that the stimulation is unaffected by actinomycin, which has reduced the incorporation of orotic acid into nucleic acid to 5–10 per cent of the control values. The results presented here do not enable us to report any effect of growth hormone on nucleic acid synthesis, although experiments designed to study this more closely may reveal such effects.

During the writing of this communication Goodman[16] reported that Knobil has also evidence that the action of growth hormone in stimulating protein biosynthesis still occurs when the production of new nucleic acid in the muscle cell is inhibited.

We thank Dr. A. Stockell Hartree for purified human growth hormone, Miss M. B. Thomas for the hypophysectomies and Mr. D. C. Gardiner for general assistance.

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Long-spacing Segments from Renatured α1 Sub-units of Collagen

The mammalian collagen molecule is thought to be composed of two α1 and one α2 peptide chains. Alpha 1 and α2 have approximately the same molecular weight but differ in their amino-acid composition[1]. A mixture of α1 and α2 in the ratio of 2:1 (produced by the denaturation of neutral salt-soluble rat skin collagen) re-forms, under certain conditions suitable for renaturation, as much as 50 per cent of the rod-like collagen molecules[2]. It is of interest to learn whether the triple helix of the collagen molecule can also be formed from three α1 chains. Recently, Piez and Carrillo[3] have investigated the renaturation of the isolated sub-units (α1, α2, and β_{12}) of collagen at 15°. According to our experience these conditions are not optimal for the formation of the triple helix[4].

We isolated the α1 component from neutral salt-soluble calf skin collagen according to the method of Piez *et al.*[1]. Its homogeneity was checked by means of ultracentrifugation and starch-gel electrophoresis. Renaturation was achieved by incubating at a collagen concentration of 2·6 mg/ml. for 100 h at 22° in 0·25 M sodium citrate buffer, *p*H 3·7. The denaturation curve of the renatured solution gave a value of 30° for T_m (defined according to v. Hippel *et al.*[5]). Renatured acid-soluble collagen from calf skin has a T_m of 33° after renaturation under identical conditions.

When examined in the ultracentrifuge the renatured material showed, in addition to α1, a second, slower sedimenting peak which, during the course of the renaturation, grew at the expense of the α1 peak. Following the addition of pepsin (weight ratio of collagen: enzyme of 10:1; temp., 25°) the α1 component disappeared while the slower, pepsin-resistant peak, although not quite so sharp as the native molecule (Fig. 1), sedimented at approximately the same rate.

It was possible to produce long-spacing segments (Fig. 2) from the pepsin-resistant material, which accounted for almost 40 per cent of the starting material. The cross-striation pattern appeared to be the same with respect to the position of the individual cross-striations when compared with normal collagen. There appears to be some difference in the intensities of the individual bands. An exact photometric evaluation of the cross-striation pattern remains to be done.

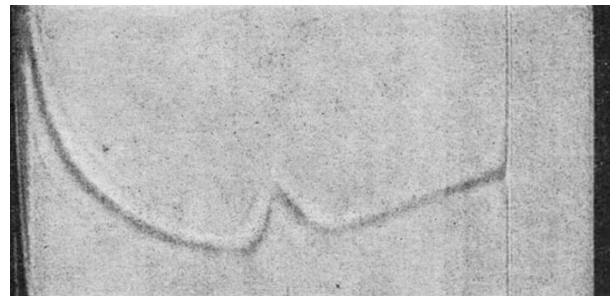

Fig. 1. Sedimentation diagram of renatured α1 after 18-h pepsin treatment; concentration prior to pepsin addition 2·6 mg collagen/ml. in 0·25 M citrate buffer, *p*H 3·7; temp., 22°; 59,000 r.p.m.; phase angle, 66°; photographed after 150 min

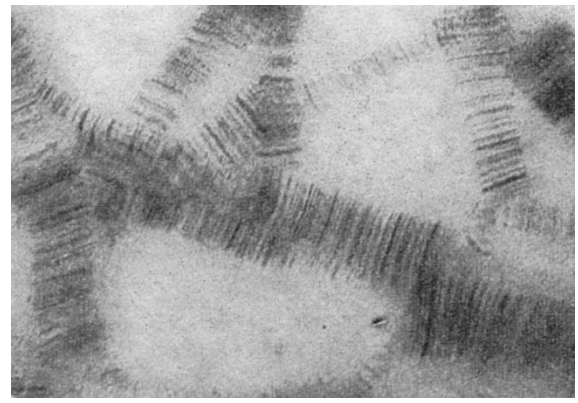

Fig. 2. Long-spacing segments from renatured α1 sub-units, produced by dialysis of the pepsin-treated solution in 0·05 per cent acetic acid against 0·4 per cent ATP solution, *p*H 3·5; stained with phosphotungstic acid and counterstained with uranylacetate

These preliminary experiments indicate that it is possible to build a native-like triple helix from three α1 chains. The cross-striation pattern of the segments thus obtained from α1 argues against the collagen model of Hodge[6] in which the α1 chain would be built up from five identical sub-sub-units and the α2 chain from seven identical sub-sub-units. If this were the case, then the cross-striation pattern of the long-spacing segments formed from α1 should be divided into five equal periods. The fact that no repeating period at all is evident suggests that it is very unlikely that α1 is composed of several identical smaller sub-units.

This investigation was supported by the Deutsche Forschungsgemeinschaft.

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