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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(12) Geller Laboratories, Hackensack, N. J.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CINCINNATI
CINCINNATI 21, OHIO
LAWRENCE KIRCH
MILTON ORCHIN

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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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DEAE-cellulose chromatography of the crude acidic or alkaline potassium chloride extracts.

Approximately 100 mg. of growth hormone can be obtained from 1 g. of acetone powder by these procedures. This yield is very near the value reported by Li,[1] and is several times that obtained by Raben.[3]

Either column chromatography of human growth hormone at *p*H 10 or standing in solution for short periods at *p*H 4 caused alteration in the electrophoresis patterns. At *p*H 10, an asymmetry was noted without resolution into components; a more definite separation was apparent at *p*H 4. No significant change in biological activity was detected as a result of this evident alteration, nor was any asymmetry or separation into components observed during analytical ultracentrifugation of the altered material. A similar situation has been reported for bovine growth hormone.[5,6]

The electrophoretic mobilities at *p*H 10 and 4 for the unaltered human hormone were -4.7×10^{-5} and $+3.4 \times 10^{-5}$ cm.^{2}/volt/sec., respectively. The altered material had a slightly greater negative mobility at *p*H 10 and a somewhat lower positive value at *p*H 4. A sedimentation constant of 2.4 *S* was calculated. These values are in agreement with those already reported.[1,2] Assayed in hypophysectomized rats, the human hormone had approximately the same growth activity as pure bovine growth hormone.

We wish to thank Dr. D. E. Williams and Mr. J. Ruscica for the physical measurements, Dr. R. H. Silber for the bio-assays and Mrs. Elizabeth Hagan for her able technical assistance.

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MERCK SHARP & DOHME RESEARCH LABORATORIES
MERCK & CO., INC. U. J. LEWIS
RAHWAY, NEW JERSEY NORMAN G. BRINK

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ENZYMATIC CONVERSION OF URIDINE DIPHOSPHATE D-GLUCURONIC ACID TO URIDINE DIPHOSPHATE GALACTURONIC ACID, URIDINE DIPHOSPHATE XYLOSE, AND URIDINE DIPHOSPHATE ARABINOSE[1,2]

Sir:

Results of previous investigations indicate that D-glucuronic acid serves as a precursor of the D-galacturonic acid moiety of pectin,[3] and that the D-xylose and L-arabinose constituent units of pentosans originate from uronic acid precursors by loss of C-6.[4-9] It has been postulated that uridine diphosphate (UDP) sugars are involved in these conversions.[7,8]

In the present communication evidence is presented that particulate preparations from *Phaseolus aureus* (mung bean) seedlings are capable of catalyzing the formation of UDP galacturonic acid and UDP pentose from UDP glucuronic acid.

One hundred grams of mung bean seedlings was homogenized in 70 ml. of 0.01 *M* sodium–potassium phosphate buffer of *p*H 7.0, and the particulate material which sedimented at 18,000 × *g* for 30 minutes, after removal of coarse debris, was suspended in 0.5 ml. of 0.1 *M* tris-(hydroxymethyl)-aminomethane chloride buffer, *p*H 7.5.

The electrophoretic separations were carried out in 0.2 *M* ammonium formate, *p*H 3.6, or in 0.2 *M* ammonium acetate, *p*H 5.8. Other methods used in this investigation have been described in a previous paper.[10]

The particulate suspension (0.2 ml.) was incubated at 23° with 0.27 μmoles (10^6 c.p.m.) of UDP glucuronic acid[11] in a total volume of 1.2 ml., and after one hour the components were separated by paper electrophoresis at *p*H 5.8. Residual UDP glucuronic acid and four new radioactive bands, representing 50% of the total radioactivity, were present in the mixture.

Band I, containing 34% of the total radioactivity, was shown to consist mainly of UDP pentose by co-electrophoresis with authentic UDP arabinose[10] at *p*H 3.6 ($M_{picrate}$, 1.20) and at *p*H 5.8 ($M_{picrate}$, 1.08). The radioactive compounds liberated by hydrolysis with 1 *N* HCl for 15 minutes at 100° co-chromatographed with authentic D-xylose and L-arabinose. The ratio of radioactive xylose to arabinose was 6:1.

Band II, containing 6% of the total radioactivity, was identified as UDP galacturonic acid by co-electrophoresis with authentic UDP galacturonic acid[12,13] at *p*H 3.6 ($M_{picrate}$, 1.26) and at *p*H 5.8 ($M_{picrate}$, 1.45), and by coelectrophoresis with authentic galacturonic acid at *p*H 3.6 ($M_{picrate}$, 0.54) of the radioactive compound liberated by hydrolysis in 1 *N* HCl for 15 minutes at 100°.

Band III, containing 1% of the total radioactivity, and Band IV, containing 9% of the total radioactivity, consisted of galacturonic acid 1-phosphate and glucuronic acid 1-phosphate, respectively.

Neither C^{14}-labeled D-glucuronic acid 1-phosphate nor D-glucuronic acid was converted to galacturonic acid, pentose, or to their phosphorylated derivatives when incubated with the particulate suspension.

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(2) It has not been determined whether the carbohydrate moieties of these nucleotides are the D or L forms. However, since the galacturonic acid appears to arise from the glucuronic acid by epimerization of the 4-hydroxyl, it is assumed that the galacturonic acid is the D optical isomer. The xylose is assumed to be derived by decarboxylation of C-6 of D-glucuronic acid, and is therefore D-xylose. The arabinose is formed either by similar decarboxylation of D-galacturonic acid, or through epimerization of D-xylose, and is assumed to be L-arabinose.

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