of cyclic AMP and ATP in different regions of these very early chick embryos cannot be easily determined. For example, further study is needed to indicate whether the concentration of cyclic AMP from fragments B and C (see Table 1) at the head process stage reflects the concentration of cyclic AMP and morphogenetic activity from an earlier stage, for example, at the primitive streak. The effects of cell movements and trauma of embryonic dissection are but two problems that have to be considered. Our study does represent a novel approach at examining specific embryonic regions of known morphogenesis obtained from eggs of a single flock sampled at a single stage of development.

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6. The head process stage was defined as follows: a short head process extended anteriorly from the anterior end of the streak, which was no less then 65 to 80 percent of the length of the area pellucida.
7. After centrifugation the supernatant was cleared of acid by four washes with ether. Portions were removed for ATP assays and polyethyleneimine cellulose (PEI) chromatography. The remainder was adsorbed on charcoal and the cyclic AMP was eluted with 2 percent ammoniacal alcohol. After removal of the alcohol the remainder of nucleotides were assayed for cyclic AMP by PEI chromatography and by Gilman's method (*9*). Between 15 to 20 embryos were pooled for each dissected fragment in each experiment and represented from 20 to 80 μg of protein for sampling 0.2 to 4 pmole of cyclic AMP. In experiment 2, cyclic AMP (0.5 pmole) was added to each sample as internal standard. Samples for cyclic AMP, fragments G to J, were lost in this experiment. The protein kinase from rat muscle was used for these assays. The assay was linear between 0.1 to 40 pmole of cyclic AMP. The major portion of the acid supernatant was used for determination of radioactivity by PEI chromatography as detailed by M. Reporter [*Biochem. Biophys. Res. Commun.* **48**, 598 (1972)]. We had obtained a similar distribution of nucleotides in cruder cuts of embryos explanted by the method of Spratt as well as in organ cultures from precardiac head fold and somite regions of the chick blastoderm.
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Proparathyroid Hormone: Biosynthesis by Human Parathyroid Adenomas

Abstract. *Biosynthesis of a precursor (proparathyroid hormone) to human parathyroid hormone was demonstrated during incubation of tissue from parathyroid adenomas. The proparathyroid hormone is labeled more rapidly than parathyroid hormone during incubation with amino acids labeled with carbon-14 and is progressively converted to the hormone. Apparent differences in the relative rate of conversion of precursor to hormone found in different tumors suggest that proparathyroid hormone may accumulate in some of the tumors and be secreted into the circulation.*

It has become evident that many proteins are initially synthesized by the cell as larger precursor molecules that are subsequently cleaved to form the final active protein. This phenomenon is not restricted to any particular functional class of protein since the existence of precursors has been demonstrated or suggested for enzymes (*1*), structural proteins (*2*), and polypeptide hormones (*3*). We have identified a precursor to parathyroid hormone (PTH) in bovine tissue (*4*), and independent evidence in agreement with this has also been obtained by Hamilton *et al.* (*5*). Immunological and clinical studies have indicated that certain adenomas of human parathyroids may secrete a form of parathyroid hormone with a molecular weight higher than that of the hormone extracted from the adenoma tissue (*6*). We now report the identification of a biosynthetic precursor to human PTH that is analogous to the precursor to bovine PTH. Furthermore, a survey of several parathyroid adenomas indicates considerable variation in the rate of conversion of the precursor to PTH among the tumors. This suggests that a variable loss of this cleavage function may occur as a result of transformation from normal to tumor cells.

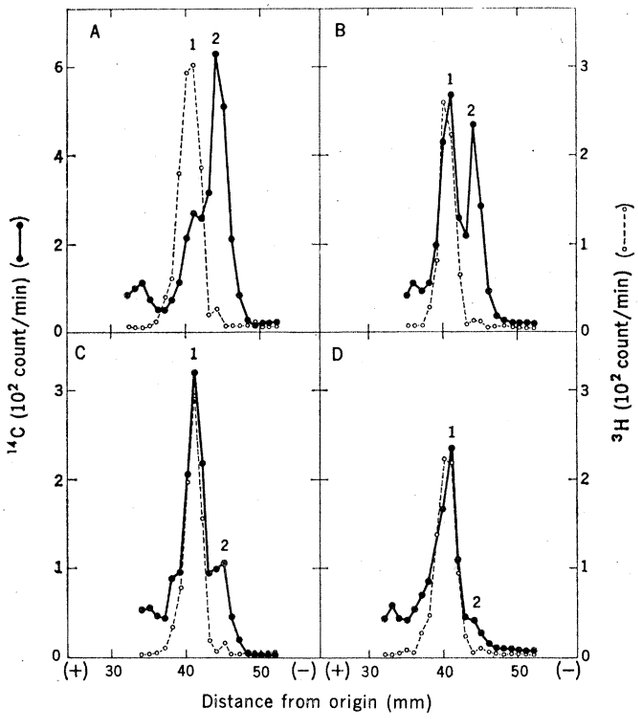
Parathyroid adenomas were obtained from six patients, and parathyroid tissue showing the histological criteria of "clear cell hyperplasia" (*7*) was obtained from a seventh patient. At the time of surgery, the excised tissue was immediately chilled and portions of minced tissue were incubated at 37°C in medium containing ^{14}C-labeled amino acids. After incubation, acid-urea extracts (*4*) of the tissue were analyzed by electrophoresis on 10 percent polyacrylamide gels containing 8*M* urea at *p*H 4.4 (*8*) or containing 8*M* urea at *p*H 7.2 in 0.1 percent sodium dodecyl sulfate (SDS) (*9*). Slices of the gels were assayed for radioactivity (*4*) and for immunoreactive PTH by means of a competitive-binding radio-

immunoassay (*10*). Two major peaks of radioactivity were detected after electrophoresis of the extracts on *p*H 4.4 gels in the region of the gels containing PTH (Fig. 1). During the 30-minute incubation (Fig. 1A) peak 2 contained most of the radioactivity. After the tissue was incubated for 60 minutes, about equal amounts of radioactivity were present in peak 2 and in peak 1, which comigrated with bovine PTH (Fig. 1B). Furthermore, the radioactivity in peak 2 declined while radioactivity in peak 1 (PTH) increased during an incubation with unlabeled amino acids (Fig. 1C) or if additional protein synthesis was inhibited by puromycin (Fig. 1D). These experiments, therefore, support a precursor product relation between peak 2 and peak 1 (PTH) as has been demonstrated with bovine tissue (*4*).

Most of the immunoreactive human PTH comigrates with peak 1 (PTH) in two different gel systems (Fig. 2). In this particular tumor most of the radioactivity is present in peak 2 after 60 minutes of incubation. A portion of the same protein extract was analyzed on both the *p*H 4.4 (Fig. 2A) and the SDS gels (Fig. 2B). The protein with the highest radioactivity (peak 2) that has a faster mobility in the *p*H 4.4 gel migrates more slowly on the SDS gel when compared to immunoreactive PTH. This suggests that the peak 2 protein has both a greater positive charge and a higher molecular weight than PTH. By measuring the mobilities of several proteins of known molecular weight as standards, we are able to estimate a molecular weight of $11,500 \pm 1,000$ for the peak 2 protein compared to 9,500 for PTH.

As in bovine parathyroid tissue (*4*) we have detected in human tissue a basic protein with a higher molecular weight than PTH that contains common antigenic determinants. The new protein is synthesized earlier than PTH and appears to be converted to PTH. We conclude that this protein is a biosynthetic precursor to PTH and refer to it as proparathyroid hormone (pro-PTH).

To investigate the activity of the cleavage system responsible for converting pro-PTH (peak 2) to PTH, the parathyroid tissues were given a standard 60-minute incubation with labeled amino acids, and the relative amounts of radioactivity appearing in the electrophoretic peaks corresponding to PTH (peak 1) and the precursor (peak 2) were measured (Table 1). The amount of precursor found in normal bovine tissues and in human clear cell hyperplasia varied from 43 to 58 percent. However, in the six adenomas,



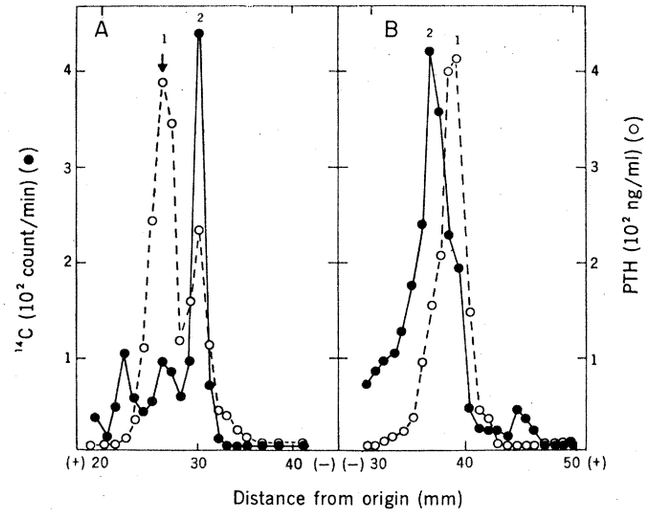


Fig. 1 (left). Electrophoretic analysis of extracts from parathyroid adenoma tissue after different incubations. Acid-urea extracts of tissue were analyzed by polyacrylamide gel electrophoresis in 8*M* urea at *p*H 4.4 (*8*) after incubation of 100 mg of tissue at 37°C under the following conditions: (A) 30-minute and (B) 60-minute incubation in ^{14}C-labeled amino acids. (C) After a 30-minute incubation, the medium containing ^{14}C-labeled amino acids was removed by centrifugation and replaced with a medium containing 20 unlabeled amino acids at 1 m*M* concentration. The incubation with the unlabeled amino acids was continued for an additional 30 minutes. (D) Puromycin (1 m*M*) was added to the incubation mixture after a 30-minute incubation with labeled amino acids, and the tissue was incubated for an additional 90 minutes. Further incorporation of labeled amino acids was completely inhibited by the addition of unlabeled amino acids and puromycin. The radioactivity plotted has been normalized to the same protein content of the extracts as determined by the Lowry method (*19*). (○) The ^{3}H-labeled bovine PTH, previously purified by gel electrophoresis, was added to the extracts prior to electrophoresis to mark the mobility of PTH. Fig. 2 (right). Polyacrylamide-gel electrophoresis of protein extracted from a human parathyroid adenoma after incubation in ^{14}C-labeled amino acids. Finely minced tissue (300 mg) from a human parathyroid adenoma was incubated for 60 minutes at 37°C in 2 ml of Hanks balanced salt solution containing 5 percent fetal bovine serum (Grand Island Biological) and 4 μc/ml of a mixture of 13 ^{14}C-labeled amino acids (New England Nuclear, standard 15, ^{14}C-labeled amino acid mixture with arginine and threonine omitted) and 8 additional unlabeled amino acids (1 m*M*). The tissue was homogenized in cold 8*M* urea–0.2*N* HCl (*4*) and centrifuged. The supernatant was precipitated with 10 percent trichloroacetic acid and twice dissolved and precipitated in 0.1*N* NaOH and 10 percent trichloroacetic acid, respectively. The final trichloroacetic acid precipitate was extracted in 8*M* urea–0.1*N* acetic acid, and portions of the extract were analyzed by polyacrylamide-gel electrophoresis in 8*M* urea at *p*H 4.4 (A) or in 8*M* urea containing 0.1 percent sodium dodecyl sulfate at *p*H 7.2 (B). The gels were cut into 1-mm slices. One half of each slice was assayed for radioactivity, and the other half was extracted in 0.1*N* acetic acid and assayed for immunoreactive PTH by a competitive-binding radioimmunoassay (*10*) as described (*4*). (●), ^{14}C-radioactivity; (○), immunoreactive PTH.

the precursor varied from 17 to 87 percent of the radioactivity. The results suggest the possibility that the rate of conversion of the precursor to PTH differed among the adenomas, although alternative possibilities of altered synthesis or degradation of pro-PTH are not ruled out.

Adenoma 3 in Table 1, which slowly converted the precursor to PTH, was analyzed further. This tissue was found to accumulate immunoreactive pro-PTH (peak 2) unlike normal tissue. A distinct peak of immunoreactive pro-PTH is present and represents about 15 percent of the total immunoreactive material (Fig. 2A). It was further shown by an immunoprecipitation study that the radioactivity comprising peak was incorporated into a protein structurally related to PTH. An antiserum to bovine PTH specifically bound 92 percent of the ^{14}C-labeled precursor (peak 2), and the addition of bovine PTH competitively reduced the binding to about 15 percent. These results support the hypothesis that precursor accumulates in this adenoma. In contrast to the studies of the adenoma shown in Fig. 2, in extracts of normal bovine tissue and in adenoma 6 in Table 1, which rapidly converts pro-PTH to PTH, only one peak of immunoreactive material was found corresponding to peak 1 (PTH). We do not know whether the amount of pro-PTH in the rapidly converting adenomas is extremely small or whether it does not cross-react well with PTH. However, Cohn *et al.* have reported that the pro-PTH in bovine glands comprises only 3 percent of the total immunoreactive PTH in tissue extracts (*11*). In addition, they have found that the bovine pro-PTH does react less well, compared to PTH, with at least one antiserum to PTH (*11*).

The biological significance of pro-PTH is unknown. The amounts of human pro-PTH available have been insufficient for direct testing of its biological activity. However, two lines of evidence would suggest that it may be a biologically inactive precursor. (i) Electrophoretic and chromatographic analyses of peptide fragments of bovine pro-PTH have revealed that the additional amino acids present are added to the amino end of the hormone (*12*), and (ii) studies on the structural requirements for biological activity of chemically synthesized bovine PTH indicate that extension of the hormone at the amino terminus lowers biological activity (*13*). In addition, Cohn *et al.* have shown that although bovine pro-PTH is biologically active, the activity is only one-third that of native PTH (*11*). Whether the intact prohormone is itself active or instead must be activated by conversion to PTH or some other fragment is not known (*11*).

Table 1. Relative amounts of radioactive parathyroid hormone (PTH) and proparathyroid hormone (pro-PTH) in various parathyroid tissues. Tissue was incubated with labeled amino acids for 60 minutes, except for human adenoma 2 which was incubated for 90 minutes. Protein was extracted and analyzed on polyacrylamide gels. Values under counts per minute are the total radioactivity in the peaks of PTH (peak 1) and pro-PTH (peak 2).

Tissue sample (No.)	PTH (count/min)	Pro-PTH (count/min)	Pro-PTH (%)
	Human adenoma		
1	30	194	87
2	216	783	78
3	154	504	77
4	1098	1362	55
5	1364	1390	50
6	673	139	17
	Human clear cell hyperplasia		
1	434	410	49
	Bovine glands		
1	1660	2287	58
2	1291	1536	54
3	8984	7240	45
4	1642	1254	43

The apparent variability of the activity of the mechanism that cleaves pro-PTH to PTH in the different tumors resulting in an accumulation of pro-PTH in some tumors may explain some clinical observations. Immunoreactive PTH secreted in vivo by some parathyroid adenomas eluted from gel filtration columns earlier than that of hormone extracted from human parathyroids (*6*). The elution position corresponded to a molecular weight of 11,000 to 12,000 (*6*). Our findings show that pro-PTH has an apparent molecular weight of approximately 11,500, is immunoreactive with antiserums to bovine PTH, and constitutes up to 15 percent of the total immunoreactive hormone in certain adenomas whereas it is undetectable in others. This suggests that the tumors secreting "large" PTH in vivo convert pro-PTH to PTH slowly and may accumulate and secrete pro-PTH. Thus, in addition to fragments of secreted PTH, pro-PTH and its degradation products may also contribute to the heterogeneity of immunoreactive PTH that exists in the circulation of patients with parathyroid adenomas (*6*, *14*). Secretion of pro-PTH by parathyroid adenomas would be analogous to the secretion of proinsulin by islet cell tumors of the pancreas (*15*). We have not carried our tissue incubations beyond 2 hours, the time required for the first appearance of radioactive protein in the medium (*16*) and, thus, have not determined whether the adenomas secrete pro-PTH into the medium. However, T. J. Martin has reported that during incubation of human parathyroid adenomas in vitro for longer times a few of the tumors appear to secrete radioactive and immunoreactive PTH with the properties of the prohormone (*17*).

We now know that human PTH undergoes at least two specific cleavages from the point of initial cellular biosynthesis to ultimate disappearance from the circulation. The first of these cleavages occurs in the cell when pro-PTH is converted to PTH (molecular weight, 9500), the predominant species of the hormone that is secreted into the circulation (*6*). After secretion the hormone rapidly undergoes a second cleavage; a biologically inactive fragment (molecular weight, 7500) is detected in the general circulation (*18*). These specific cleavage steps may provide important points of metabolic control to regulate the amount of biologically active hormone available for secretion and the concentrations of hormonally active polypeptides that are available for interaction with receptors in bone and kidney.

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Cholesterol Dissolution Rate in Micellar Bile Acid Solutions: Retarding Effect of Added Lecithin

Abstract. *In vitro studies on the dissolution rate of cholesterol monohydrate crystals in micellar bile acid solutions showed that the addition of lecithin decreases the dissolution rate even though lecithin increases the equilibrium solubility of cholesterol in these solutions. The reduction in rates caused by lecithin was attributed to a large crystal-solution interfacial barrier. An effective permeability coefficient for the interfacial barrier was calculated to be around* 1.5×10^{-5} *centimeter per second for the transport of cholesterol molecules.*

It has been generally assumed that the solubilizing capacity of bile for cholesterol is critically related to dissolution of cholesterol gallstones in vivo (*1*). However, solubility and dissolution kinetics both appear to be important (*2, 3*), as both may be limiting factors in in vivo dissolution of cholesterol gallstones. Danzinger *et al.* (*2*) have recently reported that ingestion of chenodeoxycholic acid causes gallstone dissolution in some patients. These investigations showed that, in women with cholelithiasis, chenodeoxycholic acid causes bile to become unsaturated with respect to cholesterol. They suggested, however, that stone dissolution might be limited by the kinetics of dissolution, since duodenal bile samples obtained after gallbladder contraction were significantly undersaturated with respect to cholesterol.

A great deal of research over the past few years has emphasized the role of lecithin in increasing cholesterol solubility in bile mediums (*1, 3*). Yet little is known of the influence of lecithin on the kinetics of cholesterol dissolution.

The purpose of this report is to present in vitro findings that show the existence of an important rate-determining interfacial barrier for dissolution of cholesterol monohydrate in bile acid–lecithin mediums. Lecithin shows a strong inhibitory effect on the rate of cholesterol dissolution in cholate or taurocholate mediums. This effect was quantitatively evaluated in terms of an effective permeability coefficient of around 1.5×10^{-5} cm sec^{-1} for the interfacial barrier.

The primary process of dissolution involves the disengagement and transport of molecules from the crystal surface into the bulk solution. Equation 1 was derived to express the rate of dissolution J as a function of the diffusion coefficient D, the solubility C_s, the bulk concentration C_b, and the surface area A for a solid, when both an interfacial barrier with an effective permeability coefficient P and a Nernst diffusion layer (*4*) of thickness h are important (*5*).

$$J = \frac{A(C_s - C_b)}{h/D + 1/P} \qquad (1)$$

Either of the transport barriers may be rate-determining for the process. When the interfacial resistance $1/P$ is negligible compared to the diffusional resistance h/D, the dissolution rate is dif-

Table 1. Dissolution rates J of the solute measured in solvent mediums at 37°C, with solubilities C_s and diffusion coefficients D independently determined (*6*)*, and values for h/D and $(h/D + 1/P)$ calculated according to Eq. 1.

Solute	Solvent medium	$J/A \times 10^{10}$ (mole cm^{-2} sec^{-1})	$C_s \times 10^6$ (mole ml^{-1})	$D \times 10^6$ (cm^2 sec^{-1})	$(h/D)10^{-3}$ (sec cm^{-1})	$(h/D + 1/P)10^{-3}$ (sec cm^{-1})
Cholesterol monohydrate	0.0371*M* taurocholate (*p*H 7.4)	0.43	0.30	2.15*	2.33	7.0
Cholesterol monohydrate	0.0371*M* taurocholate + 0.0133*M* lecithin (*p*H 7.4)	0.185	1.26	1.24	4.03	67.7
Cholesterol monohydrate	0.0464*M* cholate (*p*H 8.0)	1.66	1.31	2.17	2.30	7.8
Cholesterol monohydrate	0.0464*M* cholate + 0.0133*M* lecithin (*p*H 8.0)	0.43	2.60	1.49	3.36	60.4
Cholesterol monohydrate	0.116*M* cholate (*p*H 8.0)	4.82	3.31	1.90	2.63	6.8
Benzoic acid	0.01*N* HCl	1072.0	38.5	14.0	0.36	0.36

* Diffusion coefficient of 2.0×10^{-6} cm^2 sec^{-1} for 20 to 100 m*M* taurocholate loaded with cholesterol was reported by F. P. Woodford (*15*).