as occurs in some coniferophytes, particularly in the saccate pollen of the Pinaceae (*15*), and pollen with a more organized, honeycomb-like network, which may be observed in members of the cycadophyte line of gymnosperms (*16*). Significantly, spores of pteridophytes such as *Lycopodium, Psilotum,* and *Archaeopteris* fundamentally have structureless spore walls (*17*) and therefore may be considered to have a basically atectate sporoderm. Figure 2 summarizes the main evolutionary trends in spore-pollen exine structure of vascular plants which we consider most likely on the basis of current data.

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Growth Hormone: Independent Release of Big and Small Forms from Rat Pituitary in vitro

Abstract. *Sequential release of big and small forms of growth hormone by perifused rat pituitaries has been demonstrated by immunoprecipitation. The results suggest that either the two forms are independently synthesized and released, or that a newly synthesized molecule of big growth hormone follows one of two paths: direct release or intracellular processing through the storage compartment with conversion to small growth hormone.*

Heterogeneity of molecular size is characteristic of several protein and polypeptide hormones (*1*). Hormone forms that are larger than the monomeric molecule, and that serve as biosynthetic precursors, have been identified for insulin (*2, 3*), Parathormone (*4*), and glucagon (*5*). The earliest detectable biosynthetic form of pituitary growth hormone (GH) is a large molecular species, excluded from a G-200 Sephadex column. This large GH is associated with ribonucleic acid, and has been proposed to represent a complex of nascent GH associated with the GH polysome (*6*). In addition to that for GH (*7, 8*), large molecular species have also been shown for human placental lactogen (*9*) and prolactin (*7*).

Growth hormone forms of intermediate size in pituitary extracts (*10*) and plasma have been described. Two plasma forms, each designated as big GH, have been reported. One form exhibits an apparent molecular weight three times that of monomeric GH (*1*); and the other, twice that of the monomer (*11, 12*). Their relationships to the predominant monomeric form of GH are not clear. Experiments on the big GH that is twice the size of monomeric GH are described in this report. The relationship of this big GH and small (monomeric) GH was investigated in rat pituitary and incubation medium using an in vitro perifusion (*13*) system.

Rat adenohypophysial quarters were first incubated for 3 hours in Krebs Ringer bicarbonate buffer (KRB) supplemented with bovine serum albumin (1 percent) and glucose (150 mg/100 ml) and containing [^{14}C]leucine (5 μc/ml). This procedure established an intracellular, stored pool of [^{14}C]GH. Labeled explants were transferred to a 0.25-ml chamber and perifused at 0.2 ml/min by KRB without radioactive precursor. Fractions of 1 ml were collected until a constant basal release of stored [^{14}C]GH was achieved (*13*). The perifusion medium was then changed to KRB supplemented with [^{3}H]leucine (3 μc/ml), and the perifusion was continued for an additional 2 hours. Individual effluent fractions were dialyzed to remove excess radioactive amino acid and were immunoabsorbed with normal guinea pig serum and with goat antiserum against guinea pig serum to reduce nonspecific background radioactivity. They were then reacted with excess specific monkey antiserum against rat GH (individual sample blanks were reacted with normal monkey serum) and precipitated with goat antiserum against monkey gamma globulin (*8, 14*). Washed precipitates were solubilized, counted, and analyzed by computer for [^{3}H]GH and [^{14}C]GH (*6*).

Accumulation of [^{14}C]GH in the perifusion effluent was expressed as percentage of the total [^{14}C]GH in the explant at the time of each collection interval (*13*). Total [^{14}C]GH was calculated by subtracting released [^{14}C]-GH from total [^{14}C]GH present at the onset of the perifusion. When expressed in this way, [^{14}C]GH accumulation in the effluent was linear once explant equilibration had occurred (Fig. 1). Release of [^{3}H]GH began immediately after explant exposure to [^{3}H]leucine, and its accumulation in medium was linear for approximately 1 hour. After 1 hour, [^{3}H]GH release remained linear but the rate was increased. The origin of released [^{14}C]GH was the cellular storage compartment, since new synthesis of [^{14}C]GH had ceased after transfer to the perifusion chamber (*13*).

Approximately 40 to 60 minutes are required for transfer of a newly synthesized GH molecule from the synthesis mechanism to its release in vitro

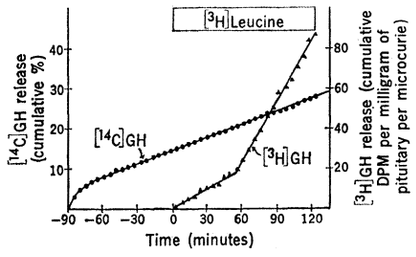

Fig. 1. Release of labeled GH in basal perifusion. Rat pituitaries were first incubated with [^{14}C]leucine to establish a pool of [^{14}]GH. The labeled explants were placed in a perifusion chamber (at − 90 minutes) through which media flowed at 0.2 ml/min. Effluent fractions of 1 ml were collected for later analysis by specific immunoprecipitation. [^{14}C]GH release, expressed as percentage per minute of pituitary content at the onset of the particular perifusion interval, quickly stabilized and remained unchanged throughout the remainder of the perifusion. [^{3}H]GH release began immediately when explants were exposed to [^{3}H]leucine (0 minute) and was linear for about 1 hour, after which the rate of release increased. The time at which [^{3}H]GH release increased corresponds with previous estimates of the time necessary for a newly synthesized molecule to pass from the synthesis mechanism through the storage pool to release from the cell; DPM, disintegrations per minute.

through the storage pool (*6*). However, the GH molecule can also be released directly after synthesis as well as from the storage pool (*6, 15*). Thus, in the continuous presence of [^{3}H]leucine, early [^{3}H]GH release (Fig. 1) appears to derive directly from the synthesis mechanism while, after about 1 hour, total [^{3}H]GH release is the sum of that released directly from synthesis plus that which has first passed into the somatotroph storage pool.

Perifusion effluent fractions collected from zero time (start of exposure to [^{3}H]leucine) through 40 minutes and from 80 through 120 minutes were pooled. Each pool was chromatographed on a Sephadex G-200 column, and the effluent fractions were subjected to immunoprecipitation. The results are shown in Fig. 2.

The ^{14}C-labeled GH, which had been synthesized at least 90 minutes previously and was therefore released from the storage pool, eluted at a V_e/V_o of 2.1 (Fig. 2A). This position corresponds to that of monomeric GH (*8*). The [^{3}H]GH released during the first 40 minutes of synthesis in the presence of radioactive precursor eluted at a V_e/V_o of 1.7, corresponding to the elution characteristics of plasma (*11, 12*) or pituitary (*10*) big GH. The immunoprecipitable radioactivity in the void volume may represent GH aggregates, GH nonspecifically attached to other macromolecules, or intracellular "large" GH [a complex of GH, protein, and RNA (*6*)] released by in vitro leakage.

The [^{14}C]GH released between 80 and 120 minutes (Fig. 2B) again migrated with a V_e/V_o of 2.1, which indicated continued release of monomeric GH from the cellular storage pool. On the other hand, [^{3}H]GH, which was still being synthesized, eluted in two peaks. One peak eluted at a V_e/V_o of 1.7, which corresponded to big GH as in Fig. 2A and indicated continued direct release from synthesis. A second peak of [^{3}H]GH was congruent with monomeric [^{14}C]GH, which suggested intracellular conversion of new [^{3}H]GH to small GH and subsequent release from the storage pool (*6*). Thus, during the second hour of GH synthesis in the presence of radioactive precursor, [^{3}H]GH is released by both the direct path from synthesis and the indirect path through the storage compartment.

The possibility that big GH represents only an extraction artifact has been considered because of an apparent molecular weight for big GH twice that of monomeric GH (*11, 12*) and because the big GH in plasma can spontaneously convert to small GH (*11, 12*). Although it is possible that big GH is simply a dimer of native, monomeric, small GH, such an explanation would require that any tendency toward dimerization be a random process, affecting all GH molecules. Further, the likelihood of dimer formation by a particular GH molecule during extraction should be independent of the elapsed time since its synthesis. Big [^{14}C]GH should have been demonstrable in both A and B of Fig. 2 if artifactual dimerization were sufficient to explain big GH formation, since [^{3}H]GH and [^{14}C]GH were present in the two pools of effluent. The fact that only [^{3}H]GH was present as big GH suggests that artifactual dimerization is an insufficient explanation and that demonstration of the big GH form is in some way related to its concurrent synthesis. On the basis of these results, big GH appears to represent either an incompletely processed and possibly immature form of GH, released directly from a rat somatotroph actively engaged in GH synthesis, or an independently synthesized and released molecule. Small GH, the predominant GH form in tissue storage and outside the cell, represents a more mature GH form resulting from the cellular processes of hormone packaging for storage before release.

These results support the concept that more than one path exists for GH release from the somatotroph (*6*). The characteristics of these two paths of hormone release may be investigated by separating the forms of released hormones before assay. It is possible that examination of GH release from cells such as the GH_1 and MtT4 tumors (*8*) or fetal pituitary (*10*), which show high rates of GH production but decreased hormone storage, will reveal a higher proportion of GH released as the big form; these cells may be an appropriate model for studying the direct path of hormone release after syn-

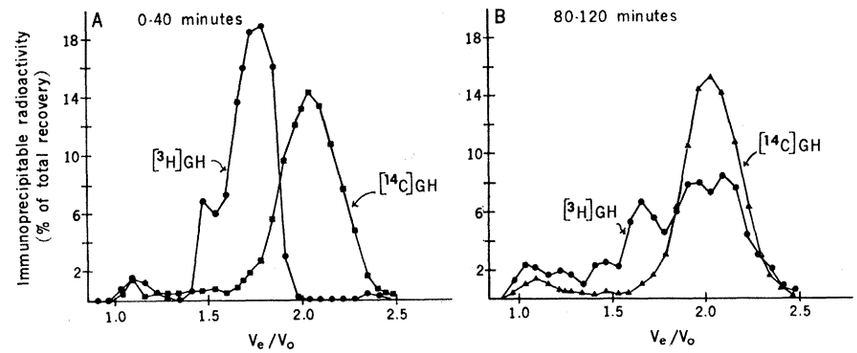

Fig. 2. Sephadex G-200 chromatography of released GH in effluent fractions collected in the experiment shown in Fig. 1: between 0 and 40 minutes (A) and 80 and 120 minutes (B). The fractions were pooled, concentrated, and filtered over a Sephadex G-200 column (1 by 90 cm) in 0.05*M* phosphate buffer, *p*H 7.5. Portions of the 1-ml column effluent fractions were subjected to immunoprecipitation for GH. Ovalbumin (molecular weight, 45,000) migrated at V_e/V_o 1.6 and chymotrypsinogen A (molecular weight, 25,000) migrated at V_e/V_o 2.0. [^{125}I]GH and immunoassayable GH elute in the same position as [^{14}C]GH.

thesis. The big GH component may (*16, 17*) or may not (*18*) show decreased binding activity in the radioreceptor assays. Discrepancies between estimates of immunoreactive hormone and expected biologic activity may result from a disproportionate increase in the big GH component, although other explanations, such as a monomeric GH with reduced or absent biological activity (*17*), must be considered. Such an increase could be produced by hypothalamic or other extrapituitary agents which may differentially alter the release of newly synthesized big and stored small GH (*15, 19*). Finally, a subcellular defect along the path from hormone synthesis to storage could result in an enhanced secretion of big GH, since release of this form appears to be related to concurrent GH synthesis.

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Endogenous Cyclic Adenosine Monophosphate in Tissues of Rabbits Fed an Atherogenic Diet

Abstract. *Rabbits fed a high cholesterol diet experienced a significant increase in plasma adenosine 3′,5′-monophosphate (cyclic AMP), which was simultaneous with the increase in plasma cholesterol. The content of cyclic AMP in atherosclerotic lesion areas of rabbit aortic intima-media was significantly higher (0.24 picomole per microgram of DNA) than that in adjacent nonlesion areas or in aortic intima-media from control animals (0.09 picomole per microgram of DNA). The cyclic AMP content of heart, liver, skeletal muscle, and diaphragm showed no significant elevation in animals fed cholesterol.*

Adenosine 3′,5′-monophosphate (cyclic AMP) has been suggested as a regulator of lipid metabolism, membrane transport, and cell proliferation. Several investigators have reported its action on these cell processes to be either stimulatory or inhibitory, depending on the concentration used (*1*).

The selective susceptibility of focal regions of the arterial vasculature to the development of atherosclerotic lesions is well established. This selective susceptibility, coupled with the fact that atherosclerotic lesions are characterized by an increase in cell proliferation and lipid accumulation, suggested that the cyclic AMP in the plasma of animals on atherogenic diets might vary from that of controls. A study of rabbits fed cholesterol showed such an increase in the cyclic AMP concentration of plasma and in lesion areas of the aortic intima-media.

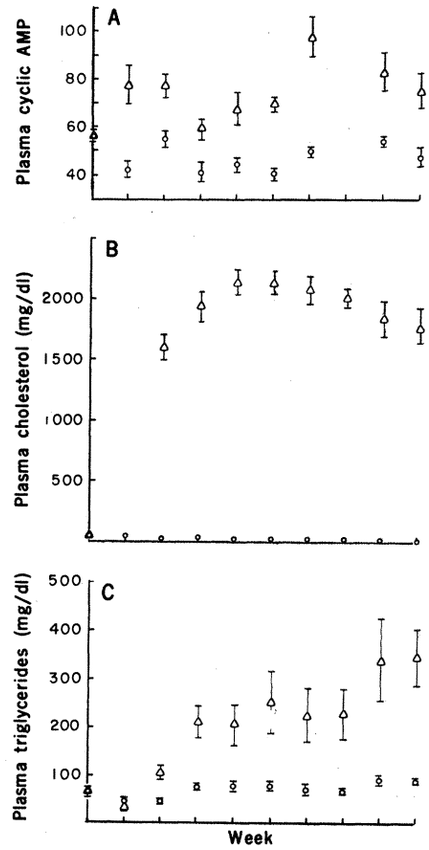

Fig. 1. Cyclic AMP, cholesterol, and triglyceride in plasma of rabbits on high cholesterol (△) and control (○) diets from one experiment. Data are given as the mean ± the standard error of the mean.

For each of two experiments, 16 male New Zealand white rabbits, 6 to 8 weeks of age, were randomly divided into two groups of eight animals each. Atherosclerotic lesions were induced in one group by feeding them Purina Rabbit Chow supplemented with 2 percent cholesterol in a corn oil vehicle. The control group was maintained on Purina Rabbit Chow only, and water was freely available to both groups. Plasma concentrations of cyclic AMP (*2*), cholesterol (*3*), and triglycerides (*4*) were determined weekly on both groups of rabbits. After 9 weeks, all animals were killed by cervical fracture. Portions of heart, liver, skeletal muscle, and diaphragm were quickly excised from randomly selected animals, briefly washed in saline, teased into small pieces, and fixed in 6 percent trichloroacetic acid for 10 minutes. The entire aorta was removed from each animal. Approximately 60 to 70 percent of the total aortic surface of rabbits on the cholesterol-supplemented diet was covered with macroscopically identifiable lesions which were stainable with Sudan dye. This extent of involvement precluded meaningful correlation between plasma cyclic AMP levels and the severity and extent of lesions. In addition, attempts at correlating the plasma concentrations of cyclic AMP and cholesterol in individual rabbits, monitored over the 9-week period, with the severity of lesions at the time the animals were killed proved inconclusive. For consistency, only the upper half of the thoracic portion of the aorta was fixed in trichloroacetic acid. After fixation, the adventitia was stripped, and macroscopically identified lesion areas were carefully dissected from