cal with that of an authentic sample in acidic, basic, and neutral solutions.

Electrical discharges through mixtures of N_2 and hydrocarbons give hydrogen cyanide as the major product (*7*). In a number of cases, the second most abundant nitrogen-containing product is now shown to be cyanoacetylene. Hydrogen cyanide is the starting point for relatively well-understood syntheses of glycine and the purines, and also syntheses for alanine, aspartic acid, and possibly other α-amino acids where the mechanism is less clear.

Miller's work demonstrated that these α-amino acids as well as serine and glutamic acid are produced by the hydrolysis of products formed in discharges through mixtures of methane, ammonia, and water, probably by a Strecker synthesis (*5*). We have now shown that under conditions similar to those used for purine synthesis cytosine may be obtained from cyanoacetylene; under other conditions aspartic acid and asparagine are formed.

These results suggest that a unified, laboratory synthesis of the major nitrogen-containing biochemicals from the products of electrical discharges may be possible, although this is by no means established. Even if this proves to be the case, other proposed modes of prebiotic synthesis would not be implausible, for acetylene and hydrogen cyanide are likely to be major products both of the prolonged photolysis or the thermal cracking of almost any reducing mixture containing carbon, hydrogen, and nitrogen compounds. Thus hydrogen cyanide, cyanogen, and cyanoacetylene are versatile starting materials in the synthesis of biologically important nitrogen-containing organic compounds.

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Retention of the Biological Potency of Human Pituitary Growth Hormone After Reduction and Carbamidomethylation

Abstract. *The reduced alkylated derivative of human pituitary growth hormone was prepared by reacting the native hormone in 8M urea solution, first with mercaptoethanol and then with iodoacetamide. On the basis of amino acid analyses, the product isolated after gel filtration on Sephadex G-100 is completely devoid of unreduced cystine but otherwise has a composition identical to that of the native hormone. When assayed in both the rat tibia test and the pigeon crop-sac assay, it appears that the full potency of the native hormone is retained in the reduced alkylated derivative.*

During the course of determining the sequence of the 188 amino acid residues in the human growth hormone (HGH) molecule (*1*), the reduced-alkylated derivative of the hormone (RA-HGH) was prepared. When RA-HGH was submitted to bioassay for growth-promoting and pigeon crop-sac stimulating activities, it was found that the derivative possesses a potency comparable with that of the native hormone. As far as we are aware, this is the first instance where cystine residues in biologically active proteins can be completely reduced and alkylated without resulting in loss of physiological potency.

For the preparation of RA-HGH, the procedure of Anfinsen and Haber (*2*) was employed. Briefly, the hormone (100 mg) was dissolved in an 8*M* urea solution (5 ml) freshly prepared from recrystallized urea. The solution was adjusted to *p*H 8.5 with 5 percent methylamine solution, freed of air by flushing with nitrogen gas, and allowed to react with mercaptoethanol (0.1 ml) in a stoppered flask for 6 hours at 23°C. After dilution with water to give a urea concentration of 2*M*, a tenfold excess (with respect to mercaptoethanol) of iodoacetamide was added; the *p*H of 8.5 was maintained by the addition of 5 percent methylamine solution and the solution was allowed to sit for 15 minutes. At the end of this period the remaining unreacted alkylating agent in the mixture was removed by reaction with an excess of mercaptoethanol. After 1 hour the products of the reaction were separated from the reagents by gel filtration in 0.005*M* NH_4OH solution on a Sephadex G-50 column.

In order to assess the homogeneity of the reduced alkylated HGH, we submitted it to exclusion chromatography on Sephadex G-100, using 0.01*M* NH_4HCO_3 solution as the eluant. For comparison, a sample of native HGH that had been allowed to stand at room temperature for 6 hours in an 8*M* urea solution of *p*H 8.5 was chromatographed on Sephadex G-100 under identical conditions. In the case of the urea-treated hormone (U-HGH), it was first freed from urea by passage in 0.005*N* NH_4OH solution through the Sephadex G-50 column and recovered by lyophylization before chromatography on the Sephadex G-100.

Figure 1 is a representation of the elution patterns obtained when each of these preparations (HGH, U-HGH, and

Table 1. Effect of various treatments on the biological activities of HGH.

Treatment	Bioassay					
	Tibia test			Pigeon crop-sac stimulation*		
	Total dose (μg)	No. of rats	Tibia width (μ)†	Total dose (μg)	Reaction	Mean
Untreated	20	5	244 ± 5	1	+2, +1, +2, +1, +1, 0, +2, +1	1.3
	60	6	278 ± 6	0.5	+1, +1, +1, +1, +1, 0, +1, +1	0.87
Urea (U-HGH-2)‡	20	6	236 ± 2.2	1	+2, +1, +1, +1, +1, +2, +2, +1	1.4
	60	6	285 ± 1.9	0.5	+1, +1, +1, 0, +1, +1, +1, +1	0.88
Reduced-alkylated (RA-HGH-4)‡	20	6	233 ± 4	1	+2, +1, +1, +1, +1, +1, +1, +1	1.1
	60	6	276 ± 2	0.5	+1, +1, 0, 0, +1, +1, +1, +1	0.75

* No stimulation, 0; moderate stimulation, + 1; good stimulation, + 2. † Mean ± standard error. ‡ See Fig. 1.

RA-HGH) was submitted to fractionation by gel filtration on Sephadex G-100 in 0.01*M* NH_4HCO_3 solution. While the HGH appeared to be homogeneous (Fig. 1, top), the treatment with urea introduced some heterogeneity (Fig. 1, middle), and further treatment during the reduction and alkylation reactions resulted in a heterogeneous mixture (Fig. 1, bottom). The various fractions from the gel filtration separations were pooled as shown in Fig. 1, and the materials were recovered by lyophylization. Samples of HGH-1 and U-HGH-2 were submitted for amino acid analyses (*3*). The composition of the two samples was identical and indistinguishable from that of the material used for the amino acid sequence studies (*1*). Moreover, the U-HGH-2 was fully active when assayed in the rat tibia test (*4*) or in the local crop-sac assay (*5*), as shown in Table 1.

Table 2. The amino acid composition of a sample of reduced alkylated human growth hormone.

Amino acid residues	Residues per mole (No.)	
	RA-HGH-4*	HGH†
Tryptophan	1	1
Lysine	8.8	9
Histidine	3.2	3
Arginine	10.3	10
S-Carboxymethyl-cysteine	4.2	
Aspartic acid	19.8	20
Threonine	10.0	10
Serine	18.2	18
Glutamic acid	26.1	26
Proline	7.7	8
Glycine	7.9	8
Alanine	7.1	7
Half cystine		4
Valine	7.3	7
Methionine	3.1	3
Isoleucine	7.8	8
Leucine	24.7	25
Tyrosine	7.8	8
Phenylalanine	12.9	13

* See Fig. 1. † From Li *et al.* (*1*).

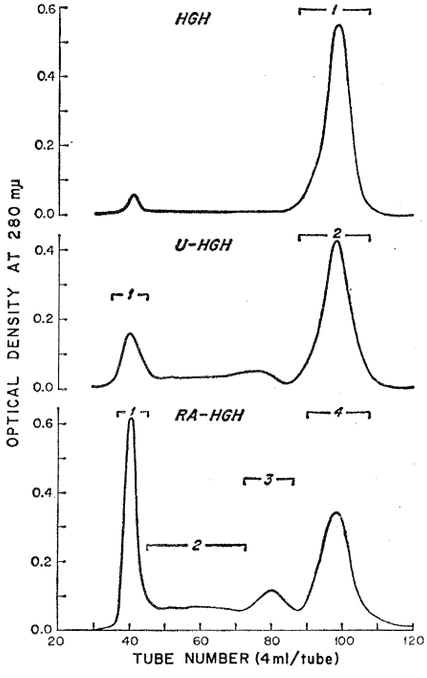

Fig. 1. Elution patterns obtained when 25 mg samples of HGH (top), U-HGH (middle), and RA-HGH (bottom) were submitted to gel filtration on a Sephadex G-100 column (75 by 3 cm) in 0.01*M* NH_4HCO_3; flow rate, 30 ml/hour. Fractions were pooled as indicated and recovered by lyophylization.

Samples of RA-HGH-1, RA-HGH-3, and RA-HGH-4 (see Fig. 1, bottom) were also submitted for amino acid analyses (*3*), and the results showed that their composition was identical with that of HGH with one exception: no free cystine was present in any of the samples, but from each a quantitative recovery (4 moles per mole of protein) of *S*-carboxymethylcysteine was obtained. Table 2 gives the amino acid composition of RA-HGH-4 compared with that of HGH (*1*). The RA-HGH-4 was further characterized by digestion with carboxypeptidase A (Worthington COA-DFP lot No. 6130); 1 mole of phenylalanine and about 0.2 mole of glycine per mole of protein (molecular weight 21,500) were liberated during 24 hours of the digestion with an enzyme-to-substrate ratio of 1/25 by weight in 0.5 percent $NaHCO_3$ solution at 37°C.

When RA-HGH-4 and U-HGH-2 were assayed for growth-promoting activity by the rat tibia test, the statistical evaluation of these results (Table 1) indicated parallelism of the slopes of the log-dose response plots and equal potency in the 95 percent confidence limit. Moreover, the results of the assay of RA-HGH-4 in the pigeon crop-sac test (as shown in Table 1) indicate full lactogenic potency in this derivative of HGH. Thus, the biological potency is retained in the reduced alkylated derivative of HGH even though it is completely devoid of —S—S— bridges.

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Ribosomal RNA Synthesis and Processing in a Particulate Site in the HeLa Cell Nucleus

Abstract. *A particulate fraction has been isolated from detergent-prepared HeLa cell nuclei. The fraction consists largely of organelles that resemble the nucleoli of intact cells. The 45S RNA that is precursor to 28S and 18S ribosomal RNA is associated with the fraction. The 32S RNA that is labeled after the 45S RNA and is the apparent precursor to 28S RNA is also associated with the fraction. The nucleoplasm contains 28S RNA that behaves as an intermediate between the 32S nucleolar RNA and the 28S cytoplasmic RNA.*

Advances in the knowledge of the RNA metabolism of mammalian cells have been extensive, particularly with respect to the synthesis and processing of ribosomal RNA. The initial event in the synthesis of the 18*S* and 28*S* ribosomal RNA species is the formation of a precursor RNA molecule with a sedimentation coefficient of 45*S* (*1*). Recent results indicate that approximately 25 minutes after formation, the 45*S* ribosomal precursor is cleaved to form 18*S* RNA and a species whose sedimentation coefficient is 35*S*. The 35*S* RNA is the precursor to the 28*S* RNA found in mature ribosomes (*2, 3*).

The location of the events in ribosomal RNA formation in subfractions of the HeLa cell has been studied (*3*). A nuclear preparation has been described which is substantially free of cytoplasmic contamination when viewed by either light or electron microscopy (*4*). This nuclear fraction contains over 90 percent of the cellular DNA. The mixed detergent used in preparing the