Normal nutrient homeostasis depends on the balance between the effects of insulin and those of the so-called contrainsulin or counterregulatory hormones. These hormones include glucagon, epinephrine, growth hormone, and cortisol. Although thyroxine and triiodothyronine affect various aspects of metabolism, circulating thyroid hormone levels are not acutely affected by nutrient signals, and fluctuations in their daily secretion do not alter metabolic processes. For these reasons, thyroxine and triiodothyronine are not generally considered among the classical contrainsulin hormones. Regulating factors in addition to insulin and the contrainsulin hormones affect metabolic balance. These factors include various neurotransmitters (noradrenaline from sympathetic postganglionic neurons, acetylcholine from parasympathetic neurons and neuropeptides) and substrates including glucose. Detailed discussion of the latter regulatory factors is beyond the scope of this chapter. Their roles have been reviewed elsewhere.

Glucagon
Glucagon, a 29-amino-acid polypeptide with a molecular weight of 3485 daltons, was discovered as a "contaminant" hyperglycemic factor in pancreatic extracts by Kimball and Murlin in 1923 and finally sequenced by Bromer et al. during the late 1950s. Studies of its mechanism during the 1960s by Sutherland et al. led to the discovery of the second messenger cyclic adenosine monophosphate (cAMP). Full appreciation of its importance for normal fuel homeostasis in humans and diabetes mellitus did not come until the 1970s when the availability of somatostatin, an inhibitor of glucagon secretion, permitted investigation of its lack under various experimental conditions.

Biosynthesis
Glucagon is secreted by A cells of pancreatic islets. Normally, these cells constitute approximately 15-20% of the total islet cell mass. In most species A cells are located at the periphery of islets juxtaposed to B cells, which secrete insulin, and D cells, which secrete somatostatin. Glucagon is synthesized initially as a large molecule of approximately 12,000 daltons. This peptide undergoes cleavage to a 9000-dalton molecule, which is cleaved to a 4900-dalton molecule that is finally cleaved to yield the 3485-dalton molecule. The whole process takes about 90 minutes. All of these peptides are immunoreactive, but only the 3485-dalton molecule is biologically active.

Plasma Glucagon
Values for plasma glucagon vary considerably from individual to individual. The main factors responsible are the specificity of the antiserum used in the immunoassay and the relative proportion of the total immunoreactivity accounted for by the 3485-dalton molecule. In the dog and humans the pancreas is not the sole source of glucagon. A cells, similar to those in pancreatic islets, have been found in the stomach and in the small and large intestines. These cells contain a peptide that is immunologically and physicochemically similar or identical to pancreatic A cell glucagon and that has a glucagon-like biological activity. These observations could explain the presence of circulating glucagon immunoreactivity following total pancreatectomy in humans and other species. The relative contribution of pancreatic and extrapancreatic A cells to plasma glucagon and substrate homeostasis remains to be established. Control of extrapancreatic glucagon secretion seems to differ from that of pancreatic glucagon.

Normally in humans and most other mammalian species, arterial and peripheral venous plasma immunoreactive glucagon concentrations range between 35 and 200 pg/ml (1.0 x 10^-8 to 6.0 x 10^-8 M) after a 12- to 16-hour fast. Portal venous levels can average 1.5-3.0 times those present in arterial blood because of extraction of glucagon by the liver. As with other peptide hormones, circulating glucagon immunoreactivity is heterogeneous. By using chromatog-
raphy, four immunoreactive species with apparent molecular weights of more than 40,000, 9000, 3500, and 2000 have been found (Fig. 6.2). There is considerable individual and species variation in the proportions of each component found in plasma.\(^\text{27,28}\) In humans the 3500-dalton species, the only fraction unequivocally demonstrated to be biologically active, usually comprises only about 25% of total plasma glucagon immunoreactivity. The 9000-dalton molecule, which has similar immunoreactivity but substantially less bioactivity than the 3500-dalton molecule, can be converted by trypsin to a smaller immunoreactive peptide of approximately 3500 daltons;\(^\text{33}\) it is thought to represent the biosynthetic precursor of glucagon (proglucagon?) found in the pancreas, which is convertible to glucagon by trypsin.\(^\text{34}\) Increased amounts of the 9000-dalton molecule are found in the plasma of patients with the glucagonoma syndrome,\(^\text{35}\) renal failure,\(^\text{33}\) or hepatocellular damage or carcinoma of the pancreas.\(^\text{8}\) The 2000-dalton molecule probably represents an inactive degradation product of glucagon.

The heterogeneity of plasma glucagon can complicate the interpretation of in vivo studies of glucagon secretion and metabolism. Changes in plasma glucagon immunoreactivity during stimulation or suppression of \(A\) cell secretion are due almost exclusively to changes in the 3500-dalton fraction.\(^\text{33,36,37}\) Although the overall distribution of plasma glucagon is not altered in diabetes and most other pathological conditions in which the study of \(A\) cell function might be of interest, the relative contribution of the fractions can vary considerably among individuals.\(^\text{9}\) Comparisons based on absolute levels of total plasma glucagon immunoreactivity may be misleading.

The pancreatic content of glucagon varies considerably among species; the human pancreas contains approximately 700–1000 \(\mu\)g of glucagon. Glucagon is stored within \(A\) cells in distinctive granules and is secreted by a process called emiocytosis,\(^\text{38}\) which involves migration of secretory granules to the periphery of cells, fusion of granules with the plasma membrane, and extrusion of granule contents into the extracellular space. Like insulin secretion, secretion of glucagon probably involves a CAMP–calcium interaction,\(^\text{39,40}\) is dependent on the presence of extracellular calcium,\(^\text{41}\) and is influenced by the concentration of ions such as potassium, calcium, and magnesium.\(^\text{42}\)

In vivo secretion of glucagon is the net result of the influence of substrate and neural, ionic, hormonal, and local factors on islet \(A\) cell function. The plasma concentration of glucagon depends on the balance between rates of secretion and degradation and on the sampling site (e.g., peripheral venous versus portal venous blood). Basal (nonstimulated) secretion rates of glucagon can be estimated from data on portal venous-arterial differences and portal venous plasma flow rates. Secretion rates of glucagon may be estimated on the basis of the clearance of glucagon under steady-state conditions; such estimation yields a value of approximately 1400 pg·kg\(^{-1}\)·min\(^{-1}\) in man.\(^\text{33}\) It should be pointed out, however, that these values underestimate secretion of glucagon and merely represent posthepatic delivery of glucagon. From what is known of the pancreatic content of glucagon and secretory rates of glucagon, it can be estimated that at least 25% (probably more) of the pancreatic content of glucagon is secreted each day.

**Glucagon Catabolism**

In normal humans the metabolic clearance rate of glucagon is independent of the prevailing plasma glucagon concentration; estimates range between 7 and 14 ml·kg\(^{-1}\)·min\(^{-1}\).\(^\text{43,44}\) Normal values have been reported in patients with diabetes\(^\text{44}\) or liver disease,\(^\text{45}\) whereas decreases have been found with renal failure\(^\text{46}\) and starvation.\(^\text{43}\) The liver and kidney seem to be the major sites of glucagon catabolism, but the relative contribution of each remains controversial.\(^\text{45,46}\)

Initial reports suggested that the liver is not a major site of glucagon degradation.\(^\text{25,29,31}\) The conflicting results reported may be reconciled if the heterogeneity of circulating glucagon immunoreactivity is taken into account. When portal venous and peripheral venous plasma is subjected to gel filtration, it seems that the liver does not appreciably extract the biologically inactive 9000- and more than 40,000-dalton plasma glucagon immunoreactivity.\(^\text{29}\) The portal/ peripheral gradient of glucagon immunoreactivity is almost totally accounted for by extraction of the biologically active 3500-dalton molecule; it averages approximately 60% and results in a portal/peripheral ratio of 2.5–3.0 for the biologically active molecule.

Although it has long been known that the kidney is capable of degrading exogenous glucagon, only