from intact somatostatin. The role of various tissues in clearing and inactivating these peptides is not known.

E. Anterior Pituitary Peptide Hormones

The anterior pituitary and the pars intermedia contain several protein and glycoprotein hormones and a series of structurally related peptide hormones, namely adrenocorticotrophin, α- and β-melanocyte stimulating hormones, and the so-called lipotrophins (261). Of these peptide hormones, information is available only for α-MSH and ACTH, which will be discussed here; protein and glycoprotein hormones will be considered in a later section.

1. α-Melanocyte-stimulating hormone (α-MSH). α-MSH is a peptide 13 amino acids in length. In primitive vertebrates it causes melanophore expansion and thus produces skin darkening. Its function, if any, in mammals is not known. This peptide is protected from aminopeptidase attack by an N-acetyl group at the amino-terminus and from carboxypeptidase attack by amidation of the carboxyl-terminus.

α-MSH has been found to be stable in vitro in dog serum for up to an hour (271). However, it has been found by bioassay to have a half-life in vivo, after intravenous injection, of 1.6 min (97). In the frog it has been suggested that α-MSH has an unusually long half-life of 2 h (107). This may be a reflection of its biological function in this species.

In the rat, Dupont et al. (77) showed by whole body autoradiography and direct measurement that high levels of radioactivity are found in the pineal, kidney, thyroid, stomach, and oesophagus after intravenous injection of [^{131}I]-labelled α-MSH. Shizume and Irie (271) used a bioassay to study the fall in blood levels of α-MSH after intravenous injection in intact and hepatectomized dogs. They concluded that the liver was a major site of inactivation of α-MSH. They did not determine how much accumulation by the liver contributed to the disappearance of the peptide as it passed through the

liver. Waring and Kirk (316) and Shizume and Irie (271) have shown that liver, kidney, and muscle homogenates can inactivate α-MSH.

Despite protection at the amino- and carboxyl-termini against exopeptidase attack, α-MSH has a short half-life in vivo in dogs and the liver appears to be a major organ for clearance. The role of other tissues has not been determined.

2. Adrenocorticotrophin (ACTH). This peptide hormone is 39 amino acids in length and its main function is to stimulate the adrenal cortex to secrete corticosteroids. Preparations of ACTH and its analogue Synacthen (corticotrophin-(1–24)-tetracosapeptide) are used clinically to treat rheumatoid arthritis and asthma and to test adrenal function. Biological potency is substantially reduced in sequences containing less than the first 18 amino acids (196). Loss of any of the residues from the amino-terminus leads to biological inactivation.

DECAY OF BLOOD CONCENTRATIONS OF ACTH IN VITRO AND IN VIVO. Some early reports indicated that ACTH preparations are unstable in blood in vitro (see Meakin, et al., (197)). However, more recently both Matsuyama (188) and Besser et al. (26) have found, by bioassay and radioimmunoassay, that human ACTH is relatively stable in fresh heparinized human plasma. These authors emphasize how important it is to use fresh blood or plasma samples in experiments on stability. Several other workers have demonstrated by bioassay that ACTH preparations are stable in vitro in heparinized blood and plasma (136, 239, 288). Synthetic human ACTH, corticotrophin-(1–24)-tetracosapeptide and [D-Ser^{1}, Lys^{17,18}]-corticotrophin-(1–18)-octadecapeptide amide have long half-lives in rat blood in vitro by bioassay (194).

Numerous reports have shown that the circulating half-life of ACTH in vivo is short and it is evident that degradation by the blood cannot contribute substantially to this. Table 2 shows the half-lives determined for both endogenous and exogenous ACTH in the rat, dog, sheep, pig, and man. They indicate that in general ACTH has a short half-life in vivo. The values obtained for physiological concentrations of endogenous ACTH are similar to those obtained for exogenous ACTH. In the examples cited, the exogenously applied hormone gave rise to levels of peptide that were much higher than those found under phys-

TABLE 2

Estimates of in vivo half-lives of ACTH in the rat, dog, sheep, pig, and man

ACTH Preparation	Species of Animal	Method of Injection	Method of Assay	Half-life (min)	Reference
Endogenous rat ACTH	Rat		Bioassay	1.0	288
Endogenous rat ACTH	Rat		Bioassay	1.7	190
			Radioimmunoassay	3.6	190
Exogenous natural human ACTH	Rat	Bolus	Bioassay	2.7	190
			Radioimmunoassay	4.1	190
Synthetic human ACTH	Rat	Infusion	Bioassay	2.3*	196
Endogenous canine ACTH	Dog		Bioassay	3.5	87
Exogenous porcine ACTH	Sheep	Bolus	Immunoprecipitation	1.0	142
Endogenous porcine ACTH	Pig		Radioimmunoassay	7.0	205
Exogenous synthetic porcine ACTH (radiolabelled with [^{131}I])	Pig	Bolus	Immunoprecipitation	13.0	205
			Radioimmunoassay	5.0*	205
Endogenous human ACTH	Man		Bioassay	10.4	236
Endogenous human ACTH	Man		Bioassay	6.75–7.0	62
Endogenous human ACTH	Man		Radioimmunoassay	10.0–15.0	319
Exogenous natural porcine ACTH; human ACTH	Man	Bolus	Bioassay	7.6–18.2	26
			Radioimmunoassay	11.0–24.6	26
Synthetic porcine ACTH	Man	Infusion	Bioassay	4.9–10.0	26
			Radioimmunoassay	17.1–28.6	26

* Authors' estimates.

iological conditions. The similar half-lives indicate that high and low levels of circulating peptide are metabolized in a similar manner. There is a trend towards longer half-lives in larger mammals, which probably reflects slower circulation times as well as slower metabolism. Radioimmunoassay gives higher values than bioassay, which indicates relatively slow destruction of immunologically active fragments that are biologically inactive. Clearance mechanisms in the dog are not saturated during intravenous infusion. The metabolic clearance rate for porcine ACTH in the dog has been found to be independent of the rate of infusion (58).

Although after injection, infusion, or the cessation of pituitary secretion the initial drop in plasma corticotrophin levels is rapid, several workers have found that if sampling is continued at later times a second slower phase of decay is observed. Everson (87) showed that after hypophysectomy, corticotrophic activity in the circulation of the dog fell with an initial half-life of 3.2 min followed by a slow decay with a half-life of 45 min. After injection of cortisol into a normal human subject, blood corticotrophin levels fell with an initial half-life of 10.4 min followed by a slow phase with a half-life of about 90 min (236). The latter study employed a sensitive cytochemical bioassay and the slow decay curve was manifested at blood levels well below normal values.

Biphasic decay curves based on radioimmunoassays have also been reported (26, 190, 205), but are less conclusive since the second phase might represent circulating fragments that are immunologically reactive but biologically inactive.

Bioassay of blood concentrations of corticotrophic activity in rats after the infusion of corticotrophin-(1–24)-tetracosapeptide, human 1–39 corticotrophin, or [D-Ser^{1}, Lys^{17,18}]-corticotrophin-(1–18)-octadecapeptide amide confirmed the presence of biphasic decay curves in each case (194). In the first 20 min, after infusion had been stopped, the levels declined with respective half-lives of approximately 1.3, 2.5, and 4 min, and by this time the concentrations of the three peptides differed substantially. However, at later times the rates of decay of all three peptides became much slower in each case, with an identical half-life of 20 min. These findings explain differences between the potencies of the three peptides observed in vivo and in vitro (196). The slow phase of decline is of interest from a pharmacological point of view since it helps to explain the prolonged steroidogenic properties of the octadecapeptide amide observed in vivo. As would be predicted, prolonged steroidogenic effects are also found with the tetracosapeptide when sufficiently large doses are administered (172). These prolonged effects are not caused by overloading of degradative processes since the biphasic property is not affected by infusion rates (194) and is also found at very low corticotrophin concentrations (236). Persistence of bound corticotrophin at the adrenal receptor site may also contribute to the prolonged steroidogenic effect.

The differences in biological properties of the tetracosapeptide and natural corticotrophin suggest that the 25–39 carboxyl-terminal portion has a protective function. The greater stability of the natural hormone in vivo does not, however, lead to significantly increased potency in vivo, because its potency at the receptor (determined with isolated adrenal cells) is approximately 7 times less than that of the tetracosapeptide (196). The observed similarity of potencies of the two peptides in vivo is therefore not an indication that the carboxyl-terminal portion is without function but is a result of a similar inhibitory influence of this part of the molecule on its interaction with inactivating processes in vivo and its interaction with the receptor.

ROLE OF TISSUES. Corticotrophin-(1–24)-tetracosapeptide is not taken up or degraded in a single passage through the lungs in vitro or in vivo (130). This finding, based on direct evidence, indicates that the earlier suggestion of degradation in the lung is

incorrect (194) and it seems unlikely that the lung would play any role in clearing endogenous corticotrophic activity since the 1–24 sequence comprises the active part of the molecule. Meakin et al. (197) and Everson (87) showed by bioassay that clearances of corticotrophin in a single passage through liver, kidney, adrenals, and hindquarters must be small. Since corticotrophic activity is undoubtedly cleared from the circulation these results could indicate small but significant amounts of clearance in many major tissues; for example, 20% total clearance per circulation with a 15-sec circulation time would give a half-life of about 1 min.

Distribution studies confirm the involvement of many tissues. Richards and Sayers (239) found that 5 min after injection, 20% of the administered biological activity was in the kidney. After injection of radioiodinated corticotrophin, radioactivity appeared in many tissues including liver, skin and muscle (50, 189, 278). However, it is not clear whether label was taken up before or after degradation and, in addition, the iodinated hormone may differ from the natural one in its properties.

Investigations of the distribution and degradation of corticotrophin, (1–24)-tetracosapeptide, and [D-Ser^1, Lys^{17,18}]-corticotrophin-(1–18)-octadecapeptide amide, specifically labelled with tritium, have provided information about the handling of these analogues in the rat. One minute after intravenous administration, 70% to 75% of the dose had left the circulation (16, 22). At this time after injection of the tetracosapeptide, circulating radioactivity was almost entirely in the form of intact peptide and a small but variable amount of the Met^5-sulphoxide (131). Thus cleavage does not occur in transit through tissues and uptake of label into organs must represent clearance of intact peptide. Although kidney and liver had the highest concentrations at 1 min, muscle and skin, by virtue of their large mass, contained the greatest amount (40%) of the injected dose. In muscle and skin the tetracosapeptide was extensively degraded while the octadecapeptide amide was stable in comparison; 0.5% compared to 20% of the radioactivity was in the form of intact peptide (22). Degradation in liver and kidney was about 70% for both peptides. The difference in degradation in muscle and skin could explain the higher plasma levels found with the octadecapeptide amide (194). Intact peptide persisted in these tissues for long periods (22) and it is possible that stored peptide can return to the circulation thus maintaining plasma concentrations. Evidence that peptides can return to the circulation from muscle and skin is provided by the observation that labelled peptide fragments found in muscle and skin at 1 min, but not in other tissues, are similar to fragments that appear in the circulation at 2 min and later times (132). Some of the circulating fragments are large, e.g., (3–24) corticotrophin. Identification of these fragments indicates cleavage after Ser^1, Tyr^2, Phe^7, Lys^{15}, Lys^{16}, Arg^{17}, Pro^{19}, Val^{20}, Lys^{21} with major attack at the amino- and carboxyl-termini (21, 131).

The role of the kidney appears to be quite different from that of muscle and skin. Autoradiographic studies (15) show that rapid uptake occurs by pinocytosis from the brush border of the proximal tubules adjacent to the glomerulus. The peptide is presumably filtered and sequestered too rapidly for the brush border enzymes to be able to degrade it because at 1 min the peptide in the kidney is mainly intact (132). Peptide is then transferred to the lysosomes where it is likely that extensive degradation occurs before any material is released. Confirmation of this suggestion is provided by results that suggest that the octadecapeptide amide is extensively degraded to [D-Ser^1-Tyr^2] within renal lysosomes in vivo (22).

OTHER ADRENOCORTICOTROPHIN ANALOGUES. Comparison of the potencies in vivo and in vitro (in a bioassay free of peptide inactivation) of corticotrophin fragments confirms the sensitivity of the amino-terminus to primary attack (196).

Investigation of a highly modified (4–9) corticotrophin analogue with activity on behaviour, [Met^4 (O^2), D-Lys^8, Phe^9]-corticotrophin-(4–9) hexapeptide, has shown that it can be absorbed from the gastrointestinal tract and cross the blood-brain barrier (303). The molecule was specifically labelled with tritium. Products in brain and the circulation 1 min after intravenous injection showed that 50% of the circulating peptide had been degraded and that cleavage of the His^6-Phe^7 and Phe^7-D-Lys^8 bonds had taken place. The second cleavage is unusual and demonstrates that an adjacent D-residue does not necessarily completely stabilize a peptide bond.

SUMMARY. Although stable in blood in vitro the initial circulating half-lives of corticotrophins are short. At later times a slow phase of decline can be observed. These characteristics and certain pharmacological features of corticotrophin analogues, i.e., potency and duration of action, are consistent with a model for distribution and metabolism that has emerged from studies with two corticotrophin analogues. According to this model the handling of these peptides can be understood in terms of two very different types of metabolic process that occur in muscle and skin on the one hand and in kidney on the other. Corticotrophins are extensively degraded by aminopeptidases on entering muscle and skin but a portion survives and is stored at sites where it resists further attack. It seems likely that this peptide can return to the circulation. Amino-terminal protection (replacement of L-Ser^1, by D-Ser^1) increases the quantity of peptide that survives the initial attack and by allowing larger amounts of peptide to return to the circulation enhances the potency and duration of action of analogues. While degradation, storage, and release are taking place in muscle and skin, circulating peptide and peptide fragments are continuously filtered from blood and taken up by proximal tubules of the kidney. This material is not extensively degraded initially but is transferred to lysosomes where it is metabolized to amino acids or very small fragments that can then return to the circulation.

F. Gastrointestinal Hormones

The stomach and intestinal tract are major endocrine organs. The three best established gastrointestinal hormones, gastrin, cholecystokinin, and secretin, will be discussed here. Several other intestinal peptides have been isolated but their physiological roles are as yet not clear. The subject of the gut as an endocrine organ has been reviewed recently (110, 141).

1. Gastrin. Gastrin is secreted into the blood stream by the antrum of the stomach after feeding; it is also found in the duodenum. Its long-recognized functions are to stimulate gastric hydrochloric acid and pepsin secretion. It also stimulates calcitonin release from the C cells of the thyroid gland. Gastrin has been shown to exist in at least five different molecular weight forms. Three of these, consisting of 13 (G-13), 17 (G-17), and 34 amino acid residues (G-34), have been isolated. They are structurally related by possession of a common carboxyl-terminal sequence of which the carboxyl group itself is amidated. Each form in turn has been found to circulate with or without a sulphate-ester group on the tyrosine residue in position 12. The sulphated and nonsulphated forms are found in equimolar quantities and apparently share identical biological properties. At least two higher molecular weight plasma gastrin components have been observed. G-17 and G-34 are regarded as the principal circulating forms of gastrin. The predominant form in the stomach antrum is G-17 whilst in plasma it is thought that G-34 or a G-34-like material is the major form. The carboxyl-terminal tetrapeptide of the gastrins possesses full intrinsic biological activity. The synthetic carboxyl-terminal pentapeptide analogue, pentagastrin, is used to test gastric acid secretion and as a provocative test for calcitonin in the diagnosis of medullary thyroid carcinoma. The physiology and metabolism of gastrin have been reviewed recently (228, 309–311).

DECAY OF BLOOD PEPTIDE CONCENTRATIONS IN VITRO AND IN VIVO. By bioassay, gastrin preparations were not degraded in serum and plasma in vitro. Blair et al. (32) showed that gastrin (G-17) and pentagastrin were stable in cat plasma. Table 3 shows the circulation half-lives in vivo of pentagastrin, minigastrin (G-13), gastrin (G-17), big gastrin (G-34), and big, big gastrin (high molecular weight form) in various species after intravenous injection or infusion. Since most of endogenous circulating gastrin exists in the plasma in two distinct forms (G-17 and G-34) the half-lives of exogenous peptides listed in table 3 are difficult to equate fully with the decay of endogenous gastrin activity in vivo. However, it is clear that in the dog at least, G-17 has a plasma half-life that is about 5 times shorter than that of G-34. In addition, Walsh et al. (312) have shown that the metabolic clearance rate of G-17 is 5 times faster than that of G-34 in the dog. This may explain in part why the main form of gastrin in the plasma is G-34 whilst in the stomach antrum it is mainly G-17. The half-life of endogenous gastrin activity is a reflection of the decay of both G-17 and G-34 (305). The progression from pentagastrin to the higher molecular weight gastrins is accompanied, as shown in table 3, by a concomitant increase in half-life values.

ROLE OF TISSUES IN DECAY OF BLOOD PEPTIDE CONCENTRATIONS. Measurement of arteriovenous differences of gastrin biological activity across the dog kidney has shown that this organ reduces the levels of both endogenous and exogenous gastrin (G-17) by 30% to 40% in a single passage (53, 63, 125). After the intravenous injection of synthetic human gastrin (G-17) radiolabelled with [^{125}I] into dogs and rats, large amounts of radioactivity were found in the kidney (204, 212). This suggests that the kidney may reduce blood gastrin by sequestration of the peptide, probably in the renal cortex. Since very little intact endogenous or exogenous gastrin is excreted in the urine of dogs (53, 63) it seems likely that the kidney both sequesters and degrades gastrin or its fragments. The importance of the kidney in gastrin metabolism is emphasized

TABLE 3

Estimates of in vivo half-lives of the gastrins in the cat, dog, and man

Gastrin Preparation	Species of Animal	Method of Injection	Method of Assay	Half-life (min)	Reference
Synthetic pentagastrin	Cat	Bolus	Bioassay	1.50	32
Natural human gastrin (G-13)	Dog	Infusion	Radioimmunoassay	1.75	65
Synthetic human gastrin (G-17)	Cat	Bolus	Bioassay	2.65	32
Synthetic human gastrin (G-17)	Dog	Infusion	Radioimmunoassay	1.3	235
Synthetic human gastrin (G-17)	Dog	Infusion	Radioimmunoassay	3.7	258
Synthetic human gastrin (G-17)	Dog	Infusion	Radioimmunoassay	2.5–2.8	54
Natural human gastrin (G-17)	Dog	Infusion	Radioimmunoassay	2.9	312
Natural porcine gastrin (G-17)	Dog	Infusion	Radioimmunoassay	3.1	312
Synthetic human gastrin (radiolabelled with [^{125}I]) (G-17)	Dog	Bolus	Immunoprecipitation	10.3	212
Natural porcine gastrin (G-17)	Dog	Bolus	Radioimmunoassay and gel filtration chromatography	3.0	287
Synthetic human gastrin (G-17)	Man		Radioimmunoassay	8.0	98
Synthetic human gastrin (G-17)	Man	Infusion	Radioimmunoassay	7.5	259
Natural human gastrin (G-34)	Dog	Infusion	Radioimmunoassay	15.0	312
Natural human gastrin (radiolabelled with iodine) (G-34)	Dog	Bolus	Gel filtration	9.0	287
Natural human big, big gastrin (radiolabelled with iodine)	Dog	Bolus	Gel filtration	90	287
Endogenous human gastrin	Man		Radioimmunoassay	~7	318

by the finding that the circulating life of synthetic human gastrin (G-17) is prolonged in nephrectomized dogs and rats compared with intact animals (54, 214) and that in some patients with chronic renal failure elevated plasma gastrin levels are observed (153).

The study of the arteriovenous gastrin difference across the dog small intestine during infusion of synthetic human gastrin (G-17) has shown that this organ considerably reduces blood hormone concentrations (289). In the same study a similar technique showed that the liver did not degrade gastrin itself but showed considerable ability to inactivate pentagastrin. The hepatic degradation of pentagastrin has been confirmed (198).

Korman et al. (153) observed that the isolated perfused lung from dogs and sheep inactivated synthetic human gastrin (G-17) and suggested that the pulmonary circulation was a major route of inactivation of this hormone. However, in similar experiments Dent et al. (71) were unable to confirm this result. Since gastrin released from the stomach antrum in vivo must negotiate the pulmonary circulation before reaching its site of action, the lung seems an inappropriate site of gastrin inactivation. Whilst investigating whether or not the isolated perfused rat lung inactivated the adrenocorticotrophin analogue, Synacthen, Hudson (130) showed that the perfusate itself was capable of rapidly degrading the peptide and care had to be taken to avoid artifactual results. Inactivation of gastrin in the perfusion medium might also explain the results of Korman et al. (153).

IN VITRO STUDIES. The in vitro inactivation of penta- and tetragastrin by homogenates of liver and kidney tissue from various species has been investigated (157, 191, 262). Destruction of biological activity was found to be brought about by removal of the carboxyl-terminal amide group although other peptidase activity was also observed. Seelig et al. (262) found that synthetic human gastrin was also inactivated by amidase activity in rat kidney and liver homogenates but that the process was very much slower than the inactivation of pentagastrin.

2. *Cholecystokinin* (CCK). CCK is found in the duodenal mucosa. It has been isolated in two molecular weight forms, one with 33 amino acid residues and the other with 39. They have been found to be equipotent. The structure of only the porcine hormone has been elucidated and natural porcine CCK is used in most studies. The carboxyl-termini of both forms are amidated. The principal physiological functions of CCK are to stimulate the secretion of pancreatic enzymes into the duodenum and to cause contraction of the gall bladder. CCK has also been found to inhibit emptying of the stomach and to augment the stimulation of bicarbonate secretion from the pancreas by secretin. The five carboxyl-terminal amino acid residues are identical to those of the gastrin molecule. The carboxyl-terminal heptapeptide sequence is the minimum required for biological activity.

In vitro, CCK has been found by bioassay to be stable in whole dog blood for up to 30 min (165). Measured by radioimmunoassay the half-life in vivo of disappearance of CCK from the circulation in man was 2.4 min after cessation of an intravenous infusion (22.7); that of endogenous CCK in man was 5 to 7 min (122); the half-life of CCK in dogs after intravenous injection was approximately 2.5 min (291) and 1.8 min by bioassay (164).

Lehnert et al. (165) and Thompson et al. (291) concluded that the short half-life of CCK in vivo primarily reflected either inactivation or sequestration of the hormone by the kidney. They found that the loss in biological or immunological activity in the blood owing to the kidney was of the order of 50% to 60% in a single passage. Lehnert et al. (165) also observed that the liver does not inactivate CCK in a single passage.

3. *Secretin.* Like CCK, secretin is found in the duodenal mucosa. There is apparently only one form of secretin. It consists of 28 amino acid residues and no fragments

have been found to possess biological activity. Only the structure of the porcine hormone is known and the natural preparation is used in most studies. Secretin is related structurally to glucagon and two other peptides of the intestinal mucosa, gastric inhibitory peptide (GIP) and vasoactive intestinal peptide (VIP). Like gastrin and CCK, the carboxyl-terminus of secretin is amidated and therefore it is protected against carboxypeptidase attack. Secretin stimulates the release of water and bicarbonate from the biliary tract and pancreas and has been found to augment the stimulation of the secretion of pancreatic enzymes by CCK.

By bioassay, secretin is stable for up to 30 min in vitro in whole blood from dogs (165). The circulating half-life of secretin in vivo in dogs after intravenous injection is 3.2 min, again by bioassay (165). As with gastrin and CCK, secretin blood concentrations are drastically reduced by the kidney. Lehnert et al. (165) found that in the dog renal degradation or uptake was responsible for reducing blood concentrations of biologically active peptide by about 75% in a single passage. No secretin activity was found in the urine during such experiments. Nephrectomy significantly prolonged the half-life of secretin, which confirms the importance of the kidney in the metabolism of secretin.

Summary. The in vivo half-lives of gastrin, cholecystokinin, and secretin in the species studied are short despite their stability in plasma in vitro. This is due to a large degree to efficient renal destruction of these hormones. To judge by the behaviour of other peptide hormones the reduction of blood hormone concentrations results in part from uptake by the proximal tubule cells of the renal cortex. However, this process follows glomerular filtration, which can account for only about 20% of the dose in a single passage and some of the observed clearances are appreciably higher than this. Thus a second mechanism of clearance must be operating but the results do not indicate whether this involves sequestration or degradation in transit.

G. Calcium Regulating Hormones of the Thyroid and Parathyroid

1. Calcitonin. This peptide hormone is 32 amino acids in length and is secreted by the parafollicular cells (C cells) of the thyroid. It lowers blood calcium levels by inhibiting outflow of calcium from bone. Synthetic human and salmon calcitonin preparations are used clinically to treat Paget's disease of bone, in which bone turnover is abnormally high.

DECAY OF BLOOD PEPTIDE CONCENTRATIONS IN VITRO AND IN VIVO. Estimates of the half-life of synthetic human calcitonin in vitro range from approximately 2 h in human and rat plasma measured by bioassay, to 30 to 43 h in human plasma by radioimmunoassay (7, 116). For natural porcine calcitonin the estimates range from 1 to 3 h in human and rat plasma by bioassay (211) and in the dog, pig, and human plasma by radioimmunoassay (116, 249, 317). Synthetic salmon calcitonin has been found to be almost completely stable in plasma in vitro; it has been found to have a half-life of approximately 48 h in dog plasma (116) and of over 30 h in human plasma (117). It is clear that salmon calcitonin is much more stable than either the porcine or human hormones in plasma in vitro; however, the inactivation in every case is too slow to account to any great extent for the short plasma half-lives observed in vivo.

Table 4 shows the half-lives determined in vivo for natural and synthetic preparations of salmon, chicken, porcine, and human calcitonin in the rat, dog, pig, and man. After bolus injection or cessation of infusion, human and porcine calcitonin concentrations fall rapidly. However, salmon and chicken calcitonin concentrations decline much more slowly, which suggests greater stability of these molecules. Results from different methods give similar values. This indicates that any biologically inactive frag-

TABLE 4

Estimates of in vivo half-lives of calcitonin preparations in the rat, dog, pig, and man

Calcitonin Preparation	Species of Animal	Method of Injection	Method of Assay	Half-lives (min)		Reference
				Initial	Final	
Natural porcine, salmon, and chicken calcitonins	Rat	Bolus	Bioassay	2 (porcine)		211
				10–15 (salmon)		211
				7 (chicken)		211
Natural porcine calcitonin	Dog	Infusion	Radioimmunoassay	2.2	150	116
Natural and synthetic salmon calcitonin	Dog	Infusion	Radioimmunoassay	21.0	115	116
Synthetic human calcitonin	Dog	Bolus	Radioimmunoassay	3	60	52
Synthetic human calcitonin (radiolabelled with [^{131}I])	Dog	Bolus	TCA precipitation	3	60	52
Natural porcine calcitonin (radiolabelled with [^{131}I])	Pig	Bolus	Chromatoelectrophoresis	5	45	317
Natural porcine calcitonin	Pig	Infusion	Bioassay	2–3	33–37	317
Synthetic human calcitonin (radiolabelled with [^{131}I])	Man	Bolus	Chromatoelectrophoresis	2.8	317.8	7
Synthetic porcine calcitonin	Man	Bolus	Radioimmunoassay	2.4	40.9	249

ments that are in the circulation must also have lost their immuno-crossreactivity.

In one of the studies (317) there was evidence for an even more rapid very early phase of distribution of calcitonin in the pig. In the first 1 to 2 min, 80% to 90% of the dose appeared to have left the circulation. This loss must to some extent represent reversible distribution because otherwise the calcitonin concentrations would continue to fall with a half-life of less than 1 min.

Evidence presented so far indicates that salmon calcitonin is much more stable than the porcine or human hormones in vivo. This has been considered to be the explanation for its greater hypocalcaemic activity (211). However, salmon calcitonin has also been found to have greater affinity for isolated rat kidney membranes and calvaria than the human or porcine hormones (187). Thus increased binding at its site of action may also account for the enhanced biological properties of salmon calcitonin. This is the view of Ardaillou et al. (6), who found no evidence that salmon calcitonin had greater metabolic stability than the human hormone in man. They found by use of chromatoelectrophoresis that [^{125}I]-labelled salmon calcitonin was cleared from the circulation faster than [^{131}I]-labelled human calcitonin. In view of the convincing evidence that salmon calcitonin is resistant to peptidase cleavage in many systems in vivo and in vitro, it may be that iodination significantly altered the properties of the molecule. Salmon calcitonin therefore appears to owe its enhanced biological activity to a combination of stability in vivo and high affinity for the appropriate target tissue receptors.

In most studies cited in table 4, between 20 min and 1 h after intravenous injection or infusion of calcitonins into various species, a slow phase of decline in plasma hormone concentration levels begins with half-lives ranging from 41 min to 318 min. In all cases but one, radioimmunoassay was employed and it may be that immunologically active but biologically inactive fragments are cleared from the circulation more slowly than the intact hormone. However, Habener et al. (118) found no evidence of circulating immunoreactive porcine or human calcitonin fragments in the dog by gel filtration chromatography and concluded that the levels of calcitonin fell slowly in the dog because of the binding of hormone to plasma proteins. Evidence for binding of calcitonin to plasma proteins has also been reported by others (163, 199). However, the efficient clearance of calcitonins by the kid-

ney, probably by glomerular filtration (see below), suggests that strong binding to plasma proteins does not take place. The binding of hormone to peripheral tissue beds has been suggested as an explanation for the slow phase of decline of plasma levels observed for ACTH (194), and this possibility cannot be ruled out for calcitonin. Whatever the explanation, it is interesting to note that Habener et al. (116) observed similar half-lives for both salmon and porcine calcitonins during this slow phase of decay in the dog, which indicates similar efficiency of protein or tissue binding in each case.

ROLE OF TISSUES IN DECAY OF BLOOD CALCITONIN CONCENTRATIONS. Salmon, porcine, and human calcitonins differ markedly in their tissue clearances after intravenous injection. Singer et al. (272) and Clark et al. (52), by cannulating the appropriate blood vessels, were able to measure the arteriovenous differences across various organs by radioimmunoassay during the infusion of salmon, porcine, and human calcitonins into the dog. Their results, summarized in table 5, provide direct evidence for the relative stability of salmon calcitonin to degradation, which has already been suggested by its prolonged plasma half-life. Only the kidney significantly reduces the blood levels of salmon calcitonin, probably by filtration at the glomerulus and concentration of peptide by the kidney cortex as has been shown for radioiodine-labelled human calcitonin, by extraction (52), and by whole body autoradiography (124). The data in table 5 indicate that all three calcitonins share a common fate in the kidney. The pulmonary circulation plays little part in reducing blood salmon or porcine calcitonin levels whilst the hindquarters and liver show considerable ability to degrade or sequester porcine calcitonin. These clearance studies do not differentiate between degradation and sequestration.

The tissue distribution of radioiodine-labelled calcitonins in the rat after intravenous injection (68, 69) shows that the liver and kidney reduce blood levels by accumulating calcitonin. The findings are in agreement with the results in table 5. Only the injection of [^{131}I]-labelled porcine calcitonin produced high concentrations of radioactivity in the liver whilst the radiolabelled human and salmon hormones gave rise to high levels in the kidney. De Luise et al. (68) found surprisingly low concentrations of radioactivity in the kidney after the intravenous injection of [^{131}I]-labelled porcine calcitonin. This may be because of the very rapid clearance by the liver and rapid degradation elsewhere in the body. The conclusions drawn from this work, concerning the relative affinities of the various calcitonins for the liver, have been confirmed in vitro by Greenberg et al. (108), who used an isolated perfused pig liver preparation.

Indirect evidence has confirmed the importance of the kidney in the metabolism of human calcitonin. Ardaillou et al. (5) showed that in patients with renal failure the half-lives of human calcitonin were considerably longer than those found in normal subjects. The mean metabolic clearance rate was 5 times higher in the normal subjects. Recently, Ardaillou et al. (4) observed high plasma concentrations of endogenous immunoreactive calcitonin in patients with acute renal failure. Clark et al. (52) showed that the disappearance of human calcitonin from the circulation of nephrectomized dogs was slower than from intact animals.

IN VITRO STUDIES. De Luise et al. (68, 69) have shown that rat kidney, liver, spleen, and muscle homogenates show consider-

TABLE 5

Arteriovenous differences in radioimmunoassayable calcitonin concentrations across organs in the dog

Calcitonin	Lung	Kidney	Liver	Muscle and/or Bone (Hindquarter)	Reference
Salmon	−3%	−17%	−3%	−2%	272
Porcine	−1%	−26%	−22%	−32%	272
Human		−30%	−8%		52

able ability to degrade [^{131}I]-labelled porcine and human calcitonins in vitro. Similarly dog kidney homogenate degrades human calcitonin (52) and extracts of rat kidney and liver degrade porcine calcitonin (211). However, salmon calcitonin was remarkably resistant to all rat tissue homogenates and extracts except those of the kidney. Although homogenization of a tissue releases intracellular peptidases that these peptides might not encounter in vivo, these experiments do confirm that salmon calcitonin is considerably more stable metabolically than either the human or porcine hormones. De Luise et al. (68) took their experiments in vitro a step further and employed subcellular fractionation to determine which component of rat kidney and liver was responsible for inactivation of their [^{131}I]-labelled calcitonins. They found that all three calcitonins were degraded by the kidney microsomal fraction whilst the porcine and human forms were also degraded by a soluble liver enzyme or enzymes.

Although stability of calcitonin preparations in tissues peripheral to the target organ determines to a large degree their blood concentrations and hence their relative potencies, the behaviour of the hormone at the appropriate target tissue must also be considered. As has previously been discussed, salmon calcitonin has greater affinity for receptors in rat renal membranes and calvaria than the porcine or human hormones (187). Recently, Hsu and Haymovits (129) have shown that isolated rat renal cells, but not bone cells, readily and apparently specifically degrade various calcitonins. The part the target tissues play in determining the potency of calcitonins in vivo is not fully known.

CALCITONIN ANALOGUES. Considerable effort has gone into the synthesis of calcitonin analogues. Much of the work has been aimed at determining which sequences of amino acids in the salmon calcitonin molecule confer on this hormone its high hypocalcaemic potency (173–176).

SUMMARY. Salmon calcitonin is known to possess greater hypocalcaemic potency than either human or porcine calcitonins. This greater potency can be explained in part by its metabolic stability, which is reflected in a long plasma half-life. Salmon calcitonin is more stable when incubated with tissue homogenates in vitro. Increased target tissue receptor affinity may also contribute to the greater potency. After intravenous injection all three calcitonins are sequestered and degraded by the kidney although the salmon hormone is resistant to degradation by the other tissues. Porcine calcitonin is very efficiently concentrated by the liver. It is probable that peripheral tissues such as muscle and skin also inactivate human and porcine calcitonins and thus contribute to the short half-lives of these peptides in vivo.

2. Parathyroid hormone (PTH). PTH contains 84 amino acids and is largely responsible for calcium homeostasis. It is secreted by the parathyroid gland in response to lowered blood calcium concentrations. It has direct effects on bone and kidney, and an indirect effect on intestine. Two precursors of PTH have been isolated and they are extended at the amino-terminus by 6 and 25 residues (146). The extra residues are cleaved within the cell during transport and storage in preparation for secretion (112). Full biological activity is retained in the synthetic amino-terminal fragment, 1–34 bovine PTH. Removal of residues from the amino-terminus, but not the carboxyl-terminus, of PTH leads to complete biological inactivation (222).

HALF-LIFE IN BLOOD IN VITRO AND IN VIVO. Results for PTH have been obtained by use of [^{125}I]-labelled hormone and radioimmunoassay. As will become clear in the section on circulating fragments of PTH, radioimmunoassays often detect biologically inactive carboxyl-terminal fragments of the molecule and data obtained by radioimmunoassay must be interpreted accordingly. [^{125}I]-PTH appears to be stable in ox, human, dog, chicken, and rat serum in vitro (113, 210). Although these results are less conclusive than findings for plasma,

since significant changes in many proteolytic enzymes take place during clotting, it seems likely that PTH is stable in plasma. Half-lives of PTH in vivo have not been determined by totally specific methods. Berson and Yalow (23) found that half-lives depend on the antiserum used in the assay. The shortest value for a half-life presumably results from the most specific antiserum and in rabbits values of about 10 min were obtained. Singer et al. (273), with an antiserum directed towards amino- and carboxyl-termini, found initial half-lives of 4 to 8 min after the infusion of bovine PTH in dogs. They also observed a second slower phase of decay but it is almost certain that this can be attributed largely to carboxyl-terminal fragments of PTH that are more stable than the intact molecule. Neuman et al. (210) reported initial half-lives of 2 to 7 min for radioactivity levels in dogs after intravenous administration of [^{125}I]-PTH.

CIRCULATING FORMS OF PTH. The presence of more than one form of immunoreactive PTH was first reported by Berson and Yalow (23) in plasma from hyperparathyroid patients and in the circulation of rabbits after injection of bovine PTH. Habener et al. (114, 115) found two forms in human plasma. One form corresponded to (1–84) PTH (about one-tenth of total immunoreactivity). The second major form was about two-thirds of the size of (1–84) PTH and lacked the amino-terminal portion (114). The latter would, therefore, be expected to be biologically inactive. The formation of a carboxyl-terminal fragment from injected PTH has been demonstrated in the dog, rat, and calf (113, 263, 265, 266). PTH disappears rapidly from the circulation and the fragments appear slowly and consist of (34–84) PTH in dogs at 6 to 8 min while at later times (37–84) PTH and (43–84) PTH become more prominent. Fragments (34–84) and (37–84) were also found in the circulation of the rat. These findings do not prove that cleavage does not occur closer to the amino-terminus than residue 33 but they are compatible with the suggestion that a biologically active amino-terminal fragment is released. In the references cited amino-terminal fragments were found to be absent from the circulation. This is not because of rapid clearance; by injection, (1–34) PTH has a long half-life of 20 min in the calf (113). Taken together, the results suggest that PTH leaves the vasculature and is converted into a carboxyl-terminal fragment that can reenter the circulation. A biologically active amino-terminal fragment might be formed but if this is the case it must be locally since it does not appear in plasma.

These conclusions, however, must be qualified since other groups of workers have reported the presence of amino-terminal fragments in human and dog serum (47, 48, 127). The amino-terminal fragment in human serum was biologically active (47). These findings also are not conclusive since the fragments in serum could have resulted from cleavage during the clotting process. Although PTH is stable in serum, during clotting transient activation of many proteolytic enzymes takes place (171). A curious feature of the findings of Canterbury and Reiss (48) was that the concentration of the fragment with biological activity (47) did not decrease after calcium infusion whereas the product corresponding to (1–84) decreased as expected. Thus the physiological role of the amino-terminal fragment is obscure.

Although the question of the existence and significance of a circulating amino-terminal fragment is unresolved it is clear that fragments found in the circulation must be formed after release of intact PTH from the parathyroid since hormone collected from the parathyroid vein in man is homogeneous according to gel filtration and immunochemical criteria (264).

ROLE OF INDIVIDUAL TISSUES IN CLEARANCE AND CONVERSION OF PTH AND ITS FRAGMENTS. The lung and hindquarters do not clear immunoreactive PTH in the dog (273). Kidney and liver are the major tissues involved in the clearance of immunoreactive PTH from the circulation of rats and dogs (89, 186, 273). In the dog the liver

has a 40% clearance for (1-84) PTH but not for the carboxyl-terminal fragments or (1-34) PTH (183). This is confirmed by studies showing negligible arteriovenous differences across the liver in man of carboxyl-terminal immunoreactivity and high differences (50% clearance) of endogenous amino-terminal immunoreactivity (57). The kidneys clear both amino- and carboxyl-terminal reactivity in man (57) and in the dog they clear (1-84) PTH, carboxyl-terminal fragments, and (1-34) PTH with an efficiency of about 20% in a single passage (183).

Martin et al. (184) have shown that in the dog kidney glomerular filtration is necessary for clearance of carboxyl-terminal fragments but accounts for only part of the clearance of (1-34) and (1-84) PTH. Although Martin et al. (184) explain this finding by postulating a mechanism of peritubular uptake it is equally possible that the peptides are degraded in transit to products with altered immunochemical reactivity. Hruska et al. (127) have shown that (1-84) PTH is converted to carboxyl-terminal fragments and a small amount of amino-terminal fragments in the perfused kidney and these fragments appear in the perfusate. Canterbury et al. (46) also found that perfused rat liver generates carboxyl- and amino-terminal fragments of PTH in the perfusion medium and that the peak containing amino-terminal fragments is biologically active. It is not clear whether these findings account for the appearance of fragments in the circulation in vivo, but it seems possible that the kidney and liver in addition to being the main tissues responsible for clearance of PTH may also play a major part in its conversion to circulating fragments.

IN VITRO STUDIES. Bovine PTH has been found to be extensively degraded in vitro by rat kidney slices (217), homogenates (296), and an isolated enzyme (185). Vajda et al. (296) also found that liver and muscle homogenates have considerable ability to degrade PTH and that the kidney peptidase activity was associated with the microsomal fraction. Although tissue incubations in vitro probably expose PTH to peptidase activity it would not otherwise encounter, some results do agree with those found from experiments in vivo. For example, Catherwood and Singer (49) found by radioimmunoassay that incubation of bovine PTH with a dog renal plasma membrane preparation gave rise to a carboxyl-terminal fragment similar to the one previously found in the bovine and human circulation (155). Also Fujita et al. (97), by use of [^{125}I] -labelled bovine PTH and its synthetic amino-terminal (1-34) fragment, showed that rat kidney homogenates degrade the (1-84) sequence very much more readily than the (1-34) portion. This is consistent with findings in the circulation of the calf (113).

SUMMARY. Parathyroid hormone, although stable in serum in vitro, is rapidly degraded in vivo after injection or secretion. The liver and kidney are mainly responsible for degradation and in man, dog, and ox may give rise to a circulating biologically inactive carboxyl-terminal fragment, about two-thirds the size of the parent molecule. The precise fate of the biologically active amino-terminus of PTH is not known.

H. Hormones of the Pancreas

The endocrine pancreas secretes two peptide hormones, glucagon and insulin, that control the release and utilization of body glucose and carbohydrate. Both peptides are secreted directly into the portal vein and thus reach one of their target organs very quickly. The liver is also a site of inactivation of both hormones.

1. Glucagon. Glucagon is secreted by the α-cells of the islets of Langerhans and is comprised of 29 amino acid residues. It mobilizes hepatic glycogen, thus releasing glucose into the blood stream in response to hypoglycaemia. Glucagon preparations are sometimes used to treat cases of insulin hypoglycaemia. Natural porcine glucagon is usually used in metabolism studies.

Interpretation of radioimmunoassays for measurements of endogenous glucagon is

complicated by the fact that several higher molecular weight immunoreactive forms are present in the circulation (138).

Narahara and Williams (209) found [^{131}I]-labelled glucagon to be stable in rat plasma upon incubation in vitro whilst Mirsky et al. (201) and Assan (13) found similar radioactive glucagon preparations to be slowly degraded by human plasma. However, this inactivation was far too slow to account for the half-life of this hormone in vivo. After intravenous injection, the half-life of glucagon in man has been estimated to be approximately 10 min (295) and 4.8 min (2) by radioimmunoassay. The half-life of [^{125}I]-labelled glucagon in the dog after intravenous injection was somewhat longer, 22 min, when measured by trichloroacetic acid-precipitable radioactivity (90).

Cox et al. (59) and Narahara et al. (208) studied the tissue distribution of [^{131}I]-labelled glucagon in the rat after intravenous injection. They found that the kidney concentrated the most radioactivity, the liver, somewhat less. Skeletal muscle, because of its large mass, accounted for the largest percentage of the injected dose even though the radioactivity was diffuse. This agrees with the results of Berson et al. (25), who found that [^{131}I]-labelled glucagon, injected intravenously, has an extremely high volume of distribution in the rabbit. Light microscopic autoradiography revealed that the site of kidney concentration of radioactivity in the rat after the injection of radioiodinated glucagon is the proximal tubule cells of the renal cortex (208).

That radioactive products rapidly appeared in the circulation of the rabbit after the intravenous injection of [^{131}I]-labelled glucagon led Berson et al. (25) to conclude that this hormone is degraded very quickly in vivo. The importance of the kidney in the reduction of endogenous glucagon concentrations in the blood has been demonstrated by measurement by radioimmunoassay of arteriovenous differences across the renal circulation. Assan et al. (14) and Lefebvre et al. (162) found that in man and the dog, renal clearances were about 50% and 30%, respectively. In nephrectomized dogs and patients with renal failure the circulating life of both endogenous and exogenous glucagon as measured by radioimmunoassay is prolonged compared with intact animals and normal subjects (14, 161). Little endogenous or exogenous glucagon is excreted intact in human urine, which indicates that this hormone is reabsorbed efficiently by the kidney from the glomerular filtrate and subsequently degraded (13, 161). Rojdmark et al. (246) have shown that endogenous and exogenous glucagon (during infusion) are not significantly cleared by the liver in the dog. They found, however, that at the start of an infusion of glucagon, the hormone is taken up by the liver and after the end of infusion it is released into the circulation.

Rat tissue homogenates degrade [^{131}I]-glucagon in vitro. Liver, kidney, and muscle possess greatest inactivating capacity (209). A rat liver plasma membrane fraction has been found to inactivate glucagon (221). Glucagon-degrading enzymes have also been isolated from bovine and rat liver (143, 193) and rat muscle (76). The degradation of glucagon in vitro has been reviewed by Kenny et al. (147). The failure of the liver in the dog to clear glucagon (246) suggests that the liver peptidases observed in vitro do not play a role in vivo, at least in this species.

Both endogenous and exogenous glucagon are rapidly cleared from the circulation. The kidney is mainly responsible for this and it sequesters peptide through reabsorption from the glomerular filtrate. The high renal clearance in man indicates that a second mechanism of clearance must also exist.

2. Insulin, proinsulin, and C-peptide. Insulin is secreted by the β-cells of the islets of Langerhans in response to a number of stimuli including elevated blood glucose concentrations. Insulin has a well defined three-dimensional structure (34) and is made up of two peptide chains, the A and B chains, which are 21 and 30 amino acid residues long, respectively, and are joined

by disulphide bridges. Insulin extracted from animal pancreas has proved to be a lifesaving hormone in the treatment of insulin-deficient diabetes mellitus. Porcine and bovine insulins are closely related structurally to human insulin and preparations from these two species have been used in most experimental studies. The structure of proinsulin, the biological precursor of insulin, has been determined for several species (64). In proinsulin the carboxyl-terminus of the B chain is linked to the amino-terminus of the A chain by a connecting peptide sequence of 35 residues, known as the C-peptide.

The secretion of the pancreas mainly consists of equimolar quantities of insulin and C-peptide with about 5% to 10% of unconverted proinsulin (247). Proinsulin has low biological activity and is not converted to insulin once released into the circulation. The C-peptide is biologically inactive but radioimmunoassay of this peptide can give useful information on pancreatic function since, unlike insulin, C-peptide is not cleared during passage through the liver. In addition, C-peptide measurements can provide an indication of pancreatic function in insulin-treated diabetics in whom it is not possible to distinguish between exogenous and endogenous hormone.

A further factor that influences studies on insulin is the presence in the circulation of insulin-like biological activity that is immunologically distinct from insulin (96). This activity, which is not suppressed by insulin antibodies, is known as NSILA (nonsuppressible insulin-like activity). It can be 10 times higher than insulin activity and its presence invalidates the use of bioassay for many insulin studies. However, radioimmunoassays appear to be specific for insulin and proinsulin and there is no evidence that other major immunoreactive species circulate.

CIRCULATING HALF-LIFE AND CLEARANCE. Radioiodine-labelled insulin is only slowly inactivated in rat, dog, and human plasma in vitro (24, 137); and it is unlikely that inactivation in plasma accounts for the short half-life of insulin in vivo.

Studies have shown that the half-life of iodine-labelled insulin in vivo is considerably longer than that of unlabelled hormone. Typical estimates of half-life of unlabelled insulin and proinsulin are shown in table 6. Sönksen et al. (282) have shown that in man clearance of unlabelled insulin depends on blood concentration and decreases by a factor of 3 when the concentration is increased from 0.2 ng/ml to 10 ng/ml. This phenomenon has also been observed in the dog over an extended concentration range (1–100 ng/ml) by Franckson and Ooms (94), who found in addition that iodine-labelled insulin was cleared more slowly than unlabelled insulin and that more heavily labelled insulin was cleared more slowly than that lightly labelled.

Table 6 shows single estimates of half-lives but there is evidence for two phases; a more rapid phase of decline at early times and a slower phase at later stages. Izzo et al. (137) found that, with low doses of lightly labelled insulin, 80% of the radioac-

TABLE 6

Estimates of in vivo half-lives of insulin and proinsulin in the pig, baboon, and man

Insulin Preparation	Species of Animal	Method of Injection	Method of Assay	Half-life (min)	Reference
Natural porcine insulin	Pig	Bolus	Radioimmunoassay	6	286
Natural porcine proinsulin	Pig	Bolus	Radioimmunoassay	20	286
Natural porcine insulin	Baboon	Bolus	Radioimmunoassay	8	286
Natural porcine proinsulin	Baboon	Bolus	Radioimmunoassay	18	286
Natural human insulin	Man	Infusion	Radioimmunoassay	4.3	282
Natural porcine proinsulin	Man	Infusion	Radioimmunoassay	25.6	282
Endogenous human nsulin	Man		Radioimmunoassay	3–4	284
Endogenous human proinsulin	Man		Radioimmunoassay	18–25	284

tivity leaves the circulation after 1 min. Indirect evidence for rapid distribution was provided by Sönksen et al. (282), who found in man that insulin has an apparent distribution volume twice that of the plasma volume and concluded that distribution must occur in the first minutes after injection. They also produced evidence of a much slower phase of decline at later times and suggested that this was because of the return of insulin to the circulation from various tissues. These suggestions were corroborated by Sherwin et al. (270), whose analysis of plasma decay curves indicated two extravascular pools in man; one of small volume, which equilibrates rapidly with plasma, and the other of large volume, which equilibrates more slowly (mean residence time 50 min) and results in a phase of decay with a longer half-life.

The half-life of proinsulin (table 6) is longer than that of insulin. C-peptide is cleared more slowly than insulin in the rat (145) and in man peripheral venous C-peptide concentrations are about 4 times higher than insulin concentrations although these two peptides are secreted in equimolar proportions (247).

ROLE OF INDIVIDUAL ORGANS AND TISSUES. Franckson and Ooms (94) showed that in the dog, liver and kidney are major organs for clearing insulin. In man the liver clears 40% to 50% of endogenous and exogenous insulin in a single passage (255) and in the dog clearances of 40% to 60% have been reported (120). The hepatic clearance of insulin does not appear to depend on insulin concentration when within the physiological range, i.e., up to about 10 ng/ml in portal blood (120, 255), but in dogs it is appreciably reduced in the presence of glucagon (246) and at higher insulin levels (94). Insulin is cleared by the isolated perfused rat liver but C-peptide and proinsulin are not (285). Hepatic clearance of lightly iodinated insulin involves uptake in the dog and rat. (94, 137). In the latter study large amounts of intact insulin and fragments were found in the liver at 1 min indicating that uptake and degradation are rapid processes. In the isolated perfused rat liver, reticuloendothelial blockade reduced clearance of [^{125}I]-insulin by 50%, which suggests that Kupfer cells and parenchymal cells may be involved (39).

The kidney clears insulin, proinsulin, and C-peptide at rates greater than the glomerular filtration rate; in the rat arteriovenous differences are 30% to 40% (145). In man the arteriovenous difference for insulin is 46% (92). Glomerular filtration is partly responsible for this clearance and Bourdeau et al. (36) found that with labelled insulin radioactivity was taken up from the filtrate and concentrated in residues at the apical end of cells of the convoluted proximal tubule. Uptake or degradation must be efficient since in man only 0.1% of the daily pancreatic secretion appears in the urine (247). C-peptide is also filtered, but uptake or degradation is less efficient and 5% of the daily pancreatic secretion appears in the urine. Glomerular filtration can account for only 20% at most of the observed renal arteriovenous difference for these peptides, and Katz and Rubenstein (145) have shown that renal clearance is still substantial in the rat when glomerular filtration is impeded. Soltiurai (277) found by autoradiography after administration of iodine-labelled insulin that in addition to uptake from the lumen of the proximal tubule some radioactivity was also present in the peritubular region of the rat kidney. The proportion of grains was about 30% and it was suggested that a pertubular uptake might represent a second mechanism of renal clearance. However, Bourdeau et al. (36) did not observe peritubular uptake and it is possible that the label observed by Soltiurai (277) represented degradation products of insulin. Franckson and Ooms (94) found that clearance of labelled insulin by the kidneys was not associated with a corresponding uptake of label, and Izzo et al. (137) also found much lower quantities of label in the kidneys than in the liver of rats at 1 min. It therefore seems possible that in addition to clearance by glomerular filtration insulin is cleared by degradation in

transit, although the possibility of peritubular uptake cannot be conclusively ruled out.

Although hepatic and renal clearances are high they do not vary over the physiological range of insulin concentrations (94, 120, 255), whereas the total metabolic clearance is sensitive over this range (1–10 ng/ml) in man and dogs (94, 282). This suggests other major sites of clearance at low concentrations. Harding et al. (120) have shown in dogs that the mesenteric circulation clears insulin with an efficiency of 33%, which is, however, independent of insulin concentration. These workers also calculated that the clearance by tissues other than liver and mesentery was 13% at low insulin levels but fell to 6% at higher concentrations. Since a large part of the circulation passes through these other tissues this clearance contributes substantially to the total.

Clearances by muscle and skin, i.e., during passage of insulin through a limb, of 20% at low insulin and 10% at moderate insulin levels (about 8 ng/ml) have been reported in man (255). Muscle and skin and the lymphatic system may also play a rather different role in determining insulin kinetics. In the dog, basal insulin concentrations in hepatic, thoracic, and paw lymph are the same as those in plasma (226). After glucose infusion or insulin injection hepatic and thoracic lymph insulin concentrations take about 15 min to rise but the paw lymph insulin rises more slowly, reaching a maximum after 30 to 40 min. These findings show that insulin is stable in interstitial fluid. The clearance of insulin by the periphery is 10% to 20% (255) and part of this could be from passage into lymph. In the liver insulin is extensively cleared and the appearance of intact insulin in hepatic lymph is surprising. Presumably the sites of insulin uptake and degradation line the blood vessels and sinuses and once insulin leaves the vasculature it is no longer exposed to active clearance processes. The return of insulin from interstitial fluid to the circulation may explain the slow phase of decay of plasma levels observed by Sönksen et al. (282) and Sherwin et al. (270). In addition to its return via the lymphatics insulin can reenter the blood vessels directly. Franckson and Ooms (94) demonstrated that the latter process occurs in dogs by measuring a negative arteriovenous difference across the hind limbs after cessation of insulin infusion. In the dog, Sönksen et al. (281) showed that when injection of iodine-labelled insulin is followed by a pulse of cold insulin, label is displaced from the hind limb and appears in venous blood draining the limb.

The role of tissues such as muscle and skin in clearing insulin and acting as an extravascular depot may have an added significance since the kinetics of these processes may depend on regional blood flow, which will be enhanced under conditions of exercise. It has been shown, for instance (27), that the rate at which insulin leaves a site of injection depends on regional blood flow and that in normal man insulin is released into the circulation from peripheral tissues during exercise (72).

In addition to the investigations already mentioned there has been a single report suggesting that insulin may be cleared to the extent of 10% to 20% in a single passage through the lung in man (248).

MECHANISMS OF INACTIVATION. Although potent enzymes capable of rapidly degrading insulin exist in many tissues (290), the mechanisms of inactivation that are important in vivo remain to be established. Mirsky and Perisatti (200) showed that an enzyme in liver homogenates was specific for insulin and that concentrations of this enzyme could vary independently of other proteolytic enzymes. Insulin-specific proteases have been isolated from rat liver (41) and muscle (40). These proteases are cytoplasmic and the muscle enzyme has remarkable properties. It is apparently specific for insulin and glucagon and either substrate inhibits inactivation of the other (76). However, this specificity must be reconciled with the observation that the highly purified enzyme cleaves glucagon at many

sites characterized by a wide variety of adjacent amino acid residues (19).

A second insulin-inactivating enzyme isolated from rat liver is not a protease. It reduces the sulphur bridges of insulin in the presence of the reduced form of glutathione and is called glutathione-insulin transhydrogenase (301). This enzyme is present in the endoplasmic reticulum (3).

Either of these enzymes would require uptake of insulin into the cell before the enzyme could act and it is likely that uptake rather than degradation would be the rate-limiting step for clearance from the circulation. It is possible that insulin might be degraded by extracellular mechanisms since insulin-degrading activity is found in liver plasma membrane preparations (95). Degradation is apparently independent of receptor binding (60, 95). In view of the extensive uptake of insulin by the liver in vivo (94, 137) it is perhaps unlikely that substantial extracellular degradation occurs in vivo. However, the question of the actual functions in vivo of the various enzyme systems capable of inactivating insulin in vitro can only be conclusively resolved by further investigations.

Summary. The metabolism of insulin appears to be more subtle than that of many other peptide hormones, perhaps because insulin has multiple sites of action and plays such a key role in distribution and utilization of fuel in the body. In man the half-life appears to be 3 to 4 min but the significance of this value is doubtful since the total metabolic clearance rate of insulin is sensitive to circulating insulin concentrations. The clearance rate of basal insulin concentrations may be up to 3 times higher than that of elevated physiological concentrations of insulin. Liver and kidney are important organs of clearance but the renal mechanism is not sensitive to insulin concentration and hepatic clearance is only significantly reduced at supraphysiological concentrations of insulin. There is some evidence to suggest that peripheral tissues such as muscle and skin play an important part in clearance at basal insulin concentrations by a mechanism that is saturable in the physiological range of concentrations. Muscle and skin can also act as a depot for insulin and after there has been a rapid fall in circulating concentrations these tissues may return insulin to the circulation, which gives rise to a slow phase of decline with a half-life of 30 to 60 min. Several mechanisms for the inactivation of insulin have been demonstrated in vitro but conclusive experimental information about the processes that take place in vivo does not appear to be available.

I. Protein and Glycoprotein Hormones of the Anterior Pituitary

These hormones are much larger than the peptide hormones considered so far. The glycoprotein hormones are made up of subunits each with carbohydrate groups that have profound effects on their metabolism. They have little in common with the smaller peptide hormones and will be considered only briefly.

1. Protein hormones. A. GROWTH HORMONE (SOMATOTROPHIN). Growth hormone is present in large amounts in the anterior pituitary and has a wide range of functions including the stimulation of protein synthesis and the promotion of skeletal and general body growth. The bovine hormone consists of 188 amino acid residues.

In general the half-life of growth hormone in vivo has been found to be longer than that found for any of the peptide hormones. Following bolus intravenous injection of bovine growth hormone into rats the circulation half-life, as measured by bioassay, was found to be 26 min by Van Dyke et al. (299). However, large amounts of hormone were administered. With [^{131}I]-labelled bovine growth hormone Sonenberg et al. (279) were able to follow the decay of circulating radioactivity after the injection of a much smaller amount of hormone into the rat. They observed a half-life of about 25 min. In man exogenous human growth hormone has a half-life of about 30 min by radioimmunoassay or by following the fate of immunoprecipitable [^{131}I]-labelled hor-

mone after intravenous injection (35, 218). Endogenous circulating growth hormone in human subjects has been observed to decay with a half-life of 20 to 30 min by radioimmunoassay (104, 135). After bolus intravenous injection of [^{131}I]-labelled human growth hormone into the rabbit, plasma radioactivity fell initially with a half-life of between 2 and 10 min. Slower phases of decay followed with half-lives of decay of 17.6 min and 165 min (254). Since no rapid phase of decay in blood concentrations of endogenous growth hormone in man was observed, the short estimated initial half-life in rabbits probably reflected the mixing of the hormone in its volume of distribution as suggested by Van Dyke et al. (299).

The distribution of tritiated or radioiodine-labelled growth hormone preparations in the rat, guinea pig, rabbit, and sheep has been studied (56, 192, 254, 279, 308). After the intravenous injection of radiolabelled hormone, the highest concentrations of radioactivity in all these studies were in the kidney and to a lesser extent the liver. However, small but significant amounts of radioactivity were in many other tissues. Collipp et al. (56) calculated that, because skeletal muscle represents 40% of the body weight, the low concentrations of radioactivity in the tissue in fact constitute about 40% of the injected dose in the guinea pig and rat. Light microscopic autoradiography has localized the renal fate of radiolabelled growth hormone. With light microscopic autoradiography, Collipp et al. (56), De Kretser et al. (67), and Rabkin et al. (223) found the radioactivity to be associated with the proximal tubule cells of the kidney cortex. Similarly, fluorescent-labelled bovine growth hormone was detectable by ultraviolet light microscopy in the proximal tubule cells of the rat after intravenous injection (304). With an isolated sheep proximal tubule preparation Stacy et al. (283) showed by electron microscopic autoradiography that the intracellular fate of [^{125}I]-labelled sheep growth hormone involved uptake by the lysosomes. Rapid degradation of the hormone was observed to take place in these organelles. The fact that after intravenous injection of growth hormone no biologically active hormone is found in rat urine (299) and that the metabolic products excreted by man and sheep are of low molecular weight (218, 308) confirm that the kidney actively degrades this hormone. That the contribution of the kidney to the overall metabolism of exogenous growth hormone is large is demonstrated by the fact that nephrectomy in the rat and sheep markedly prolongs the circulating life of the hormone (307, 308). However, endogenous human growth hormone has a half-life of decay, after the intravenous administration of somatostatin, of 27 min and 17 min by radioimmunoassay, in patients with liver and renal disease respectively (220). These values are well within the normal range and indicate that despite the abnormal state of these two organs the metabolism of endogenous human growth hormone carries on unabated.

B. PROLACTIN AND PLACENTAL LACTOGEN. Prolactin has sequence homology with growth hormone and consequently is difficult to separate from this hormone. The function of prolactin is to promote full breast development in the female and to initiate milk production after parturition. The ovine hormone consists of 198 amino acid residues.

The circulating half-lives of prolactin preparations in vivo that have been reported are very variable. With [^{131}I]-labelled rat and bovine prolactin, Van der Gugten and Kura (298) found that radioactivity in the blood decayed with a half-life of about 70 min after intravenous injection into the female rat. As the authors suggest, the long circulating life may be a reflection of the high doses administered. In contrast, Grosvenor (111) found by bioassay that the circulating half-lives of various prolactin preparations in the rat were very short. He found that ovine, rat, bovine, and porcine prolactins had half-lives in the female rat of 10 to 12 min, 10 min, 3 min, and 3 min, respectively. Koch et al. (151) found that rat prolactin labelled

with [^{131}I] decayed with a half-life of 4 to 6 min in the female rat after both bolus injection and intravenous infusion. Similarly Kura et al. (155) found the half-life of bovine prolactin in female rats to be 6 min by radioimmunoassay. Intermediate half-life values, rather similar to those obtained for growth hormone, have been observed. Birkinshaw and Falconer (28) found the half-life of [^{125}I]-labelled prolactin in female rabbits to be 16 min whilst Johke (140) found that the circulating half-life of bovine prolactin in the lactating cow was 29 min by radioimmunoassay. These results suggest that there may be a correlation between the half-life of prolactin and the size of the mammal studied; a correlation that is not apparent with growth hormone.

Another lactogenic hormone, placental lactogen, is secreted by the placenta. This polypeptide hormone has structural homology with both growth hormone and prolactin itself. After delivery of the human placenta the decay of endogenous human placental lactogen in the circulation has been found to have a half-life of between 10 and 29 min by radioimmunoassay (244).

Radioiodinated prolactin preparations have been used to study the tissue distribution of this hormone in mice, rats, and rabbits either by direct measurement of tissue radioactivity or by whole-body autoradiography (28, 225, 280). Highest concentrations were in the kidney in each study with lesser amounts in the liver. With fluorescent-labelled prolactin Vilar et al. (304) showed that the hormone was concentrated in the kidney by the proximal tubule cells of the renal cortex. The importance of the kidney in the clearance of human placental lactogen from the circulation of the dog has been shown by Rochman et al. (244). They found that the circulation life of this hormone was considerably prolonged in nephrectomized dogs compared with intact animals.

SUMMARY. As a general rule the half-lives of the anterior pituitary protein hormones are longer than those found for the smaller peptide hormones. The larger molecular size appears to confer some measure of metabolic stability on these hormones. The kidney appears to play a major part in the degradation of these hormones.

2. Glycoprotein hormones. The glycoprotein hormones all have nonidentical α- and β-subunits each of about 100 amino acid residues and carbohydrate moieties. In each species the α-chain appears to be identical or nearly so in each glycoprotein hormone whilst the β-chain differs from hormone to hormone and apparently confers hormonal specificity.

The gonadotrophic hormones, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH), are involved in the stimulation and normal development of the gonads and the production of gametes. Their critical role in reproduction has led to their use to help infertile women become pregnant. Thyrotrophic hormone (TSH) is essential for proper development and function of the thyroid gland. It stimulates this gland to secrete thyroxine and triiodothyronine, which in turn raise the basal metabolic rate.

The half-lives of the glycoprotein hormones in vivo are much longer than the other hormones considered in this review. For instance, human LH has a half-life of 136 min in man by radioimmunoassay after intravenous infusion (182) and the half-lives of [^{125}I]-labelled human FSH after intravenous injection into the rat, rabbit, ewe, and cow are 94, 118, 334, and 301 min, respectively (156). The half-life of immunoreactive human FSH in man after intravenous infusion is 2.9 h (149).

The intravenous injection of radioiodinated TSH and FSH into the rat resulted in high concentrations of radioactivity being sequestered by the kidney (43, 144). The sequestration of fluorescent labelled FSH, LH, and TSH by the rat kidney after intravenous injection results from incorporation of the proteins by the proximal tubule cells of the renal cortex (304). Light microscopic autoradiography revealed similar accumulation of radioactivity after the injection of [^{125}I]-labelled LH (67). Nephrectomy considerably prolonged the circulating life of both LH and FSH in the rat

and LH in the sheep (66, 100). Measurement of the arteriovenous difference of radioactivity across the kidney during the intravenous infusion of radioiodinated TSH has shown that this organ clears 5.8% of the hormone in a single passage whilst the liver, thyroid, and hindquarters are inactive in this respect (240). Recently, Ascoli et al. (12) showed that appreciable amounts of tritiated LH are excreted in the urine by the rat without substantial modification after intravenous injection. Similar results were obtained by Coble et al. (55) when radioiodinated FSH was injected into the monkey.

The kidney appears to be mainly responsible for lowering the circulating concentrations of the glycoprotein hormones. Under certain circumstances the liver may be important in limiting the effective life of these hormones. Morell et al. (203) found that, in common with other glycoproteins, chorionic gonadotrophin and FSH are rapidly and almost completely taken up by the rat liver after intravenous injection if the sialic residues of the carbohydrate moieties have been removed previously. The sialic residues are essential for the biological activity in vivo of these two hormones.

The prolonged life in the circulation of the glycoprotein hormones follows from their resistance to breakdown by most tissues, which presumably may be attributed to their three-dimensional structure. The kidney probably lowers circulating glycoprotein hormone levels by glomerular filtration. Part of the hormone in the filtrate is sequestered by renal proximal tubule cells and part may be excreted in the urine unchanged. Finally, the liver may be important in the rapid accumulation of these hormones if the biologically important sialic acid residues are removed.

III. General Discussion: Comparison of the Metabolism of Peptide Hormones

A. General Characteristics of the Plasma Decay Curve and Clearance

The decay of plasma concentrations after intravenous injection of a peptide hormone will be discussed in terms of three phases.

First phase. The earliest phase lasts for 1 or 2 min and is characterized by very rapid disappearance from the circulation. Iodine-labelled insulin in the rat (137), LHRH in man (139), calcitonin in the pig (317), corticotrophins in the rat (22), and somatostatin in the rat (195) all leave the circulation to the extent of 70% to 80% in 1 to 2 min after intravenous injection. At later times the rate of decline for insulin, LHRH, calcitonin, and corticotrophins falls and this suggests that the early phase reflects rapid equilibration into a pool with an apparent volume greater than plasma. A peptide hormone returning from this pool will sustain the plasma concentration and thus reduce the initial rate of decay that otherwise would have resulted in a half-life in the second phase of less than 1 min. An early phase of distribution of insulin also occurs in man (270, 282). Although an early phase has not been reported for many hormones, the methods used may have been inadequate to detect it, and it is possible that the phenomenon of rapid extravascular distribution is widespread. For peptides with very short half-lives in the period after 2 min it will not be possible to distinguish first and second phases and interpretation of the processes involved in distribution and clearance will be very difficult.

Second phase. From 1 to 2 min after intravenous injection, peptide hormone levels usually decline exponentially for 20 to 40 min. In the first phase peptide concentration may fall by a factor 2 to 5 times faster than in the second more prolonged phase. Even though the disappearance rate is slower in the second phase than in the first, the peptide hormone concentration may still fall very rapidly in the second phase. This phase is therefore of great importance physiologically and it is the exponential decline of this phase that is usually reported as a half-life. Half-lives in the second phase are usually in the range of 1 to 10 min but can be more than 1 h in the case of some of the large peptide and protein hormones. Bradykinin and angiotensin have potent local effects and exceptionally

short half-lives. For these hormones it would therefore not be easy to distinguish two phases. The half-lives of peptides are usually independent of dose up to moderately high concentrations but vasopressin is an exception; its half-life decreases with concentration (61, 102).

Clearance measurements made during infusion or steady endogenous concentrations probably reflect processes that operate in the second phase of decay of blood concentrations. Clearance is usually independent of hormone concentration but insulin (94, 282) and vasopressin (170) do not conform to this rule.

Third phase. At times later than 20 to 40 min after injection or termination of infusion, a third phase of decline of plasma levels is sometimes observed that can be as much as 10 times slower than the second phase. Examples in man are LHRH (269), calcitonin (249), ACTH (236), and insulin (270, 282). It is possible that careful studies with other hormones would yield many other examples of a third phase of decline. Although concentrations of hormone have normally fallen sharply by this time, the third phase could be important when large doses of hormone are administered or when very low hormone concentrations play a physiological role. For insulin (94, 281) and ACTH (22, 132), it appears likely that the slow phase is caused by return of peptide to the circulation from tissue sites that equilibrate slowly with plasma.

Inspection of the half-lives reported in different species (tables 2-6) shows that there is considerable variation and it is not possible to generalize from one peptide hormone to another. This in itself is an indication that a multiplicity of processes are involved in clearance and degradation. It is also apparent that the half-life of a peptide does not depend in a simple way on its linear sequence. Addition of an extra portion of peptide chain frequently lengthens the half-life considerably, e.g., insulin and proinsulin (284), gastrin (G17) and gastrin (G34) (312), (1-24) ACTH and (1-39) ACTH (194). These findings suggest that the three-dimensional structure of the peptide is an important determinant of its susceptibility to uptake and degradation.

B. Mechanisms of Clearance and Distribution

The mechanisms of clearance and distribution that have emerged from studies carried out so far can be provisionally classified into five major types (see fig. 2). This classification is based on the way a mechanism affects plasma levels of peptide and peptide fragments. Ultimately one would like to be able to categorize mechanisms on molecular and morphological bases.

Type I, rapid degradation or conversion in blood or plasma, is observed with a number of smaller peptides. It can be the result of circulating aminopeptidases [enkephalins (119), angiotensin II (237), somatostatin (195)]; iminopeptidase [melanostatin (207)]; carboxypeptidase N [bradykinin (86)]; dipeptidyl carboxypeptidase [bradykinin (276), angiotensin I (213)]; or amidase [TRH (232)]. In no case has it been demonstrated that this type of process is the major mechanism of inactivation in vivo and for some of these hormones it is known

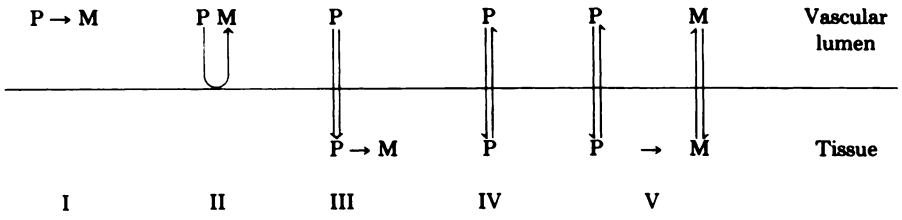

FIG. 2. Processes observed in peptide metabolism. P, peptide; M, metabolites; I, degradation in blood; II, degradation in transit; III, irreversible uptake and degradation; IV, equilibration; V, equilibration and degradation.

that clearance by tissues is even faster than degradation in the blood (91, 126, 213).

Type II, conversion or degradation in transit through a tissue bed, occurs in the lung, kidney, and probably liver. Peptide hormones do not leave the circulation and peptide fragments emerge from the tissue at the same time as a plasma protein marker. Peptide analogues that resist type I and type II processes should be longer-acting and more potent. The best examples of a type II process are the extensive conversion of angiotensin I to angiotensin II and the inactivation of bradykinin in the pulmonary circulation by the same enzyme [angiotensin converting enzyme or dipeptidyl carboxypeptidase; see Soffer (276) and section I B]. The enzyme is thought to line caveolae that lie close to and open into the vascular lumen (251). The lung does not clear angiotensin II (126), oxytocin, vasopressin (29), (1-24) ACTH (136), gastrin (G-17) (71), porcine and salmon calcitonin (272), or PTH (273). Thus although angiotensin converting enzyme has a broad specificity in vitro it does not cleave many peptides in vivo. It is possible that the carboxyl-terminal amide group of many of the small peptides prevents degradation by the lung. A type II process has been demonstrated in the kidney for angiotensin II (214) that may involve the action of an aminopeptidase. Type II processes may be responsible for converting PTH to amino- and carboxyl-terminal fragments in the liver and kidney (46, 127) but it is also possible in either of these tissues that peptide uptake precedes formation of fragments.

Type III, irreversible uptake followed by degradation, appears to be a common process in kidney and may be important for some peptides in the liver. The rate-limiting step for clearance by this type of mechanism is uptake and the degradation process is of secondary importance. Many peptide hormones are filtered by the glomerulus in the kidney and it is unlikely that they can subsequently return to the circulation. Up to 20% of plasma peptide can be cleared in a single passage in this way and the process will apply to all molecules of less than about 20,000 MW unless they are protein bound. TSH, with a molecular weight of 28,000, is cleared by the kidney with an efficiency of 6% (240) and clearance is due to filtration because glycopeptides of this sort are subsequently taken up by proximal tubule cells (304). After glomerular filtration, a peptide can be excreted unchanged [melanostatin (230)], degraded to products that are excreted [oxytocin and vasopressin (313)] or reabsorbed by the proximal tubule [corticotrophin analogues (15), calcitonin (124), glucagon (208), insulin (36), growth hormone (304), and glycopeptide hormones (304)]. After uptake peptides can be transferred to lysosomes and extensively digested (15, 22) by enzymes of broad specificity.

Uptake by glomerular filtration is obviously a process of minimal specificity. Clearance by the liver may also involve a type III process but one of considerable specificity. Examples of this specificity are: clearance of pentagastrin but not gastrin (289); clearance of porcine calcitonin (272), low clearance of human calcitonin (52), negligible clearance of salmon calcitonin (272); clearance of (1-84) PTH but not the carboxyl-terminal fragment or (1-34) PTH (183); clearance of insulin but not C-peptide or proinsulin (285). Many other peptides are cleared by the liver, but clearances of gastrin, cholecystokinin, secretin, glucagon, and TSH are low or negligible. It is often not clear whether a peptide hormone is taken up before degradation or cleaved in transit. However, uptake of peptide has been observed for insulin (94, 137), corticotrophin (22, 239), and porcine calcitonin (52). Presumably for these peptides hepatic clearance is a process of the third type. The specificity of a type III process will reflect the characteristics of uptake or binding rather than of the nature of the peptidases that may be subsequently involved in degradation.

Type IV, equilibration with tissues, has already been mentioned (Section III A 2) as an explanation of the first and third

phases in the decline of plasma hormone concentrations. Rapid equilibration is required to explain the first phase. The anatomical basis for this distribution is not known; perhaps the peptide does not leave the blood vessels but is adsorbed to the capillary wall or alternatively does not penetrate far into the tissues. Sönksen et al. (281) have shown that iodine-labelled insulin is rapidly displaced from the dog hind limb by cold insulin and this suggests rapid equilibration. The slow phase of decline of plasma concentrations at times later than 20 to 40 min suggests that mechanisms of slow equilibration are also important. Rojdmark et al. (246) found that, after infusion, glucagon was slowly released from the liver in dogs and Franckson and Ooms (94) showed a similar release of insulin from the dog hind limb after infusion. Dieterle et al. (72) found that insulin is released from peripheral stores during exercise and there is also evidence that after intravenous injection insulin slowly appears in the peripheral lymph (226). This discussion of equilibration has assumed that no degradation is taking place. It is possible that a certain amount of inactivation of insulin occurs in the periphery so that strictly speaking some of the results cited above might be better described by a process of the fifth type.

Type V, equilibration and degradation, refers to a process in which a peptide hormone can enter and leave sites within a tissue at which degradation occurs. If equilibration is fast, this process will become indistinguishable from degradation in transit. If equilibration is slow and degradation not too extensive, the tissue will act as a depot from which intact peptide hormone as well as peptide fragments will slowly enter the circulation. Findings for corticotrophin are consistent with such mechanisms. Extensive degradation of (1–24) ACTH occurs in the rat during uptake into tissues such as muscle and skin in the first minute after injection (22). Subsequently, the rate of degradation in the tissues falls and ACTH and peptide fragments return to the circulation (132). An aminopeptidase plays a major role in the degradation but endopeptidases also are active (21, 131). Replacement of the amino-terminal amino acid by a D-residue results in a peptide that at early times is more stable in muscle and skin but not in other tissues (22) and which has a very high potency and long duration of action (172, 196). Although this type of mechanism has been described only for corticotrophins, it seems likely that it will apply to other peptides.

So far, we have not considered the details of renal clearance, which frequently involves processes in addition to glomerular filtration. This has been shown for: angiotensin II (215); gastrin (G-17) (53); cholecystokinin and secretin (165); human calcitonin (52) and porcine calcitonin (272); parathyroid hormone, its carboxyl-terminal fragment and amino-terminal (1–34) fragments (183); glucagon (13); and insulin, proinsulin, and C-peptide (145). Evidence that clearance is not attributable solely to glomerular filtration includes observation either of a clearance of more than 20% or of a clearance that persists after glomerular filtration has been stopped, e.g., by tying the ureter. A type II clearance process may be responsible for angiotensin II (214) and insulin (section II G 2) but for most peptides the information available does not distinguish clearance by degradation and clearance in transit. The lack of specificity of this clearance process that does not involve glomerular filtration is in marked contrast to clearance by the liver, which is highly selective. If clearance in transit does occur in the kidney the enzymes must cover a wide range of specificity.

C. Summary and Conclusions

After an initial period of 1 to 2 min after intravenous injection, peptide hormone concentrations in plasma rapidly decline further in an exponential manner for 20 to 40 min. Half-lives are usually short (1 to 10 min) but can be as much as 1 to 2 h. In the first 1 to 2 min a more rapid decline has been observed for some peptides, which appears to be caused by distribution. At

times later than 20 to 40 min a slow phase of decline has been reported in a number of instances. For insulin and corticotrophins the slow phase seems to be return of these hormones to the circulation from tissues such as muscle and skin. For most peptides reversible processes that could give rise to the rapid early phase and slow late phase of decline of plasma concentrations have not been studied and little is known about the ultrastructural basis of the mechanisms involved.

In the intermediate phase of decline of plasma concentrations (between 1 to 2 and 20 to 40 min) several clearance mechanisms can operate and the part these play in the metabolism of the most thoroughly studied peptides is summarized in table 7. Glomerular filtration is important for every peptide for which there are adequate data and this mechanism is well understood. Pulmonary clearance, on the other hand, is a highly selective process and the enzyme responsible for clearance has been well characterized. Renal clearance by a nonspecific process that does not involve glomerular filtration is also important for many hormones although it seems likely that TSH, ACTH, and salmon calcitonin, which have comparatively low renal clearances (87, 240, 272), resist this process and oxytocin and vasopressin are probably also not cleared in this manner (313). Little is known about the nature of renal clearance of peptide hormones and it is quite possible that several mechanisms that give rise to the broad specificity are involved. Clearance by the liver is highly selective but the mechanisms of clearance and the reasons for the specificity are unknown. Clearance by muscle and skin has been inadequately studied. The proportion of the cardiac output flowing through these tissues is large and even when the proportion of peptide cleared in a single passage is low, the contribution to total metabolic clearance can be large. The information available does not enable us to assess whether clearance by these tissues is specific or not.

A large number of investigations of the

TABLE 7

*Clearance processes involved in the metabolism of peptide hormones**

Peptide	Kidney (A)	Kidney (B)	Liver	Muscle/ Skin	Lung	Plasma
Salmon calcitonin	+(272)	0(272)	0(272)	0(272)	0(272)	0(117)
Thyroid stimulating hormone	+(240)	0(240)	0(240)	0(240)	N.A.	N.A.
Gastrin (G17)	+(53)	+(53)	0(289)	N.A.	0(71)	0(32)
Oxytocin	+(102)	0(313)	+(102)	N.A.	0(29)	0(1)
Vasopressin	+(102)	0(313)	+(102)	N.A.	0(29)	0(1)
Cholecystokinin	+(165)	+(165)	0(165)	N.A.	N.A.	0(165)
Secretin	+(165)	+(165)	0(165)	N.A.	N.A.	0(165)
Glucagon	+(14)	+(14)	0(246)	N.A.	N.A.	0(201)
Proinsulin	+(145)	+(145)	0(285)	N.A.	N.A.	N.A.
Insulin C-peptide	+(145)	+(145)	0(285)	N.A.	N.A.	N.A.
Parathyroid hormone C-fragment	+(183)	+(183)	0(183)	N.A.	N.A.	N.A.
1-34 Parathyroid hormone	+(183)	+(183)	0(183)	N.A.	N.A.	N.A.
Parathyroid hormone	+(183)	+(183)	+(183)	0(273)	0(273)	0(210)
1-24 Corticotrophin	+(16)	N.A.	+(22)	+(132)	0(130)	0(194)
Human calcitonin	+(124)	+(52)	+(52)	N.A.	N.A.	0(7)
Porcine calcitonin	+(272)	+(272)	+(272)	+(272)	0(272)	0(211)
Insulin	+(36)	+(92)	+(255)	+(255)	N.A.	0(24)
Angiotensin II	N.A.	+(126)	+(126)	+(126)	0(126)	+(126)
Bradykinin	N.A.	+(91)	+(91)	+(91)	+(91)	+(91)

* +, tissue or mechanism is important; 0, contribution to clearance is small or undetectable; N.A., adequate information not available to the best of the authors' knowledge. Kidney (A) refers to clearance by glomerular filtration; kidney (B) to renal clearance by other mechanisms. The peptides are listed in order of increasing involvement of clearance processes. Numbers in parentheses are references.

metabolism of different peptides have been carried out in the past 20 to 30 years. Although the information that has been gained remains incomplete, we have tried to show that it is possible to compare and describe the metabolism of peptide hormones in terms of a limited number of basic processes of clearance and distribution. The classification adopted is, of course, provisional and inevitably raises many questions. However, with the experience gained from previous investigations and the powerful experimental methods now available, e.g., sequence-specific radioimmunoassays and specifically labelled peptides in combination with radiosequencing and chromatography, there is no reason to suppose that rapid progress will not continue to be made in this field.

REFERENCES

1. ADAMSONS, K., ENGEL, S. L., AND VAN DYKE, H. B.: Stability of neurohypophysial hormones. Endocrinology **63:** 679-687, 1958.
2. ALFORD, F. P., BLOOM, S. R., AND NABARRO, J. D. N.: Glucagon metabolism in man: Studies on the metabolic clearance rate and the plasma acute disappearance time of glucagon in normal and diabetic subjects. J. Clin. Endocrinol. Metab. **42:** 830-838, 1976.
3. ANSORGE, S., BOHLEY, P., KIRSCHKE, H., LANGNER, J., AND HANSON, H.: Metabolism of insulin and glucagon. Breakdown of radioiodinated insulin and glucagon in rat liver cell fractions. Eur. J. Biochem. **19:** 283-288, 1971.
4. ARDAILLOU, R., BEAUFILS, M., NIVEZ, M.-P., ISAAC, R., MAYAUD, C., AND SRAER, J.-D.: Increased plasma calcitonin in early acute renal failure. Clin. Sci. Mol. Med. **49:** 301-304, 1975.
5. ARDAILLOU, R., PAILLARD, F., SAVIER, C., AND BERNIER, A.: Renal uptake of radioiodinated human calcitonin in man. Rev. Eur. Etud. Clin. Biol. **16:** 1031-1036, 1971.
6. ARDAILLOU, R., PAILLARD, F., SRAER, J., AND VALLÉE, G.: Compared kinetics of salmon and human radioiodinated calcitonin in man. Horm. Metab. Res. **5:** 232-233, 1973.
7. ARDAILLOU, R., SIZONENKO, P., MEYRIER, A., VALLÉE, G., AND BEAUGAS, C.: Metabolic clearance rate of radioiodinated human calcitonin in man. J. Clin. Invest. **49:** 2345-2352, 1970.
8. ARIMURA, A., KASTIN, A. J., GONZALES-BARCENA, D., SILLER, J., WEARER, R. E., AND SCHALLY, A. V.: Disappearance of LH-releasing hormone in man as determined by radioimmunoassay. Clin. Endocrinol. **3:** 421-425, 1974.
9. ARIMURA, A., SATO, H., DUPONT, A., NISHI, N., AND SCHALLY, A. V.: Somatostatin: Abundance of immunoreactive hormone in rat stomach and pancreas. Science **189:** 1007-1009, 1975.
10. ARONSON, A. S., ANDERSSON, K. E., BERGSTRAND, C. G., AND MULDER, I. L.: Treatment of diabetes insipidus in children with DDAVP, a synthetic analogue of vasopressin. Acta Paediat. Scand. **62:** 133-140, 1973.
11. AROSKAR, J. P., CHAN, W. Y., STOUFFER, J. E., SCHNEIDER, C. H., MURTI, V. V. S., AND DUVIGNEAUD, V.: Renal excretion and tissue distribution of radioactivity after administration of tritium-labeled oxytocin to rats. Endocrinology **74:** 226-232, 1964.
12. ASCOLI, M., LIDDLE, R. A., AND PUETT, D.: The metabolism of luteinizing hormone: Plasma clearance, urinary excretion and tissue uptake. Mol. Cell. Endocrinol. **3:** 21-36, 1975.
13. ASSAN, R.: *In vivo* metabolism of glucagon. *In* Glucagon: Molecular Physiology, Clinical and Therapeutic Implications, ed. by P. J. Lefebvre and R. H. Unger, pp. 47-59, Pergamon Press, London, 1972.
14. ASSAN, R., TCHOBROUTSKY, G., AND DEROT, M.: Intervention of kidney and liver in glucagon catabolism and clearance from plasma. Acta Isotop. **10:** 289-294, 1970.
15. BAKER, J. R. J., BENNETT, H. P. J., CHRISTIAN R. A., AND MCMARTIN, C.: Renal uptake and metabolism of adrenocorticotrophin analogues in the rat: An autoradiographic study. J. Endocrinol. **74:** 23-35, 1977.
16. BAKER, J. R. J., BENNETT, H. P. J., HUDSON, A. M., MCMARTIN, C., AND PURDON, G. E.: On the metabolism of two adrenocorticotrophin analogues. Clin. Endocrinol. **5**(suppl.): 63s-72s, 1976.
17. BAKHLE, Y. S., REYNARD, A. M., AND VANE, J. R.: Metabolism of the angiotensins in isolated perfused tissues. Nature (London) **222:** 956-959, 1969.
18. BARTH, T.: Neurohyphysial hormones. Enzymic inactivation and its significance for the synthesis of metabolically more stable analogues. *In* Intracellular Protein Catabolism—Proceedings of the Symposium, ed. by H. Hanson and P. Bohley, pp. 306-318, Martin-Luther University, Halle, G. D. R., 1976.
19. BASKIN, F. K., DUCKWORTH, W. C., AND KITABCHI, A. E.: Sites of cleavage of glucagon by insulin-glucagon protease. Biochem. Biophys. Res. Commun. **67:** 163-169, 1975.
20. BASSIRI, R. M., AND UTIGER, R. D.: Metabolism and excretion of exogenous thyrotrophin-releasing hormone in humans. J. Clin. Invest. **52:** 1616-1619, 1973.
21. BENNETT, H. P. J., HUDSON, A. M., MCMARTIN, C., AND PURDON, G. E.: Use of octadecasylyl-silica for the extraction and purification of peptides in biological samples: Application to the identification of circulating metabolites of corticotrophin-(1-24)-tetracosapeptide and somatostatin *in vivo*. Biochem. J. **168:** 9-13, 1977.
22. BENNETT, H. P. J., AND MCMARTIN, C.: Distribution and degradation of two tritium labelled corticotrophin analogues in the rat. J. Endocrinol. **82:** 33-42, 1979.
23. BERSON, S. A., AND YALOW, R. S.: Immunochemical heterogeneity of parathyroid hormone in plasma. J. Clin. Endocrinol. Metab. **28:** 1037-1047, 1968.
24. BERSON, S. A., YALOW, R. S., BAUMAN, A., ROTHSCHILD, M. A., AND NEWERLEY, K.: Insulin-I^{131} metabolism in human subjects, demonstration of insulin binding globulin in the circulation of insulin treated subjects. J. Clin. Invest. **35:** 170-190, 1956.
25. BERSON, S. A., YALOW, R. S., AND VOLK, B. W.: *In vivo* and *in vitro* metabolism of insulin-I^{131} and glucagon-I^{131} in normal and cortisone treated rabbits. J. Lab. Clin. Med. **49:** 331-342, 1957.
26. BESSER, G. M., ORTH, D. N., NICHOLSON, W. E., BYYNY, R. L., ABE, K., AND WOODHAM, J. P.: Dissociation of the disappearance of bioactive and radioimmunoreactive ACTH from plasma in man. J. Clin. Endocrinol. Metab. **32:** 595-603, 1971.
27. BINDER, C.: Absorption of injected insulin. Acta Pharmacol. Toxicol. **27:** suppl. 2, 1-85, 1969.
28. BIRKINSHAW, M., AND FALCONER, I. R.: The localization of prolactin labelled with radioactive iodine in rabbit mammary tissue. J. Endocrinol. **55:** 323-334, 1972.
29. BIRON, P., AND BOILEAU, J. C.: Fate of vasopressin and oxytocin in the pulmonary circulation. Can. J. Physiol. Pharmacol. **47:** 713-717, 1969.
30. BIRON, P., AND CAMPEAU, L.: Pulmonary and extrapulmonary fate of Angiotensin I. Rev. Can. Biol. **30:** 27-34, 1971.

31. BIRON, P., MEYER, P., AND PANISSET, J. C.: Removal of angiotensins from systemic circulation. Can. J. Physiol. Pharmacol. **46:** 175–178, 1968.
32. BLAIR, E. L., FARRA, Y., RICHARDSON, D. D., AND STEINBOK, P.: The half-life of exogenous gastrin in the circulation. J. Physiol. **208:** 299–315, 1970.
33. BLAIR-WEST, J. R., COGHLAN, J. P., DENTON, D. A., FUNDER, J. W., SCOGGINS, B. A., AND WRIGHT, R. D.: The effect of the heptapeptide (2–8) and hexapeptide (2–8) fragments of angiotensin II on aldosterone secretion. J. Clin. Endocrinol. Metab. **32:** 575–578, 1971.
34. BLUNDELL, T., DODSON, G., HODGKIN, D., AND MERCOLA, D.: Insulin: The structure in the crystal and its reflection in chemistry and biology. Advan. Protein Chem. **26:** 279–402, 1972.
35. BOUCHER, B. J.: Disappearance of iodine^{131}-labelled human growth hormone from the plasma of diabetic, nondiabetic and acromegalic subjects. Nature (London) **210:** 1288–1289, 1966.
36. BOURDEAU, J. E. K., CHEN, E. R. Y., AND CARONE, F. A.: Insulin uptake in the renal proximal tubule. Amer. J. Physiol. **225:** 1399–1404, 1973.
37. BRADBURY, A., SMYTH, D., SNELL, C., BIRDSALL, N., AND HULME, E.: C fragment of lipotropin has a high affinity for brain opiate receptors. Nature (London) **260:** 793–795, 1976.
38. BRAZEAU, P., RIVIER, J., VALE, W., AND GUILLEMIN, R.: Inhibition of growth hormone secretion in the rat by synthetic somatostatin. Endocrinology **94:** 184–187, 1974.
39. BRODAL, B. P., ASSEV, S., AND EEG-LARSON, N.: Degradation studies of insulin in the isolated perfused rat liver with and without blocked reticulo-endothelial system. Horm. Metab. Res. **3:** 382–386, 1971.
40. BRUSH, J. S.: Purification and characterization of a protease with specificity for insulin from rat muscle. Diabetes **20:** 140–145, 1971.
41. BURGHEN, G. A., KITABCHI, A. E., AND BRUSH, J. S.: Characterisation of a rat liver protease with specificity for insulin. Endocrinology **91:** 633–642, 1972.
42. BÜSCHER, H. H., HILL, R. C., ROMER, D., CARDINAUX, F., CLOSSE, A., HAUSER, D., AND PLESS, J.: Evidence for analgesic activity of enkephalin in the mouse. Nature (London) **261:** 423–425, 1976.
43. BUTT, W. R., RYLE, M., AND SHIRLEY, A.: Kinetic studies with ^{125}I-labelled follicle-stimulating hormone. J. Endocrinol. **58:** 275–287, 1973.
44. CALDWELL, P. R. B., SEEGAL, B. C., AND HSU, K. C.: Angiotensin-converting enzyme: Vascular endothelial localization. Science **191:** 1050–1051, 1976.
45. CAMPBELL, W. B., BROOKS, S. N., AND PETTINGER, W. A.: Angiotensin II- and angiotensin III-induced aldosterone release *in vivo* in the rat. Science **184:** 994–996, 1974.
46. CANTERBURY, J. M., BRICKER, L. A., LEVEY, G. S., KOZLOUSKIS, P. L. RUIZ, E., ZULL, J. E., AND REISS, E.: Metabolism of bovine parathyroid hormone—immunological and biological characteristics of fragments generated by liver perfusion. J. Clin. Invest. **55:** 1245–1253, 1975.
47. CANTERBURY, J. M., LEVEY, G. S., AND REISS, E.: Activation of renal cortical adenylate cyclase by circulating immunoreactive parathyroid hormone fragments. J. Clin. Invest. **52:** 524–527, 1973.
48. CANTERBURY, J. M., AND REISS, E.: Multiple immunoreactive molecular forms of parathyroid hormone in human serum. Proc. Soc. Exp. Biol. Med. **140:** 1393–1398, 1972.
49. CATHERWOOD, B., AND SINGER, F. R.: Generation of a carboxyl-terminal fragment of bovine parathyroid hormone by canine renal plasma membranes. Biochem. Biophy. Res. Commun. **57:** 469–475, 1974.
50. CATS, A., AND KASSENAAR, A. A. H.: The distribution of ^{131}I-labelled corticotrophin preparations and proteins in rats after intravenous injection. Acta Endocrinol. **24:** 35–42, 1957.
51. CHIN, A. T., AND PEACH, M. J.: Inhibition of induced aldosterone biosynthesis with specific antagonist of angiotensin II. Proc. Nat. Acad. Sci. **71:** 341–344, 1974.
52. CLARK, M. B., WILLIAMS, C. C., NATHANSON, B. M., HORTON, R. E., GLASS, H. I., AND FOSTER, G. V.: Metabolic fate of human calcitonin in the dog. J. Endocrinol. **61:** 199–210, 1974.
53. CLENDINNEN, B. G., DAVIDSON, W. D., REEDER, D. D., JACKSON, B. M., AND THOMPSON, J. C.: Renal uptake and excretion of gastrin in the dog. Gastroenterology **132:** 1039–1043, 1971.
54. CLENDINNEN, B. G., REEDER, D. D., BRANDT, E. N., AND THOMPSON, J.: Effect of nephrectomy on the rate and pattern of the disappearance of exogenous gastrin in dogs. Gut **14:** 462–467, 1973.
55. COBLE, Y., KOHLER, P. O., TULLNER, W. W., AND CARGILLI, C. M.: Clearance of iodinated human follicle-stimulating hormone in Mucaca mulatta. J. Endocrinol. **51:** 783–784, 1971.
56. COLLIPP, P. J., PATRICK, J. R., GOODHART, C., AND KAPLAN, S. A.: Distribution of tritium-labelled human growth hormone in rats and guinea pigs. Proc. Soc. Exp. Biol. Med. **121:** 173–177, 1966.
57. CORVILAIN, J., MANDERLIER, T., STRUYVEN, J., FUSS, M., BERGANS, A., NISS, N., AND BRAUMAN, H.: Metabolism of human PTH by the kidney and liver. Horm. Metab. Res. **9:** 239–242, 1977.
58. COURAN, J. S. Constancy and linearity of the metabolic clearance of adrenocorticotrophin in dogs. Can. J. Physiol. Pharmacol. **52:** 8–13, 1974.
59. COX, R. W., HENLEY, E. D., NARAHARA, H. T., VANARSDEL, P. P., JR., AND WILLIAMS, R. H.: Studies on the metabolism of glucagon-I^{131} in rats. Endocrinology **60:** 277–284, 1957.
60. CUATRECASAS, P.: Membrane receptors. Annu. Rev. Biochem. **43:** 169–214, 1974.
61. CZACZKES, J. W., AND KLEEMAN, C. R.: The effect of various states of hydration and the plasma concentration on the turnover of antidiuretic hormone in mammals. J. Clin. Invest. **43:** 1649–1658, 1964.
62. DALY, J. R., FLEISHER, M. R., CHAMBERS, D. J., BITENSKY, L., AND CHAYEN, J.: Application of the cytochemical bioassay for corticotrophin to clinical and physiological studies in man. Clin. Endocrinol. **3:** 335–345, 1974.
63. DAVIDSON, W. D., SPRINGBERG, P. D., AND FALKINBERG, N. R.: Renal extraction and excretion of endogenous gastrin in the dog. Gastroenterology **64:** 955–961, 1973.
64. DAYHOFF, M. O.: Atlas of Protein Sequence and Structure, vol. 5, suppl. 1 and 2, National Biomedical Research Foundation, Washington, D.C., 1972, 1973, 1976.
65. DEBAS, H. T., WALSH, J. H., AND GROSSMAN, M. I.: Pure human minigastrin: Secretory potency and disappearance rate. Gut **15:** 686–689, 1974.
66. DEKRETSER, D. M., ATKINS, R. C., AND PAULSEN, C. A.: Role of the kidney in the metabolism of luteinizing hormone. Endocrinology **58:** 425–434, 1973.
67. DEKRETSER, D. M., CALT, K. J., BURGER, H. G., AND SMITH, G. C.: Radioautographic studies on the localization of ^{125}-I-labelled human luteinizing and growth hormone in immature male rats. J. Endocrinol. **43:** 105–111, 1969.
68. DELUISE, M., MARTIN, T. J., GREENBERG, P. B., AND MICHELANGELI, V.: Metabolism of porcine, human and salmon calcitonin in the rat. J. Endocrinol. **53:** 475–482, 1972.
69. DELUISE, M., MARTIN, T. J., AND MELICK, R. A.: Tissue distribution of calcitonin in the rat: Comparison with parathyroid hormone. J. Endocrinol. **48:** 173–179, 1970.
70. DELUISE, M., MARTIN, T. J., AND MELICK, R. A.: Inactivation and degradation of porcine calcitonin by rat liver and relative stability of salmon calcitonin. J. Endocrinol. **48:** 181–188, 1970.
71. DENT, R. I., LEVINE, B., JAMES, J. H., HIRSCH, H., AND

FISCHER, J. E.: Effects of isolated perfused canine lung and kidney on gastrin heptadecapeptide. Amer. J. Physiol. **225:** 1038–1044, 1973.

72. DIETERLE, P., BIRKNER, B., GUEINER, K. H., WAGNER, E., ERHARDT, F., HENNER, J., AND DIETERLE, C.: Release of peripherally stored insulin during acute muscular work in man. Horm. Metab. Res. **5:** 316–322, 1973.
73. DISALVO, J., BRITTON, S., GALVAS, P., AND SANDERS, T. W.: Effects of angiotensin I and angiotensin II on canine hepatic vascular resistance. Circ. Res. **32:** 85–92, 1973.
74. DISALVO, J., AND MONTEFUSCO, C. B.: Conversion of angiotensin I to angiotensin II in the canine mesenteric circulation. Amer. J. Physiol. **221:** 1576–1579.
75. DISALVO, J., PETERSON, A., MONTEFUSCO, C., AND MENTA, M.: Intrarenal conversion of angiotensin I to angiotensin II in the dog. Circ. Res. **29:** 398–406, 1971.
76. DUCKWORTH, W. C., HEINEMANN, M., AND KITABCHI, A. E.: Proteolytic degradation of insulin and glucagon. Biochem. Biophys. Acta **377:** 421–430, 1975.
77. DUPONT, A., KASTIN, A. J., LABRIE, F., PELLETIER, G., PUVIANI, R., AND SCHALLY, A. V.: Distribution of radioactivity in the organs of the rat and mouse after injection of ^{125}-I-α-melanocyte-stimulating hormone. J. Endocrinol. **64:** 237–241, 1975.
78. DUPONT, A., LABRIE, F., LEVASSEUR, L., AND SCHALLY, A. V.: Characteristics of the plasma disappearance of tritium-labelled thyrotrophin-releasing hormone in the rat. Can. J. Physiol. Pharmacol. **52:** 1012–1019, 1974.
79. DUPONT, A., LABRIE, F., PELLETIER, G., PUVIANI, R., COY, D. H., SCHALLY, A. V., AND KASTIN, A. J.: Distribution of radioactivity in the organs of the rat and mouse after injection of L-[^{3}H]-prolyl-L-leucyl-glycinamide. J. Endocrinol. **64:** 243–248, 1975.
80. EDWARDS, G. R. W., KITAN, M. J., CHARD, T., AND BESSER, G. M.: Vasopression analogue D.D.A.V.P. in diabetes insipidus: Clinical and laboratory studies. Brit. Med. J. **3:** 375–378, 1973.
81. ELGEE, N. J., WILLIAMS, R. H., AND LEE, N. D.: Distribution and degradation studies with insulin-I^{131}. J. Clin. Invest. **33:** 1252–1260, 1954.
82. ELISEEVA, Y. E., OREKHOVICH, V. N., PAULIKHINA, L. V., AND ALEXEENKO, L. P.: Carboxycathepsin—a key regulatory component of two physiological systems involved in regulation of blood pressure. Clin. Chim. Acta **31:** 413–419, 1971.
83. EMERSON, C. H., FORHMAN, L. A., SZABO, M., AND THAKKAR, I.: TRH immunoreactivity in human urine: Evidence for dissociation from TRH. J. Clin. Endocrinol. Metab. **45:** 392–399, 1977.
84. ERDÖS, E. G.: Hypotensive peptides: Bradykinin, kallidin and eledoisin. Advan. Pharmacol. **4:** 1–90, 1966.
85. ERDÖS, E. G.: The kinins—A status report. Biochem. Pharmacol. **25:** 1563–1569, 1976.
86. ERDÖS, E. G., SLOANE, E. M., AND WOHLER, I. M.: Carboxypeptidase in blood and other fluids: Properties, distribution and partial purification of the enzyme. Biochem. Pharmacol. **13:** 893–905, 1964.
87. EVERSON, R. A.: Kinetics of adrenocorticotrophin hormone inactivation in the dog. Ph.D. Thesis, Physiology Department, University of California, Berkeley, U.S.A. University Microfilms Ltd., High Wycomb Ltd., England, 1969.
88. FABIAN, M., FORSLING, M. L., JONES, J. J., AND PRYOR, J. S.: The clearance and antidiuretic potency of neurohyphysial hormones in man and their plasma binding and stability. J. Physiol. **204:** 653–658, 1969.
89. FANG, V. S., AND TASHJIAN, A. H., JR.: Studies on the role of the liver in the metabolism of parathyroid hormone. I. Effects of partial hepatectomy and incubation of the hormone with tissue homogenates. Endocrinology **90:** 1177–1184, 1972.
90. FEDERSPIL, G., TRISOTTO, A., SCANDELLARI, C., FREZZATO, S., AND CARDIANO, C.: I^{125}-labelled glucagon metabolism in the dog. Acta Isotop. **8:** 159–163, 1968.
91. FERREIRA, S. H., AND VANE, J. R.: Disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat. Brit. J. Pharmacol. Chemother. **30:** 417–424, 1967.
92. FINE, A., THOMSON, R. M., JONES, J. V., MURRAY, R. G., TWEDDLE, A., AND GRAY, C. E.: The renal handling of insulin and thyroid hormones in normal man. Clin. Sci. Mol. Med. **50:** 435–437, 1976.
93. FINK, G.: The development of the releasing factor concept. Clin. Endocrinol. **5:** suppl. 245s–260s, 1976.
94. FRANCKSON, J. R. M., AND OOMS, H. A.: The catabolism of insulin the dog: Evidence of two catabolic pathways. Postgrad. Med. J. **49**(suppl.)**:** 931–939, 1973.
95. FREYCHET, P., KAHN, R., ROTH, J., AND NEVILLE, D. M.: Insulin interactions with liver plasma membranes: Independence of binding of the hormone and its degradation. J. Biol. Chem. **247:** 3953–3961, 1972.
96. FROESCH, E. R., BÜRGI, H., MÜLLER, W. A., HUMBEL, R. E., JAKOB, A., AND LABHART, A.: Nonsuppressible insulin-like activity of human serum: Purification, physiochemical and biological properties and its relation to total serum ILA. Recent Progr. Hormone Res. **23:** 565–616, 1967.
97. FUJITA, T., OHATA, M., OKANO, K., AND YOSHIKAWA, M.: Differential hydrolysis of bovine parathyroid hormone and its N-terminal peptide by rat kidney. Endocrinol. Jap. **22:** 39–42, 1975.
98. GANGULI, P. C., ELDER, J. B., SMITH, I. S., AND HUNTER, W. M.: The half-life of synthetic human gastrin I in man. Brit. J. Surg. **57:** 848, 1972.
99. GARDNER, R. C., ODELL, C. W. D., AND ROSS, G. T.: Comparison of $t_{\frac{1}{2}}$ of α-MSH in the rat by three methods. Clin. Res. **14:** 280, 1966.
100. GAY, V. L.: Decreased metabolism and increased serum concentrations of LH and FSH following nephrectomy of the rat. Absence of short-loop regulatory mechanisms. Endocrinology **95:** 1582–1588, 1974.
101. GINSBURG, M.: Production of the neurohypophysial hormones. Handb. Exp. Pharmak. **23:** 338–371, 1968.
102. GINSBURG, M., AND HELLER, H.: The clearance of injected vasopressin from the circulation and its fate in the body. J. Endocrinol. **9:** 283–291, 1953.
103. GINSBURG, M., AND SMITH, M. W.: The fate of oxytocin in male and female rats. Brit. J. Pharmacol. **14:** 327–333, 1959.
104. GLICK, S. M., ROTH, J., AND LONERGAN, E. T.: Survival of endogenous growth hormone in plasma. J. Clin. Endocrinol. Metab. **24:** 501–505, 1964.
105. GONZALEZ-PANIZZA, V. M., SICA-BLANCO, Y., AND MENDEZ-BAUER, C. J.: Fate of injected oxytocin in the pregnant woman near term. *In* Oxytocin, ed. by R. Caldeyro-Barcia and H. Heller, pp. 347–357, Pergamon Press, London, 1961.
106. GOODFRIEND, T. L., AND PEACH, M. J.: Angiotensin III: (DesAspartic acid^1)-angiotensin II. Evidence and speculation for its role as an important agonist in the renin-angiotensin system. Circ. Res. **36, 37**(suppl.)**:** I-38-I-48, 1975.
107. GOOS, H. J. T., DEGRAAN, P., AND VAN OORDT, P. G. W. J.: Half-life of melanocyte-stimulating hormone and hormone content of the pars intermedia in relation to background adaptation time in xenopus laevis. J. Endocrinol. **72:** 26p–27p, 1977.
108. GREENBERG, P. B., MARTIN, T. J., MELICK, R. A., JABLONSKI, P., AND WATTS, J. M.: Calcitonin clearance in the pig and in the isolated perfused pig liver. J. Endocrinol. **54:** 125–135, 1972.
109. GRIFFITHS, E. C., HOOPER, K. C., AND HOPKINSON, C. R. N.: Evidence for an enzymic component in the rat hypothalamus capable of inactivating luteinizing hormone releasing factor (LFR). Acta Endocrinol. **74:** 49–55, 1973.
110. GROSSMAN, M. I.: Gastrointestinal hormones. *In* Peptide Hormones, ed. by J. A. Parsons, pp. 105–117,

Macmillan Press Ltd., London and Basingstoke, 1976.
111. GROSVENOR, C. E.: Disappearance rate of exogenous prolactin from serum of female rats. Endocrinology **80:** 195–200, 1967.
112. HABENER, J. F., KEMPER, B. W., RICH, A., AND POTTS, J. T.: Biosynthesis of parathyroid hormone. Rec. Progr. Hormone Res. **33:** 249–308, 1977.
113. HABENER, J. F., MAYER, G. P., DEE, P. C., AND POTTS, J. T., JR.: Metabolism of amino- and carboxyl-sequence immunoreactive parathyroid hormone in the bovine: Evidence for peripheral cleavage of hormone. Metabolism **25:** 385–395, 1976.
114. HABENER, J. F., POWELL, D., MURRAY, T. M., MAYER, G. P., AND POTTS, J. T., JR.: Parathyroid hormone: Secretion and metabolism *in vivo*. Proc. Nat. Acad. Sci. **68:** 2986–2991, 1971.
115. HABENER, J. F., SEGRE, G. V., POWELL, D., MURRAY, T. M., AND POTTS, J. T., JR.: Immunoreactive parathyroid hormone in circulation of man. Nature New Biol. **238:** 152–154, 1972.
116. HABENER, J. F., SINGER, F. R., DEFTOS, L. J., NEER, R. M., AND POTTS, J. T., JR.: Explanation for unusual potency of salmon calcitonin. Nature New Biol. **232:** 91–92, 1971.
117. HABENER, J. F., SINGER, F. R., DEFTOS, L. J., AND POTTS, J. T., JR.: Immunological stability of calcitonin in plasma. Endocrinology **90:** 952–960, 1972.
118. HABENER, J. F., SINGER, F. R., NEER, R. M., DEFTOS, L. J., AND POTTS, J. T., JR.: Metabolism of salmon and porcine calcitonin: An explanation for the increased potency of salmon calcitonin. *In* Calcium, Parathyroid Hormone and the Calcitonins, Proceedings of the Fourth Parathyroid Conference, ed. by R. V. Talmage and P. L. Munson, pp. 152–156, Excerpta Medica Foundation, Amsterdam, 1972.
119. HAMBROOK, J. M., MORGAN, B. A., RANCE, M. J., AND SMITH, C. F. C.: Mode of deactivation of the enkephalins by the rat and human plasma and rat brain homogenates. Nature (London) **262:** 782–783, 1976.
120. HARDING, P. E., BLOOM, G., AND FIELD, J. B.: Effect of insulin into portal vein on hepatic extraction of insulin in anaesthetized dogs. Amer. J. Physiol. **228:** 1580–1588, 1975.
121. HARRIS, G. W.: Humours and hormones. J. Endocrinol. **53:** ii–xxiii, 1972.
122. HARVEY, R. F., DOWSETT, L., HARTOG, M., AND READ, A. E.: A radioimmunoassay for cholecystokinin-pancreozymin. Lancet **2:** 826–828, 1973.
123. HELLER, H., AND GINSBURG, M.: Secretion, metabolism and fate of the posterior pituitary hormones. *In* The Pituitary Gland, ed. by G. W. Harris and B. T. Donovan, vol. 3 pp. 330–373, Butterworth, London, 1966.
124. HICKS, E. P., COOPER, C. W., AND WADDELL, W. J.: Whole body autoradiography of the distribution of ^{125}I-thyrocalcitonin in mice. J. Dent. Res. **50:** 1307–1331, 1971.
125. HJELMQUIST, U. B., REEDER, D. D., BRANDT, E. N., AND THOMPSON, J. C.: Effect of the kidney on endogenous gastrin. Surg. Forum **23:** 318–320, 1972.
126. HODGE, R. L., NG, K. K. F., AND VANE, J. R.: Disappearance of angiotensin from the circulation of the dog. Nature (London) **215:** 138–141, 1967.
127. HRUSKA, K. A., KOPELMAN, W. E., RUTHERFORD, W. E., KLAHR, S., AND SLATOPOLSKY, E.: Metabolism of immunoreactive parathyroid hormone in the dog. The role of the kidney and the effects of chronic renal disease. J. Clin. Invest. **56:** 39–58, 1975.
128. HRUSKA, K. A., MARTIN, K., MENNES, P., GREENWATT, A., ANDERSON, C., KLAHR, S., AND SLATOPOLSKY, E.: Degradation of parathyroid hormone and fragment production by the isolated perfused dog kidney. J. Clin. Invest. **60:** 501–510, 1977.
129. HSU, H. H. T., AND HAYMOVITS, A.: Nature of degradation of calcitonin by mammalian cells. Proc. Soc. Exp. Biol. Med. **146:** 1044–1049, 1974.
130. HUDSON, A. M.: Mechanisms of inactivation of Synacthen (corticotrophin-(1-24)-tetracosapeptide) and adrenocorticotrophic hormone in the rat *in vivo*. Ph.D. Thesis, Brunel University, Uxbridge, London, 1978.
131. HUDSON, A. M., BENNETT, H. P. J., AND MCMARTIN, C.: Metabolism of tritiated 1-24 corticotrophin in the rat: Extraction and identification of fragments in the circulation. J. Endocrinol., **72:** 58p–59p, 1977.
132. HUDSON, A. M., AND MCMARTIN, C.: Metabolism of tritiated 1-24 corticotrophin in peripheral tissues, immediately following intravenous injection in the rat. J. Endocrinol. in press, 1979.
133. HUDSON, D., PICKERING, A., MCLOUGHLIN, J. L., MATTHEWS, E., SHARPE, R., FINK, G., MACINTYRE, I., AND SZELKE, M.: The synthesis and metabolic fate of 3-[^{14}C]-Trp, 9-[^{14}C]Pro-LHRH. Vth Int. Congr. Endocrinol. Proc. Abstr. 43, p. 18., Hamburg, 1976.
134. HUGHES, J., SMITH, T. W., KOSTERLITZ, H. W., FOTHERGILL, L. A., MORGAN, B. A., AND MORRIS, H. R.: Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature (London) **258:** 577–579, 1975.
135. HUNTER, W. M., AND GREENWOOD, F. C.: A radio-immunoelectrophoretic assay for human growth hormone. Biochem. J. **91:** 43–56, 1964.
136. IMURA, H., MATSUYAMA, H., MATSUKURA, S., MIYAKE, T., AND FUKASE, M.: Stability of ACTH preparations in human plasma incubated *in vitro*. Endocrinology **80:** 599–602, 1967.
137. IZZO, J. L., BARTLETT, J. W., RONCONE, A., IZZO, M. J., AND BALE, W. F.: Physiological processes and dynamics in the disposition of small and large doses of biologically active and inactive ^{131}I-insulin in the rat. J. Biol. Chem. **242:** 2343–2355, 1967.
138. JASPAR, J. B., AND RUBENSTEIN, A. H.: Circulating glucagon: Plasma profiles and metabolism in health and disease. Diabetes **26:** 887–904, 1977.
139. JEFFCOATE, S. L., GREENWOOD, R. H., AND HOLLAND, D. T.: Blood and urine clearance of luteinizing hormone releasing hormone in man measured by radioimmunoassay. J. Endocrinol. **60:** 305–314, 1974.
140. JOHKE, T.: Radioimmunoassay for bovine prolactin in plasma. Endocrinol. Jap. **16:** 581–589, 1969.
141. JOHNSON, L. R.: Gastrointestinal hormones and their functions. Annu. Rev. Physiol. **39:** 135–158, 1977.
142. JONES, C. T., LUTHER, E., RITCHIE, J. W. K., AND WORTHINGTON, D.: The clearance of ACTH from the plasma of adult and fetal sheep. Endocrinology **96:** 231–234, 1975.
143. KAKIUCHI, S., AND TOMIZAWA, H. H.: Properties of glucagon-degrading enzyme of beef liver. J. Biol. Chem. **239:** 2160–2164, 1964.
144. KASSENAAR, A. A. H., LAMEYER, L. D. F., AND QUERIDO, A.: The distribution of injected heterologous thyrotrophic hormone in the rat. Acta Endocrinol. **21:** 32–36, 1956.
145. KATZ, A. I., AND RUBENSTEIN, A. H.: Metabolism of proinsulin, insulin and C-peptide in the rat. J. Clin. Invest. **52:** 1113–1121, 1973.
146. KEMPER, B., HABENER, J. F., ERNST, M. D., POTTS, J. T., JR., AND RICH, A.: Pre-proparathyroid hormone: Analysis of radioactive tryptic peptides and amino acid analysis. Biochemistry **15:** 15–19, 1976.
147. KENNY, A. J., FULLARTON, J. R., GEORGE, S. G., AND KERR, M. A.: The degradation of peptide hormones. *In* Intracellular Protein Catabolism, Proceedings of the Symposium, ed. by H. Hanson and P. Bohley, pp. 291–305, Martin Luther University, Halle, G. D. R., 1976.
148. KEYE, W. R., JR., KELCH, R. P., NISWENDER, G. D., AND JAFFE, R. B.: Quantitation of endogenous gonadotrophin-releasing hormone by radioimmunoassay. J. Clin. Endocrinol. Metab. **36:** 1263–1267, 1973.
149. KJELD, J. M., HARSOULIS, P., KUKU, S. F., MARSHALL, J. C., KAUFMAN, B., AND FRASER, T. R.: Infusions of LFSH and LLH in normal men. 1. Kinetics of human

follicle stimulating hormone. Acta Endocrinol. **81:** 225–233, 1976.

150. KOCH, Y., BAROM, T., CHOBSIENG, P., AND FRIDKIN, M.: Enzymic degradation of luteinizing hormone-releasing hormone (LH-RH) by hypothalamic tissue. Biochem. Biophys. Res. Commun. **61:** 95–103, 1974.
151. KOCH, Y., CHOW, Y. F., AND MEITES, J.: Metabolic clearance and secretion rates of prolactin in the rat. Endocrinology **89:** 1303–1308, 1967.
152. KORMAN, M. G., HANSKY, J., RITCHIE, B. C., WATTS, J. M., AND MALONEY, J. E.: Disappearance of gastrin across the lung. Aust. J. Exp. Biol. Med. Sci. **51:** 679–687, 1973.
153. KORMAN, M. G,, LAVER, M. C., AND HANSKY, J.: Hypergastrinaemia in chronic renal failure. Brit. Med. J. **1:** 209–210, 1972.
154. KREYE, J. A. W., AND GROSS, F.: Conversion of angiotensin I to angiotensin II in peripheral vascular beds of the rat. Amer. J. Physiol. **220:** 1294–1296, 1971.
155. KURA, H. G., FELTKAMP, C. A., VAN DER GUGTEN, A. A., AND VERHOFSTAD, F.: The rate of elimination of prolactin as a determinant factor for plasma levels assayed in rats. J. Endocrinol. **48:** 299–300, 1970.
156. LASTER, D. B.: Disappearance and uptake of iodine-125-labelled FSH in rat, rabbit, ewe and cow. J. Reprod. Fert. **30:** 407–415, 1972.
157. LASTER, L., AND WALSH, J. H.: Enzymatic degradation of the C-terminal tetrapeptide amide of gastrin by mammalian tissue extracts. Fed. Proc. **27:** 1328–1330, 1968.
158. LAUSON, H. D.: Metabolism of antidiuretic hormones. Amer. J. Med. **42:** 713–744, 1967.
159. LEARY, W. P., AND LEDINGHAM, J. G.: Removal of angiotensin by isolated perfused organs of the rat. Nature (London) **222:** 959–960, 1969.
160. LEARY, W. P., AND LEDINGHAM, J. G.: Inactivation of angiotensin analogues by isolated perfused rat liver and kidney. Nature (London) **227:** 178–179, 1970.
161. LEFEBVRE, P. J., AND LUYCKX, A. S.: Effect of acute kidney exclusion by ligation of renal arteries on peripheral plasma glucagon levels and pancreatic glucagon production in the anesthetized dog. Metabolism **24:** 1169–1176, 1975.
162. LEFEBVRE, P. J., LUYCKX, A. S., AND NIZET, A. H.: Renal handling of endogenous glucagon in the dog. Comparison with insulin. Metabolism **23:** 753–761, 1974.
163. LEGGATE, L., CARE, A. D., AND FRAZER, S. C.: Evidence for thyrocalcitonin binding to protein in plasma. J. Endocrinol. **43:** 73–81, 1969.
164. LEHNERT, P., STAHLHEBER, H., FORELL, M. M., FRITZ, H., AND WERLE, E.: Kinetics of exocrine pancreatic secretion. Digestion **6:** 9–22, 1972.
165. LEHNERT, P., STAHLHEBER, H., FORELL, M. M., FÜLLNER, R., FRÜHAUF, S., FRITZ, H., HUTZEL, M., AND WERLE, E.: Studies on the elimination of secretin and cholecystokinin with regard to the kinetics of exocrine pancreatic secretion. Digestion **11:** 51–63, 1974.
166. LEPPÄLUOTE, J., VIRKKUNEN, P., AND LYBECK, H.: Elimination of TRH in man. J. Clin. Endocrinol. Metab. **35:** 477–478, 1972.
167. LEVI, J., ROSENFELD, S., AND KLEEMAN, C. R.: Inactivation of arginine-vasopressin by the isolated rabbit kidney. J. Endocrinol. **62:** 1–10, 1974.
168. LI, C. H., AND CHUNG, D.: Isolation and structure of an untriacintapeptide with opiate activity from camel pituitary glands. Proc. Nat. Acad. Sci. **73:** 1145–1148, 1976.
169. LING, N., BURGUS, R., AND GUILLEMIN, R.: Isolation, primary structure, and synthesis of α-endorphin and γ-endorphin, two peptides of hypothalamic-hypophysial origin with morphinomimetic activity. Proc. Nat. Acad. Sci. **73:** 3942–3446, 1976.
170. LITTLE, J. B., KLEVAY, L. M., RADFORD, E. P., AND MCGANDY, R. B.: Antidiuretic hormone inactivation by isolated perfused rat liver. Amer. J. Physiol. **211:** 786–792, 1966.
171. MACFARLANE, R. G.: An enzyme cascade in the blood clotting mechanism, and its function as a biological amplifier. Nature (London) **202:** 498–499, 1964.
172. MAIER, R., BARTHE, P. L., SCHENKEL-HULLIGER, L., AND DESAULLES, P. A.: The biological activity of 1-D-serine 17,18-dilysine-β-corticotrophin-(1-18)-octadecapeptide amide. Acta Endocrinol. **68:** 458–466, 1971.
173. MAIER, R., BRUGGER, M., BRUCKNER, H., RINIKER, B., AND RITTEL, W.: Analogues of human calcitonin. V. Influence of basic amino acids in positions 11, 17 and 24 on hypocalcaemic activity in the rat. Acta Endocrinol. **85:** 102–108, 1977.
174. MAIER, R., KAMBER, B., RINIKER, B., AND RITTEL, W.: Analogues of human calcitonin. II. Influence of modifications in amino acid positions, 1, 8 and 22 on hypocalcaemic activity in the rat. Horm. Metab. Res. **7:** 511–514, 1975.
175. MAIER, R., KAMBER, B., RINIKER, B., AND RITTEL, W.: Analogues of human calcitonin. IV. Influence of leucine substitution in positions 12, 16 and 19 on hypocalcaemic activity in the rat. Clin. Endocrinol. **5**(suppl.): 327s–332s, 1976.
176. MAIER, R., RINIKER, B., AND RITTEL, W.: Analogues of human calcitonin. I. Influence of modifications in amino acid positions 29 and 31 on hypocalcaemic activity in the rat. Fed. Eur. Biochem. Soc. Lett. **48:** 68–71, 1974.
177. MAINS, R. E., EIPPER, B. A., AND LING, N.: Common precursor to corticotrophins and endorphins. Proc. Nat. Acad. Sci. **74:** 3014–3018, 1977.
178. MARKS, N., GRYNBAURN, A., AND NEIDLE, A.: On the degradation of enkephalins and endorphins by rat and mouse brain extracts. Biochem. Biophys. Res. Commun. **74:** 1552–1559, 1977.
179. MARKS, N., STERN, F., AND BENUCK, M.: Correlation between biological potency and biodegradation of a somatostatin analogue. Nature (London) **261:** 511–512, 1976.
180. MARKS, N., AND STERN, F.: Enzymatic mechanisms for the inactivation of luteinizing hormone-releasing hormone (LH-RH). Biochem. Biophys. Res. Commun. **61:** 1458–1463, 1974.
181. MARKS, N., AND STERN, F.: Inactivation of somatostatin (GH-RIH) and its analogs by crude and partially purified rat brain extracts. Fed. Eur. Biochem. Soc. Lett. **55:** 220–224, 1975.
182. MARSHALL, J. C., ANDERSON, D. C., RUSSELL-FRASER, T., AND HARSOULIS, P.: Human luteinizing hormone in man: Studies of metabolism and biological action. J. Endocrinol. **56:** 431–439, 1973.
183. MARTIN, K. J., HRUSKA, K. A., GREENWALT, A., KLAHR, S., AND SLATOPOLSKY, E.: Selective uptake of intact parathyroid hormone by the liver. Differences between hepatic and renal uptake. J. Clin. Invest. **58:** 781–788, 1976.
184. MARTIN, K. J., HRUSKA, K. A., LEWIS, J., ANDERSON, C., AND SLATOPOLSKY, E.: The renal handling of parathyroid hormone. The role of peritubular uptake and glomerular filtration. J. Clin. Invest. **60:** 808–814, 1977.
185. MARTIN, T. J., MELICK, R. A., AND DELUISE, M.: Metabolism of parathyroid hormone degradation of ^{125}I-labelled hormone by a kidney enzyme. Biochem. J. **111:** 509–514, 1969.
186. MARTIN, T. J., MELICK, R. A., AND DELUISE, M.: The effect of nephrectomy on the metabolism of labelled parathyroid hormone. Clin. Sci. **37:** 137–142, 1969.
187. MARX, S. J., WOODWARD, C. J., AND AURBACH, G. D.: Calcitonin receptors of kidney and bone. Science **178:** 999–1001, 1972.
188. MATSUYAMA, H.: Studies on the inactivation of adrenocorticotrophic hormone (ACTH). Part 1: Inactivation of ACTH in human plasma *in vitro* and relationship between structure and activity of ACTH. Folia Endocrinol. Jap. **45:** 163–172, 1969.
189. MATSUYAMA, H.: Studies on the inactivation of adrenocorticotrophic hormone (ACTH). Part 2. Distributions

in vivo of ^{131}I-labelled ACTH, ^{3}H-labelled ACTH and unlabelled natural ACTH in rats. Folia Endocrinol. Jap. **45:** 173–180, 1969.

190. MATSUYAMA, H., RUHMANN-WENNHOLD, A., JOHNSON, L. R., AND NELSON, D. H.: Disappearance rates of exogenous and endogenous ACTH from rat plasma measured by bioassay and radioimmunoassay. Metabolism **21:** 30–35, 1972.
191. MATSUYAMA, T.: Experimental research on exogenous gastrin metabolism. Hiroshima Daigaku/gaku Zasshi **22:** 569–594, 1974.
192. HAYBERRY, H. E., VAN DEN BRANDE, J. L., VAN WYK, J. J., AND WADDELL, W. J.: Early localization of ^{125}I-labelled human growth hormone in adrenals and other organs of immature hypophysectomized rats. Endocrinology **88:** 1309–1317, 1971.
193. MCDONALD, J. K., CALLAHAN, P. X., ZEITMAN, B. B., AND ELLIS, S.: Inactivation and degradation of glucagon by dipeptidyl aminopeptidase 1 (cathepsin C) of rat liver. J. Biol. Chem. **244:** 6199–6208, 1969.
194. MCMARTIN, C., AND PETERS, J.: Levels of corticotrophin analogues in the blood after infusion into rats. J. Endocrinol. **67:** 41–48, 1975.
195. MCMARTIN, C., AND PURDON, G. E.: Early fate of somatostatin in the circulation of the rat following intravenous injection. J. Endocrinol. **77:** 67–74, 1978.
196. MCMARTIN, C., PURDON, G. E. E., SCHENKEL, L., DESAULLES, P. A., MAIER, R., BRUGGER, M., RITTEL, W., AND SEIBER, P.: Differences between *in vitro* and *in vivo* potencies of corticotrophins: An interpretation in terms of metabolic stability. J. Endocrinol. **73:** 79–89, 1977.
197. MEAKIN, J. W., TINGEY, W. H., AND NELSON, D. H.: The catabolism of adrenocorticotrophic hormone: The stability of adrenocorticotrophic hormone in blood, plasma, serum and saline. Endocrinology **66:** 59–72, 1960.
198. MERCHANT, F. J., BOMBECK, C. T., PISSIDIS, A., AND NYHUS, L. M.: Hepatic destruction of synthetic gastrin pentapeptide. J. Nat. Med. Assoc. **64:** 246–249, 1972.
199. MILHAUD, G., AND SZAMKA, I.: Specific calcitonin-binding proteins in man. Experientia (Basel) **27:** 1335–1336, 1971.
200. MIRSKY, I. A., AND PERISUTTI, G.: The relative specificity of the insulinase activity of rat liver extracts. J. Biol. Chem. **228:** 77–83, 1957.
201. MIRSKY, I. A., PERISUTTI, G., AND DAVIS, N. C.: The destruction of glucagon, adrenocorticotrophin and somatotrophin by human plasma. J. Clin. Invest. **38:** 14–20, 1959.
202. MIYACHI, Y., MECKLENBURG, R. S., HANSEN, J. W., AND LIPSETT, M. B.: Metabolism of iodine ^{125}-labelled LHRH. J. Clin. Endocrinol. Metab. **37:** 63–67, 1973.
203. MORELL, A. G., GREGORIADIS, G., SCHEINBERG, I. H., HICKMAN, J., AND ASHWELL, G.: The role of sialic acid in determining the survival of glycoproteins in the circulation. J. Biol. Chem. **246:** 1461–1467, 1971.
204. MOTOKI, T., KATO., Y., KAMII, K., MIGITA, T., KAMEDO, H., AND MURAO, S.: Distribution studies with iodine-^{125}-labelled synthetic human gastrin in rats. Radioisotopes **22:** 659–661, 1973.
205. MURPHY, S. S., DONALD, R. A., AND NABARRO, J. D. N.: The half-life of porcine corticotrophin in pigs. Acta Endocrinol. **61:** 525–532, 1969.
206. NAIR, R. M. G., REDDING, T. W., KASTIN, A. J., AND SCHALLY, A. V.: Site of inactivation of melanocyte-stimulating hormone-releasing-inhibiting hormone by human plasma. Biochem. Pharmacol. **22:** 1915–1919, 1973.
207. NAIR, R. M. G., REDDING, T. W., AND SCHALLY, A. V.: Site of inactivation of thyrotrophin-releasing hormone by human plasma. Biochemistry **10:** 3621–3624, 1971.
208. NARAHARA, H. T., EVERETT, N. B., SCIMMONS, B. S., AND WILLIAMS, R. H.: Metabolism of insulin-^{131}I and glucagon-^{131}I in the kidney of the rat. Amer. J. Physiol. **192:** 227–231, 1958.
209. NARAHARA, H. T., AND WILLIAMS R. H.: Degradation of glucagon-^{131}I by rat tissues *in vitro*. Endocrinology **60:** 285–289, 1957.
210. NEUMAN, W. F., NEUMAN, M. W., LANE, K., MILLER, L., AND SAMMON, P. J.: Metabolism of labelled parathyroid hormone. V. Collected biological studies. Calcified Tissue Res. **18:** 271–287, 1975.
211. NEWSOME, F. E., O'DOR, R. K., PARKES, C. O., AND COPP, D. H.: A study of the stability of calcitonin biological activity. Endocrinology **92:** 1102–1106, 1973.
212. NEWTON, W. T., AND JAFFE, B. M.: The fate of intravenously administered radiolabelled gastrin. Surgery **69:** 34–40, 1971.
213. NG, K. K. F., AND VANE, J. R.: Fate of angiotensin I in the circulation. Nature (London) **218:** 144–150, 1968.
214. OLOWA-OKORUN, M. O., AND AMURE, B. O.: The role of the kidney in gastrin metabolism in the rat. Experientia (Basel) **30:** 1029–1030, 1974.
215. OPARIL, S., AND BAILIE, M. D.: Mechanism of renal handling of angiotensin II in the dog. Circ. Res. **33:** 500–507, 1973.
216. OPARIL, S., SANDERS, C. A., AND HABER, E.: In-vivo and in-vitro conversion of angiotensin I to angiotensin II in dog blood. Circulation **26:** 511–519, 1970.
217. ORIMO, H., FUJITA, T., MORII, H., AND NAKAO, K.: Inactivation *in vitro* of parathyroid hormone activity by kidney slices. Endocrinology **76:** 255–258, 1965.
218. PARKER, M. L., UTIGER, R. D., AND DAUGHADAY, W. H.: Studies on human growth hormone. II. Physiological disposition and metabolic fate of human growth hormone in man. J. Clin. Invest. **41:** 262–268, 1962.
219. PIMSTONE, B., EPSTEIN, S., HAMILTON, S. M., LEROITH D., AND HENDRICKS, S.: Metabolic clearance and plasma half disappearance time of exogenous gonadotrophin releasing hormone in normal subjects and in patients with liver disease and chronic renal failure. J. Clin. Endocrinol. Metab. **44:** 356–360, 1977.
220. PIMSTONE, B. L., LEROITH, D., EPSTEIN, S., AND KRONHEIM, S.: Disappearance rates of plasma growth hormone after intravenous somatostatin in renal and liver disease. J. Clin. Endocrinol. Metab. **41:** 392–395, 1975.
221. POHL, S. L., KRANS, H. M. J., BIRNBAUMER, L., AND RODBELL, M.: Inactivation of glucagon by plasma membranes of rat liver. J. Biol. Chem. **247:** 2295–2230, 1972.
222. POTTS, J. T.: Chemistry and physiology of parathyroid hormone. Clin. Endocrinol. **5:** 307–315S, 1976.
223. RABKIN, R., PIMSTONE, B. L., EALES, L., AND CHRISTIE, A.: Autoradiographic demonstration of glomerular filtration and proximal tubular absorption of iodine-^{125}-labelled growth hormone in the mouse. Horm. Metab. Res. **5:** 172–175, 1973.
224. RADO, J. P., MAROSI, J., SZENDE, L., BORBELY, L., TAKO, J., AND FISCHER, J.: The antidiuretic action of 1-desamino-8-O-arginine vasopressin (DDAVP) in man. Int. J. Clin. Pharmacol. Biopharmacol. **13:** 199–209, 1976.
225. RAJANIENI, H., OKSANEN, A., AND VANHA-PERTTULA, T.: Distribution of iodine^{125}-labelled prolactin in mice and rats. Studies with whole-body and microautoradiography. Horm. Res. **5:** 6–20, 1974.
226. RASIO, E. A., MACK, E., EGDAHL, R. H., AND HERRERA, M. G.: Passage of insulin and insulin across vascular membranes in the dog. Diabetes **17:** 668–672, 1968.
227. RAYFORD, P. L., FENDER, H. R., RANUS, N. I., REEDER, D. D., AND THOMPSON, J. C.: Release and half-life of CCK in man. *In* Gastrointestinal Hormones, ed. by J. C. Thompson, pp. 301–318, University of Texas Press, Austin, 1975.
228. RAYFORD, P. L., AND THOMPSON, J. C.: Gastrin. Surg. Gynecol. Obstet. **145:** 257–268, 1977.
229. REDDING, T. W., KASTIN, A. J., GONZALEZ-BARZENA, D., COY, D. H., COY, E. H., SCHALCH, D. S., AND SCHALLY, A. V.: The half-life, metabolism and excretion of tritiated luteinizing hormone-releasing hormone (LH-RH). J. Clin. Endocrinol. Metab. **37:** 626–631, 1973.

230. REDDING, T. W., KASTIN, A. J., GONZALEZ-BARZENA, D., COY, D. H., HIROTSU, Y., RUELAS, J., AND SCHALLY, A. V.: Disappearance, excretion and metabolism of tritiated prolyl-leucyl-glycinamide in man. Neuroendocrinology **16:** 119–126, 1974.

231. REDDING, T. W., KASTIN, A. J., NAIR, R. M. G., AND SCHALLY, A. V.: Distribution, half-life and excretion of carbon-14-and tritium-labelled L-prolyl-L-leucyl-glycinamide in the rat. Neuroendocrinology **11:** 92–100, 1973.

232. REDDING, T. W., AND SCHALLY, A. V.: Studies on the inactivation of thyrotrophin-releasing hormone (TRH). Proc. Soc. Exp. Biol. Med. **131:** 415–420, 1969.

233. REDDING, T. W., AND SCHALLY, A. V.: Half-life of thyrotrophin-releasing hormone in rats. Neuroendocrinology **9:** 250–256, 1972.

234. REDDING, T. W., AND SCHALLY, A. V.: The distribution, half-life and excretion of tritiated luteinizing hormone-releasing hormone (LH-RH) in rats. Life Sci. **12:** 23–32, 1973.

235. REEDER, D. D., JACKSON, B. M., BRANDT, E. N., JR., AND THOMPSON, J. C.: Rate and pattern of disappearance of exogenous gastrin in dogs. Amer. J. Physiol. **222:** 1571–1574, 1972.

236. REES, L. H., RATCLIFFE, J. G., BESSER, G. M., KRAMER, R., LANDON, J., AND CHAYEN, J.: Comparison of the redox assay for ACTH with previous assays. Nature New Biol. **241:** 84–85, 1973.

237. REGOLI, D., RINNIKER, B., AND BRUNNER, H.: The enzymatic degradation of various angiotensin II derivatives by serum, plasma or kidney homogenate. Biochem. Pharmacol. **12:** 637–646, 1963.

238. REGOLI, D., PARK, W. K., AND RIOUX, F.: Pharmacology of angiotensin. Pharmacol. Rev. **26:** 69–123, 1974.

239. RICHARDS, J. B., AND SAYERS, G.: Fate and excretion of adrenocotricotrophic hormone. Proc. Soc. Exp. Biol. Med. **77:** 87–93, 1951.

240. RIDGWAY, E. C., SINGER, F. R., WEINSTAUB, B. D., LORENZ, L., AND MALOOF, F.: Metabolism of human thyrotrophin in the dog. Endocrinology **95:** 1181–1185, 1974.

241. RIGGS, L., ARNAUD, C. D., GOLDSMITH, R. S., TAYLOR, W. F., MCCALL, J. T., AND SESSLER, A. D.: Plasma kinetics and acute effects of pharmacological doses of porcine calcitonin in man. J. Clin. Endocrinol. Metab. **33:** 115–127, 1971.

242. RIVIER, J., BROWN, M., RIVIER, C., LING, N., AND VALE, W.: Hypothalamic hypophysiotrophic hormones. Review on the design of synthetic analogues. *In* Peptides, 1976, ed. by A. Loffet, pp. 427–461, University of Brussels, Belgium, 1976.

243. RIVIER, J., BROWN, M., AND VALE, W.: D-Trp^{8}-somatostatin: An analog of somatostatin more potent than the native molecule. Biochem. Biophys. Res. Commun. **65:** 746–751, 1975.

244. ROCHMAN, H., VARNAVIDES, L. A., AND HAWLEY, P. R.: The degradation of human placental lactogen in normal and nephrectomized dogs. J. Endocrinol. **53:** 407–417, 1972.

245. ROEMER, D., BUESCHER, H. H., HILL, R. C., PLESS, J., BAUER, W., CARDINAUX, F., CLOSSE, A., HAUSER, D., AND HUGUENIN, R.: A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. Nature (London) **268:** 547–549, 1977.

246. ROJDMARK, S., BLOOM, G., CHOU, M. C. Y., AND FIELD, J. B.: Hepatic entraction of enogenous insulin and glucagon in the dog. Endocrinology **102:** 806–813, 1978.

247. RUBENSTEIN, A. H., STEINER, D. F., HORWITZ, D. L., MAKO, M. E., BLOCK, M. B., STARR, J. I., KUZUYA, H., AND MELANI, F.: Clinical significance of circulating proinsulin and C-peptide. Recent Progr. Hormone Res. **33:** 435–475, 1977.

248. RUBENSTEIN, A. H., ZWI, S., AND MILLER, R.: Insulin and the lung. Diabetes **4:** 236–238, 1968.

249. RYAN, J. W., ROBLERO, J., AND STEWART, J. M.: Inactivation of bradykinin in rat lung. Advan. Exp. Med. Biol. **8:** 263, 1970.

250. RYAN, J. W., SMITH, V., AND NIEMEYER, R. S.: Angiotensin I: Metabolism by plasma membrane of lung. Science **176:** 64–66, 1972.

251. RYAN, J. W., RYAN, V. S., SCHULTZ, D. R., WHITAKER, C., CHUNG, A., AND DORER, F. E.: Subcellular localisation of pulmonary angiotensin-converting enzyme (kininase II). Biochem. J. **146:** 497–499, 1975.

252. SAAMELI, K., AND ESKES, T. K. A. B.: Bradykinin and cardiovascular system: Estimation of half-life. Amer. J. Physiol. **203:** 261–265, 1962.

253. SAKUMA, M., NAKAZATO, F., KIMURA, M., FUGIWARA, M., SATO, Y., MIYAI, K., AND KUMAHARA, Y.: Biological fate of thyrotrophin-releasing hormone. Nippon Naibumpi Gukkai Zasshi **49:** 1186–1197, 1973.

254. SALMON, S., UTIGER, R., PARKER, M., AND REICHLIN, S.: The fate of ^{131}I-labelled human growth hormone in the rabbit. Endocrinology **70:** 459–464, 1962.

255. SAMOLS, E., AND RYDER, J. A.: Studies on tissue uptake of insulin in man using a differential immunoassay for endogenous and enogenous insulin. J. Clin. Invest. **40:** 2092–2102, 1961.

256. SANDOW, J., ENZMANN, F., SCHRODER, H. G., AND VOGEL, H. G.: Inactivation of LH-RH by the plasma of various species. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **274:** suppl. R95, 1972.

257. SANDOW, J., HEPTNER, W., AND VOGEL, H. G.: *In vivo* inactivation of synthetic LH-RH luteinizing hormone-releasing hormone. *In* Hypothalmic Hypophysiotrophic Hormones, Proceedings of the Conference. 1972, ed. by C. Gual and E. Rosenberg, pp. 64–67, Excerpta Medica, Amsterdam, 1973.

258. SCHRUMPF, E., AND SENB, L. S.: The metabolic clearance rate and half-life of synthetic human gastrin in dogs. Scand. J. Gastroenterol. **8:** 203–207, 1973.

259. SCHRUMPF, E., SENB, L. S., AND VOLD, H.: Metabolic clearance and disappearance rates of synthetic human gastrin in man. Scand. J. Gastroenterol. **8:** 731–734, 1973.

260. SCHWARTZ, I. L.: Neurohypophysial hormones: Old and new slants on the relationship of chemical structure to biological activity. *In* The Chemistry of Polypeptides, ed. by P. G. Katsoyannis, pp. 297–313, Plenum Press, New York and London, 1973.

261. SCOTT, A. P., RATCLIFFE, J. G., REES, L. H., LONDON, J., BENNETT, H. P. J., LOWRY, P. J., AND MCMARTIN, C.: Pituitary peptide. Nature New Biol. **244:** 65–67, 1973.

262. SEELIG, H. P., MÜLLER, U., AND LINTHE, H.: Inactivation of gastrin II by rat tissue homogenates, human serum and liver amidases. Acta Hepato-Gastroenterol. **20:** 142–149, 1973.

263. SEGRE, G. V., D'AMOUR, P., AND POTTS, J. T., JR.: Metabolism of radioiodinated bovine parathyroid hormone in the rat. Endocrinology **99:** 1645–1652, 1976.

264. SEGRE, G. V., HABENER, J. F., POWELL, D., TREGEAR, G. W., AND POTTS, J. T.: Parathyroid hormone in human plasma: Immunochemical characterization and biological implications. J. Clin. Invest. **51:** 3163–3172, 1972.

265. SEGRE, G. V., NIALL, H. D., HABENER, J. F., AND POTTS, J. T., JR.: Metabolism of parathyroid hormone. Physiologic and clinical significance. Amer. J. Med. **56:** 774–784, 1974.

266. SEGRE, G. V., NIALL, H. D., SAUER, R. T., AND POTTS, J. T., JR.: Edman degradation of radioiodinated parathyroid hormone: Application to sequence analysis and hormone metabolism *in vivo*. Biochemistry **16:** 2417–2427, 1977.

267. SEMPLE, P. F., BOYD, A. S., DAWES, P. M., AND MORTON, J. J.: Angiotensin II and its heptapeptide (2-8), hexapeptide (3–8) and pentapeptide (4–8) metabolites in arterial and venous blood in man. Circ. Res. **39:** 671–678, 1976.

268. SEMPLE, P. F., AND MORTON, J. J.: Angiotensin II and

angiotensin III in rat blood. Circ. Res. **38:** suppl. 2, 122-126, 1976.
269. SEYLER, L. E., JR.: Disappearance of biological activity of synthetic LRH in normal and hypogonadal men. J. Clin. Endocrinol. Metab. **41:** 1155-1160, 1975.
270. SHERWIN, R. S., KRAMER, K. J., TOBIN, J. D., INSEL, P. A., LILJENQUIST, J. E., BERMAN, M., AND ANDRES, R.: A model of the kinetics of insulin in man. J. Clin. Invest. **53:** 1481-1492, 1974.
271. SHIZUME, K., AND IRIE, M.: The role of the liver in the inactivation of the melanocyte-stimulating hormone in the body. Endocrinology **61:** 506-513, 1957.
272. SINGER, F. R., HABENER, J. F., GREENE, E., GODIN, P., AND POTTS, J. T., JR.: Inactivation of calcitonin by specific organs. Nature New Biol. **237:** 269-270, 1972.
273. SINGER, F. R., SEGRE, G. V., HABENER, J. F., AND POTTS, J. T., JR.: Peripheral metabolism of bovine parathyroid hormone in the dog. Metabolism **24:** 139-144, 1975.
274. SJÖHOLM, I., AND RYDÉN, G.: Uptake of oxytocin in tissues after intravenous injection of tritiated oxytocin in rats. Acta Endocrinol. **61:** 432-440, 1969.
275. SKILLMAN, J. T., SILEN, W., AND HARPER, H. A.: Role of the liver in secretin inactivation. Amer. J. Physiol. **202:** 347-348, 1962.
276. SOFFER, R. L.: Angiotensin-converting enzyme and the regulation of vasoactive peptides. Annu. Rev. Biochem. **45:** 73-94, 1976.
277. SOLTIURAI, V. A.: The role of the convoluted segment of the proximal tubule in the disposal of ^{131}I-insulin in the rat. Amer. J. Anat. **140:** 181-190, 1974.
278. SONENBERG, M., KESTON, A. S., AND MONEY, W. L.: Labelled anterior pituitary preparations—adrenocorticotrophin. Endocrinology **48:** 148-161, 1951.
279. SONENBERG, M., MONEY, W. I., DORANS, J. F., LUCAS, V., AND BOURQUE, L.: The distribution of radioactivity in the administration of radioactive growth hormone preparations. Endocrinology **55:** 709-720, 1954.
280. SONENBERG, M., MONEY, M. L., KESTON, A. S., FITZGERALD, P. J., AND GODWIN, J. T.: Localization of radioactivity after administration of labelled prolactin preparations to the female rat. Endocrinology **49:** 709-719, 1951.
281. SÖNKSEN, P. H., MCCORMICK, J. R., EGDAHL, R. H., AND SOELDNER, J. S.: Distribution and binding of insulin in the dog hindlimb. Amer. J. Physiol. **221:** 1672-1680, 1971.
282. SÖNKSEN, P. H., TOMPKINS, C. V., SRIVASTAVA, M. C., AND NABARRO, J. D. N.: A comparative study on the metabolism of human insulin and porcine proinsulin in man. Clin. Sci. Mol. Med. **45:** 633-654, 1973.
283. STACY, B. D., WALLACE, A. L. C. GREMMELL, G. T., AND WILSON, B. W.: Absorption of ^{125}I-labelled sheep growth hormone in single proximal tubules of the rat kidney. J. Endocrinol. **68:** 21-30, 1976.
284. STARR, J. I., AND RUBENSTEIN, A. H.: Metabolism of endogenous proinsulin and insulin in man. J. Clin. Endocrinol. Metab. **38:** 305-308, 1974.
285. STOLL, R. W., TOUBER, J. L., MENAHAN, L. A., AND WILLIAMS, R. H.: Clearance of porcine insulin, proinsulin, and connecting peptide by the isolated rat liver. Proc. Soc. Exp. Biol. Med. **133:** 894-896, 1970.
286. STOLL, R. W., TOUBER, L. A., AND WILLIAMS, R. H.: Hypoglycemic activity and immunological half-life of porcine insulin and proinsulin in baboons and swine. Endocrinology **88:** 714-717, 1971.
287. STRAUS, E., AND YALOW, R. S.: Studies on the distribution and degradation of heptadecapeptide, big and big, big gastrin. Gastroenterology **66:** 936-943, 1974.
288. SYDNOR, K. I., AND SAYERS, G.: Biological half-life of endogenous ACTH. Proc. Soc. Exp. Biol. Med. **83:** 729-733, 1953.
289. TEMPERLEY, J. M., STAGG, B. H., AND WYLIE, J. H.: Disappearance of gastrin and pentagastrin in the portal circulation. Gut **12:** 372-376, 1971.
290. THOMAS, J. H.: The role of 'insulinase' in degrading insulin. Postgrad. Med. J. **49:** 940-944, 1973.
291. THOMPSON, J. C., FENDER, H. R., RAMUS, N. I., VILLAR, H. V., AND RAYFORD, P. L.: Cholecystokinin metabolism in man and dogs. Ann. Surg. **182:** 496-504, 1975.
292. TSENG, L. F., LOH, H. H., AND LI, C. H.: β-Endorphin as a potent analgesic by intravenous injection. Nature (London) **263:** 239-240, 1976.
293. TUPPY, H., AND NESVADBA, H.: Aminopeptidase activity in the serum of pregnant women and its ability to inhibit oxytocin. Monatsh. Chem. **88:** 977-988, 1957.
294. UNGER, R. H., AND POTTS, R. E.: Insulin, glucagon and somatostatin secretion in the regulation of metabolism. Annu. Rev. Physiol. **40:** 307-343, 1978.
295. UNGER, R. H., EISENTRAUT, A. M., MCCALL, M. S., AND MADISON, L. L.: Glucagon antibodies and an immunoassay for glucagon. J. Clin. Invest. **40:** 1280-1289, 1961.
296. VAJDA, F. J. E., MARTIN, T. J., AND MELICK, R. A.: Destruction of bovine parathyroid hormone labelled with ^{131}I by rat kidney tissue. Endocrinology **84:** 162-164, 1969.
297. VALE, W., BRAZEAU, P. RIVIER, C., BROWN, M., BOSS, B., RIVIER, J., BURGUS, R., LING, N., AND GUILLEMIN, R.: Somatostatin. Recent Progr. Hormone Res. **31:** 365-397, 1975.
298. VANDER GUGTEN, A. A., AND KURA, H. G.: Disappearance rate of bovine prolactin from plasma of female rats studied at intervals of up to 160 minutes by radioimmunoassay. Acta Endocrinol. **64:** 718-725, 1969.
299. VAN DYKE, D. C., SIMPSON, M. E., LI, C. H., AND EVANS, H. M.: Survival in the circulation of the growth and adrenocorticotrophic hormone as evidenced by parabiosis. Amer. J. Physiol. **163:** 297-309, 1950.
300. VANE, J. R.: The release and fate of vaso-active hormones in the circulation. Brit. J. Pharmacol. **35:** 209-242, 1969.
301. VARANDINI, P. T., AND NAFZ, M. A.: Insulin degradation. I. Purification and properties of glutathione-insulin transhydrogenase of rat liver. Biochem. Biophys. Acta **286:** 126-135, 1972.
302. VAURA, I., MACHOUA, A., HOLECEK, V., CORT, J. H., ZAORAL, M., AND SORM, F.: Effect of a synthetic analogue of vasopressin in animals and in patients with diabetes insipidus. Lancet **i:** 948-952, 1968.
303. VERHOEF, J., AND WITTER, A.: In vivo fate of a behaviourally active ACTH 4-9 analog in rats after intravenous administration. Pharmacol. Biochem. Behav. **4:** 583-590, 1976.
304. VILAR, O., ALVANE, B., DAVIDSON, O., AND MANCINI, R. E.: Incorporation by the kidney of fluorescent pituitary hormones. J. Histochem. Cytochem. **12:** 621-627, 1964.
305. VILLAR, H. V., REEDER, D. D., RAYFORD, P. L., AND THOMPSON, J. C.: Rate of disappearance of circulating endogenous gastrin in dogs. Surgery **81:** 404-408, 1977.
306. VIRKKUNEN, P., LYBECK, H., PARTANEN, J., RANTA, T., LEPPÄLUOTO, J., AND SEPPÄLÄ, M.: Plasma disappearance of injected synthetic LRH in man as measured by bioassay and radioimmunoassay. J. Clin. Endocrinol. Metab. **39:** 702-705, 1974.
307. WALLACE, A. L. C., AND STACY, B. D.: Disappearance of iodine-125-labelled rat growth hormone in nephrectomized and sham operated rats. Horm. Metab. Res. **7:** 135-138, 1975.
308. WALLACE, A. L. C., STACY, B. D., AND THORBURN, G. D.: Fate of radioiodinated sheep growth hormone in intact and nephrectomized sheep. Pflügers Arch. Gesamte Physiol. Menschen Tiere **331:** 25-37, 1972.
309. WALSH, J. H.: Circulating gastrin. Annu. Rev. Physiol. **37:** 81-104, 1975.
310. WALSH, J. H.: Biologic activity and disappearance rates of big, little and mini-gastrins in dog and man. *In* Gastrointestinal Hormones, ed. by J. C. Thompson, pp. 75-83, University of Texas Press, Austin, 1975.
311. WALSH, J. H.: Gastrin heterogeneity: Biological signifi-

cance. Fed. Proc. **36:** 1948–1951, 1977.

312. WALSH, J. H., DEBAS, H. T., AND GROSSMAN, M. T.: Pure human big gastrin: Immunological properties, disappearance, half-time and acid-stimulating action in dogs. J. Clin. Invest. **54:** 477–485, 1974.
313. WALTER, R., AND BOWMAN, R. H.: Mechanism of inactivation of vasopressin and oxytocin by the isolated perfused rat kidney. Endocrinology **92:** 189–193, 1973.
314. WALTER, R., AND SHLANK, H.: *In vivo* inactivation of oxytocin. Endocrinology **89:** 990–995, 1971.
315. WALTER, R., AND SHLANK, H.: Differences in the enzymic inactivation of arginine-vasopressin in oxytocin by rat kidney homogenate. Endocrinology **96:** 811–814, 1975.
316. WARING, H., AND KIRK, R. L.: The metabolism of melanophore-expanding pituitary ("B") hormone and the effect of this hormone on carbohydrate metabolism in mammals. Aust. J. Exp. Biol. Med. Sci. **30:** 409–413, 1952.
317. WEST, T. E. T., O'RIORDAN, J. L. H., AND CARE, A. D.: Clearance of homologous calcitonin. J. Endocrinol. **45:** 495–504, 1969.
318. YALOW, R. S., AND BERSON, S. A.: Radioimmunoassay of gastrin. Gastroenterology **58:** 1–14, 1970.
319. YALOW, R. S., GLICK, S. M., ROTH, J., AND BERSON, S. A.: Radioimmunoassay of human plasma ACTH. J. Clin. Endocrinol. Metab. **24:** 1219–1225, 1964.
320. YANG, H. Y. T., ERDÖS, E. G., AND LEVIN, Y.: Characterization of a dipeptide hydralase (kininase II: angiotensin I converting enzyme). J. Pharmacol. Exp. Ther. **177:** 291–300, 1971.
321. ZAORAL, M., KOLC, J., AND SORM, F.: Amino acids and peptides. LXXI. Synthesis of L-deamino-8-D-γ-aminobutyrine-vasopressin, 1-deamino-8-D-lysine-vasopressin and 1-deamino-8-D-arginine vasopressin. Collect. Czech. Chem. Commun. **32:** 1250–1257, 1967.