Incorporation of C^{14}-labeled AMP into ATP is the most direct evidence for reversibility of amino acid incorporation into RNA. No incorporation of AMP into ATP occurred when RNA plus free amino acid was used in place of amino acid-RNA, or when P–P was omitted. Calculation of the data shows that the molar rate of AMP incorporation into ATP greatly exceeds the rate of amino acid-RNA breakdown, suggesting that reactions which form unlabeled ATP occur during the incubation.

The approximate equilibrium position of these reactions was near unity, indicating the high energy nature of the amino acid-RNA linkage.

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16-ALKYLATED CORTICOIDS. I. 16α-METHYLPREDNISONE AND 16β-METHYLPREDNISONE[1]

Sir:

The recent publication by Boland[2] on 16α-methyl corticosteroids prompts us to report our studies of both 16α- and 16β-methyl steroids which possess a high order of anti-inflammatory activity without salt retention in animal and clinical trials.

Reaction of 3α-acetoxy-16-pregnene-11,20-dione[3] (I) with diazomethane gave an intermediate pyrazoline,[4] m.p. 199–200° dec., $[\alpha]_D$ +149.6° (all rotations 1% in diox.). *Anal.* Found: C, 69.51; H, 7.98; N, 6.69. Pyrolysis of this product at its melting point gave 3α-acetoxy-16-methyl-16-pregnene-11,20-dione, m.p. 163–166°, $[\alpha]_D$ +69.9°, λ_{max}^{MeOH} 248 mμ (ε 10,800). *Anal.* Found: C, 74.58; H, 8.55. Reduction with palladium yielded 3α-acetoxy-16β-methyl pregnane-11,20-dione (II) m.p. 160–163°, $[\alpha]_D$ +93.6°, no ultraviolet absorption between 220–300 mμ. *Anal.* Found: C, 74.37; H, 9.06. Enol acetylation with *p*-toluenesulfonic acid and acetic anhydride, then treatment with peracetic acid and finally alkaline hydrolysis, gave 3α,17α-dihydroxy-16β-methylpregnane-11,20-dione, m.p. 182–185°, $[\alpha]_D$ +83.6°. *Anal.* Found: C, 72.82; H, 8.92. Bromination at C-21 and then treatment with potassium acetate gave 3α,17α-21-trihydroxy-16β-methylpregnane-11,20-dione 21-acetate, m.p. 200–206°, $[\alpha]_D$ +119.6°. *Anal.* Found: C, 68.79; H, 8.39. Oxidation with N-bromosuccinimide produced the 3-ketone, m.p. 198–202°, $[\alpha]_D$ +128.0°. *Anal.* Found: C, 69.04; H, 8.10. Dibromination at positions 2 and 4, followed by dehydrobromination with dimethylformamide, produced 16β-methylprednisone 21-acetate (III) m.p. 232–235°, $[\alpha]_D$ +213.6°, λ_{max}^{MeOH} 238 mμ (ε 14,200). *Anal.* Found: C, 69.24, H, 7.21. Hydrolysis with potassium bicarbonate gave 16β-methylprednisone, m.p. 210–204°, $[\alpha]_D$ +190.2°, λ_{max}^{MeOH} 238 mμ (ε 14,700). *Anal.* Found: C, 71.19; H, 7.37

Reaction of I with methylmagnesium iodide produced 3α-hydroxy-16α-methylpregnane-11,20-dione,[5] m.p. 149–154°, $[\alpha]_D$ +100.5°, no selective absorption between 200 and 340 mμ. *Anal.* Found: C, 74.16; H, 9.41. This was converted into 16α-methylprednisone 21-acetate (m.p. 212–214° $[\alpha]_D$ +157.8°, λ_{max}^{MeOH} 238 mμ (ε 15,500). *Anal.* Found: C, 69.84; H, 7.22) by the same procedure used for the conversion of II to III (*i.e.*, enol acetylation and peroxidation, 21-bromination and acetoxylation, oxidation at C-3 and 2,4-dibromination and dehydrobromination).

A direct comparison of prednisone and its 16-methyl derivatives in human subjects utilizing (1) metabolic balance studies[6] consisting of the analysis of diet, urine and feces for calcium, phosphorus, nitrogen, sodium and potassium, and (2) the clinical response of patients indicate that 16-methylation (α or β) of the parent steroid, prednisone, is associated with an enhancement of anti-anabolic properties and an increase of 30–50% in both anti-inflammatory and sodium excreting properties. Unlike 16α-hydroxylation, 16α- or 16β-methylation contributes to anti-inflammatory potentiation. The *in vivo* conversion of the 16-methyl corticoids into urinary 17-keto steroids is limited to less than 5%: a conversion slightly less than that obtained with the parent steroids unsubstituted at position 16, and much less than that obtained with cortisone or hydrocortisone.

(1) After submission of this manuscript, a Communication appeared [G. Arth, D. Johnston, J. Fried, W. Spooncer, D. Hoff and L. Sarett, THIS JOURNAL, **80**, 3160 (1958)] describing the preparation of 16α-methylprednisone by essentially the same route. We have tried to eliminate as much of the common material as possible.

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(3) H. Slates and N. Wendler, *J. Org. Chem.*, **22**, 498 (1957).

(4) *Cf.* A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944).

(5) *Cf.* R. Marker and H. Crooks, THIS JOURNAL, **64**, 1280 (1942).

(6) E. C. Reifenstein, F. Albright and S. Wells, *J. Clin. Endocrinol.*, **5**, 367 (1945).

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THE INTERMEDIATE COBALT HYDROCARBONYL–OLEFIN COMPLEX IN THE OXO REACTION[1]

Sir:

The several mechanisms[2,3,4] which have been suggested for the oxo synthesis all involve a rate-determining displacement of a mole of carbon monoxide from a carbonyl of cobalt by the attacking olefin. The present study shows that not only does complexing occur between olefin and hydrocarbonyl[5] under room conditions without the libera-

(1) We wish to thank the Houdry Process Corp. for a generous fellowship which made this work possible.

(2) H. W. Sternberg, R. Markby and I. Wender, THIS JOURNAL, **79**, 6116 (1957); I. Wender and M. Orchin, in "Catalysis," Vol. V, Reinhold Publishing Corp., New York, N. Y. 1957, p. 124.

(3) A. R. Martin, *Chem. and Ind.*, 1536 (1954).

(4) G. Natta, R. Ercoli, S. Castellano and P. H. Barbieri, THIS JOURNAL, **76**, 4094 (1954).

(5) M. Orchin, L. Kirch and I. Goldfarb, *ibid.*, **78**, 5450 (1956).

tion of carbon monoxide but that the mixture instead absorbs carbon monoxide and complex decomposition leads to oxo aldehydes.

Dilute solutions of cobalt hydrocarbonyl were prepared from a toluene solution of dicobalt octacarbonyl at 25° under one atmosphere of carbon monoxide by disproportionation[6] with dimethylformamide (DMF) and subsequent acidification of the resulting mixture according to the equations

$$3Co_2(CO)_8 + 12DMF \longrightarrow 2Co(DMF)_6[Co(CO)_4]_2 + 8CO$$

$$Co(DMF)_6[Co(CO)_4]_2 + 2HCl \longrightarrow 2HCo(CO)_4 + 6DMF + CoCl_2$$

Addition of olefin to the hydrocarbonyl solution caused carbon monoxide absorption and resulted in the gradual disappearance of the hydrocarbonyl as measured by *o*-phenanthroline.[7] The relative rates of complex formation with 1-hexene, 2-hexene, and cyclohexene were 13:6:1. The relative rates of hydroformylation of these olefins at 110° are,[8] respectively, 11.4:3.1:1.

At 0° excess 1-hexene reacts with hydrocarbonyl and carbon monoxide to give a stable complex. At 25°, the complex begins to decompose with evolution of carbon monoxide and formation of aldehyde. Because the complex with 2-hexene decomposes at a faster rate, it is unlikely that the two isomers form the same complex.[9]

In other experiments, the acid–dimethylformamide phase was removed prior to olefin addition. Addition of excess olefin resulted in the rapid absorption of one mole of carbon monoxide per two moles of hydrocarbonyl. The composition of the complex is unquestionably $2HCo(CO)_4 \cdot olefin \cdot CO$.

Preparation of Hydrocarbonyl and Reaction with 1-Hexene.—To a flask thermostated at 25° and connected to a gas buret,[10] there was added 25 ml. of toluene and 1 ml. of dimethylformamide. After flushing with carbon monoxide, 5 ml. of toluene containing 0.51 g. (15 mmoles) of dicobalt octacarbonyl was added with stirring. Carbon monoxide liberation commenced immediately and, after one hour, 4 mmoles had been collected. Then 4 ml. (30 mmoles) of 1-hexene was injected through a serum-stoppered side-arm and the flask cooled to 0°. Two ml. of concentrated hydrochloric acid was injected and the mixture kept at 0° for 40 hours. Analysis (*o*-phenanthroline) showed that all the hydrocarbonyl had disappeared. In a blank experiment in the absence of olefin, 1.8 mmoles of hydrocarbonyl was present.

Carbon Monoxide Absorption.—Disproportionation of 3 mmoles of octacarbonyl was carried out at 25° as described above. The acid dimethylformamide phase was removed and 10 ml. (80 mmoles) of 1-hexene injected. Carbon monoxide absorption commenced immediately and was complete when two mmoles of gas was absorbed.

Aldehyde Formation.—In a disproportionation experiment using 10 ml. of dimethylformamide and 90 ml. of toluene containing 27 mmoles of octacarbonyl, 72 mmoles of gas was liberated. Then 12 ml. of 1-pentene and 20 ml. of hydrochloric acid were injected. Gas evolution began at once. After standing overnight, the reaction mixture was poured into an alcoholic solution of 2,4-dinitrophenylhydrazine. The toluene was removed by distillation and the precipitated hydrazone recrystallized from ethanol, 2 g., m.p. 103–104°; literature[11] for hexaldehyde, 103°. *Anal.* Calcd. for $C_{12}H_{16}N_4O_4$: C, 51.45; H, 5.76; N, 20.00. Found:[12] C, 51.59; H, 5.67; N, 19.99.

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(7) H. W. Sternberg, I. Wender and M. Orchin, *Anal. Chem.*, **24**, 174 (1952).

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(10) M. Orchin and I. Wender, *Anal. Chem.*, **21**, 875 (1949).

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CRYSTALLINE HUMAN GROWTH HORMONE

Sir:

We wish to report methods for the isolation in high yields of crystalline human pituitary growth hormone. Li, *et al.*,[1] Ehrenberg and Heijenskjöld[2] and Raben[3] also have recorded the preparation of human growth hormone, but not in crystalline form.

An acetone powder prepared from whole human pituitary glands was extracted with 0.3 *M* KCl at *p*H 5.5. The extract was brought to *p*H 8.5 and fractionated with ethanol in the cold,[4] material precipitated by ethanol in the concentration range of 10–30% being collected. This was dissolved in 0.1 *M* KCl at *p*H 7.5 and ethanol added to 25% concentration. During addition of ethanol, a crop of impure crystals of growth hormone formed and was retained for subsequent purification. The supernatant liquor when refractionated yielded essentially homogeneous crystals. Amounts of hormone equal to or exceeding those obtained during the acid extraction were isolated by re-extracting the acetone powder residue with 0.3 *M* KCl at *p*H 11, and then ethanol fractionation.

Column chromatography with DEAE (diethylaminoethyl)-cellulose, was used for final purification. Ellis and Simpson[5] have used this exchanger with the beef hormone, but under different conditions. In our experiments the protein sample was applied to the column in *p*H 10, 0.02 Γ/2 carbonate buffer, and the components were resolved by elution with buffer containing increasing amounts of sodium chloride. The human hormone was eluted at a salt concentration of 0.1–0.2 *M*. The columns were useful in recovering growth hormone from side fractions of the ethanol procedure. The hormone also has been obtained directly by

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