capable of catalyzing the reduction of cortisone to tetrahydrocortisone. This activity is found solely in the particle free supernatant of a sucrose or phosphate homogenate and is precipitated between 55 and 70% saturation with ammonium sulfate.

This reaction, which results in loss of the α,β unsaturation of cortisone, can be followed by the disappearance of the characteristic absorption of the steroid at 240 mμ. The hydrogen donor is TPNH; DPNH is completely inactive. Reversal cannot be observed upon the addition of TPN and either dihydrocortisone or tetrahydrocortisone. The reaction can be coupled to the oxidation of *d*-isocitrate by TPN and isocitric dehydrogenase or to the oxidation of glucose-6-phosphate by TPN with glucose-6-phosphate dehydrogenase (Table I). The reaction proceeds faster under nitrogen than air. In this system, with cortisone as the substrate, tetrahydrocortisone appears to be the sole product. The allopregnane isomer is not detected. The metabolite has been identified by (a) paper chromatography of the free alcohol and of the acetate in two solvent systems; (b) spectrophotometric comparison of the sulfuric acid chromogens of isolated and authentic tetrahydrocortisone[5]; and (c) comparison of infrared spectra with known compounds.

TABLE I

COFACTOR REQUIREMENT FOR CORTISONE REDUCTION

Reaction components: 5μM. $MgCl_2$, 5 μM. nicotinamide, 50 μM. phosphate buffer *p*H 7.4, 0.28 μM. cortisone, 7 mg. protein of ammonium sulfate fraction; final vol. 1.5 ml.; incubated 1 hour at 38°, under nitrogen.

Additions	μM. Cortisone metabolized
DPN (0.5μM.)	0.0
DPN (0.5μM.) + lactate (20μM.) + lactic dehydrogenase (0.2 mg.)	0.0
TPN (0.1μM.)	0.0
TPN (0.1μM.) + *d*-isocitrate (1μM.) + isocitric dehydrogenase (5 mg. protein)	0.22
TPN (0.1μM.) + glucose-6-phosphate (1μM.) + glucose-6-phosphate dehydrogenase (0.2 mg.)	0.18

Since the reduction of cortisone to tetrahydrocortisone involves the addition of four hydrogens, it is likely that the reaction proceeds in two steps with dihydrocortisone as the intermediate. In support of this, the same liver fraction also has been found to catalyze the conversion of dihydrocortisone to tetrahydrocortisone.

This latter reaction requires either DPNH or TPNH. It can be followed spectrophotometrically by the absorption of the reduced pyridine nucleotides at 340 mμ and is readily reversible with the equilibrium toward the reduced product (Fig. 1). The reaction is completely inhibited by *p*-chloromercuribenzoate (5×10^{-4} *M*) and the inhibition can be reversed by glutathione or cysteine (5×10^{-3} *M*).

It is probable, therefore, that the formation of tetrahydrocortisone from cortisone proceeds thus

$$\text{Cortisone} \xrightarrow{\text{TPNH}} \text{Dihydrocortisone} \underset{\text{TPN or DPN}}{\overset{\text{TPNH or DPNH}}{\rightleftharpoons}} \text{Tetrahydrocortisone}$$

(5) A. Zaffaroni, THIS JOURNAL, **72**, 3828 (1950).

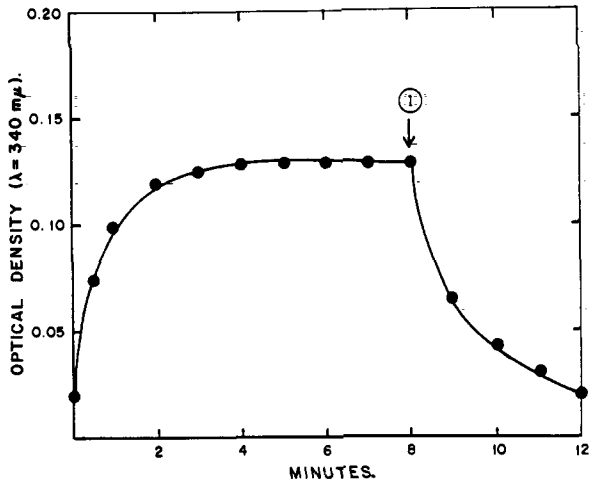

Fig. 1.—Ascending curve represents formation of TPNH as a result of the oxidation of tetrahydrocortisone on adding 1 μM. TPN, 1 μM. tetrahydrocortisone and 15 mg. protein of ammonium sulfate fraction at *p*H 7.4. Reversibility is demonstrated at (1) when 0.15 μM dihydrocortisone is added, causing decrease in optical density as dihydrocortisone is reduced.

That dihydrocortisone does not accumulate in detectable amounts when cortisone is the substrate may be explained by the observation that the reduction of dihydrocortisone to tetrahydrocortisone is more rapid than the over-all reaction. Definitive proof of this mechanism awaits the separation of the enzymes involved.

The ammonium sulfate fraction is also capable of catalyzing the disappearance of other Δ4,3-ketosteroids in the presence of TPNH. These include hydrocortisone, desoxycorticosterone, progesterone, testosterone, adrenosterone, and cholestenone. It is not yet known whether the enzymes mediating these reactions are the same as those concerned with the reduction of cortisone.

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A NEW TYPE OF METALLIC BONDING IN MOLECULAR COMPLEXES[1]

Sir:

Evidence for weak metallic bonding in planar Ni(II), Pd(II) and Pt(II) complexes has been presented.[2] It has been suggested that metallic chains may result when normal dsp^2 square bonding leaves a vacant p-orbital, allowing mixing with an octahedral state involving d^2sp^3 orbitals and metal bonds.[2] Au(III) complexes should, then, be capable of forming weak metal bonds.

Examining this possibility led to the preparation of $Au(DMG)_2{}^+AuCl_2{}^-$ (HDMG = dimethylglyoxime) which has linear gold chains with Au–Au —

(1) Work was performed in part in the Ames Laboratory of the Atomic Energy Commission.

(2) (a) S. Yamada, *Bull. Chem. Soc. Japan*, **24**, 125 (1915), and references therein. (b) L. E. Godycki and R. E. Rundle, *Acta Cryst.*, **6**, 487 (1953).

3.26 Å., nearly identical with metal distances in complexes of Ni, Pd and Pt. Here, however, the chains are alternately Au(I) and Au(III). Figure 1 shows one layer of the structure. In the layer above the one shown, cation and anion positions are interchanged and both ions rotated approximately 90° in the plane. The gold bonds are between layers. The configuration about Au(III) is octahedral, counting metal bonds, as in Ni(II), Pd(II) and Pt(II). The configuration about Au(I) is square, counting the metal bonds.

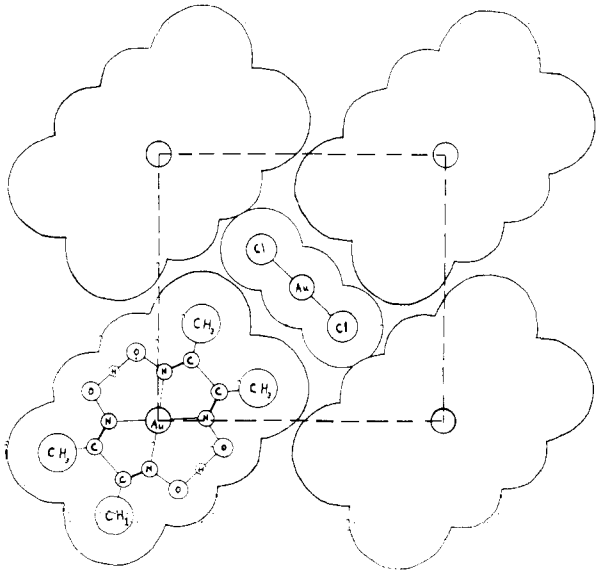

Fig. 1.—Structure for layer at $z = 0$. Layer at $z = {}^1/_2 c$ is identical except that cation and anion exchange positions are reflected through dashed line (a horizontal, b vertical).

It now appears that metals forming linear sp bonds, and possessing vacant p-orbitals, can similarly form weak metal bonds, presumably through some contribution of a dsp^2 state, illustrating anew the principle that atoms tend to use all low energy orbitals in bond formation.[3]

Though further examples of this type of interaction should be found, promotional energies are high, and the contribution of the metallic bond, small. Differences in the solubility of Ni(DMG) and $Cu(DMG)_2$[4] suggest that the Ni–Ni bond energy is approximately 10 kcal./mole. Moreover, such weak bonds can be destroyed by steric influences.

Preparation.—Aqueous $HAuCl_4$ and alcoholic dimethylglyoxime were mixed in the molar ratio 1:2. During several hours the solution deepened from yellow to red-amber, crystals separating subsequently. (Base causes immediate precipitation of lighter yellow crystals, composition as yet unknown.)

Analysis.—Au:DMG:Cl = 1:1:1. The amber crystals are notable for their stability to strong acid. (I am indebted to Dr. C. V. Banks for this analysis.)

X-Ray Data.—Orthorhombic needles, $a = 11.52$, $b = 10.59$, c (needle) = 6.52 Å. $\rho_{obsd} = 2.93$ g./cc., $\rho_{calcd} = 2.92$ for $z = 2$. Probable space group, Pnnm. 4Au at 000, $\frac{1}{2}\frac{1}{2}0$, $00\frac{1}{2}$, $\frac{1}{2}\frac{1}{2}\frac{1}{2}$.

Structural Derivation.—Infrared spectra of crystalline mull is nearly identical with that of Ni(DMG)$_2$ and Pd(DMG)$_2$, establishing $Au(DMG)_2^+$ ion. Packing considerations plus gold positions are then straightforward. The extreme importance of gold scattering renders a complete X-ray determination nearly impossible. (Dr. Marvin Margoshes kindly obtained the infrared spectrum.)

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RECEIVED APRIL 24, 1954

(3) R. E. Rundle, THIS JOURNAL, **69**, 1327 (1947); *J. Chem. Phys.*, **17**, 671 (1949).

(4) H. Christopherson and E. B. Sandell, *Anal. Chim. Acta*, **10**, 1 (1954).

CITRULLINE AS A PRECURSOR OF PYRIMIDINES[1]

Sir:

The incorporation of carbon dioxide into carbon-2 of pyrimidines in the rat was demonstrated in 1950.[2] The location of this carbon in a ureide group in the pyrimidine suggested that the mechanism of synthesis may be related to that of urea. Ratner and Pappas[3] demonstrated a condensation product of citrulline and aspartic acid (later identified as argininosuccinic acid[4]) to be an intermediate in arginine synthesis. This suggested that such a compound might be an intermediate in pyrimidine synthesis also; this would account for the appearance of carbon dioxide in carbon-2 of uracil, as well as utilization of aspartic acid.[5]

Carbamyl-labeled L-citrulline was synthesized[6] from urea-C^{14} and L-ornithine. Administration of the citrulline-C^{14} to rats and a chick resulted in some labeling of proteins, practically no label in nucleic acid components, and excretion of the greatest portion of activity. The loss of active material by conversion to arginine was avoided by using an arginine-requiring strain of *Neurospora* (46004a).[7] The mold was grown in 900 ml. of base medium[8] to which was added 54 mg. of L-arginine and 5 mg. of L-citrulline-carbamyl-C^{14}. After growth for four days at room temperature, the washed mycelium was extracted with cold trichloroacetic acid, lipid solvents, and hot sodium chloride. The nucleate precipitated from the latter extract with alcohol was grossly contaminated with polysaccharide, and could not be completely purified by reprecipitation with alcohol or by precipitation as the lanthanum salt.[9] A quantity of this material containing 14 mg. of nucleic acid was hydrolyzed with alkali[10] at room temperature, and

(1) Supported by Contract Number AT(30-1)-1351 with the U. S. Atomic Energy Commission.

(2) M. R. Heinrich and D. W. Wilson, *J. Biol. Chem.*, **186**, 447 (1950).

(3) S. Ratner and A. Pappas, *ibid.*, **179**, 1183 (1949).

(4) S. Ratner, B. Petracek and O. Rochovansky, *ibid.*, **204**, 95 (1953).

(5) U. Lagerkvist, P. Reichard and G. Ehrensvard, *Acta Chem. Scand.*, **5**, 1212 (1951).

(6) A. C. Kurtz, *J. Biol. Chem.*, **122**, 477 (1938). Urea-C^{14} was purchased from Tracerlab, Inc., on authorization of the U. S. Atomic Energy Commission.

(7) Kindly supplied by Dr. N. H. Horowitz.

(8) G. W. Beadle, *J. Biol. Chem.*, **156**, 683 (1944). Sucrose was replaced by 15 g./l. of glucose.

(9) J. N. Davidson and C. Waymouth, *Biochem. J.*, **38**, 39 (1944).

(10) G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.*, **161**, 83 (1945).