dithiohydantoin[3] and 5-hexyl-5-methyl-2,4-dithiohydantoin (m.p. 98.5–99.6°. *Anal.* Calcd.: N, 12.16; S, 27.83. Found: N, 12.20; S, 27.83.)

Doses of the order of 200–300 mg./kg./day of 5-*n*-heptyl-2-thiohydantoin were required to protect all mice infected intravenously with *M. tuberculosis* H37Rv from the lethal effects of the disease. This was true also when a strain highly resistant to streptomycin was used to infect the animals. In both cases postmortem examination of the surviving mice revealed little if any tuberculous pathology. When this drug was fed to hamsters infected with the H37Rv strain at a concentration of 0.1% in the diet a therapeutic effect equivalent to that obtained with a fourfold concentration of *p*-aminosalicylic acid was achieved. In this species, too, the tuberculous pathology in the survivors was quite small. Extensive acute and chronic toxicity studies in rodents, dogs and monkeys showed that the drug is well tolerated by these species. In view of these results it is felt that 5-*n*-heptyl-2-thiohydantoin is worthy of clinical trial as an antitubercular drug.

It seems more than a coincidence that the grouping, —NH—C(=S)—NH— or a tautomeric form thereof occurs so frequently in *in vivo* tuberculostatically active drugs. The thiosemicarbazones, the thioureas reported by Huebner,[4] the mercaptotriazinones of Hagenbach[5] and now the thiohydantoins all have in common the thioureido function.

(3) H. C. Carrington, *J. Chem. Soc.*, 681 (1947).
(4) C. F. Huebner, *et al.*, THIS JOURNAL, **75**, 2274 (1953).
(5) R. E. Hagenbach, E. Hodel and H. Gysin, *Experientia*, **10**, 620 (1954).

E. FROELICH
ALICE FRUEHAN
MARY JACKMAN
FRED K. KIRCHNER
E. J. ALEXANDER
S. ARCHER
STERLING-WINTHROP RESEARCH INSTITUTE
RENNSELAER, N. Y.

RECEIVED MAY 1, 1954

DIRECT PRODUCTION OF RADIOACTIVE ALIPHATIC HYDROCARBONS BY PILE IRRADIATION[1]

Sir:

Study of the hot atom chemistry of carbon-14 by the irradiation of nitrogenous organic materials in the heavy water pile at the Argonne National Laboratory (CP-3′) has led us to observe a method of producing saturated aliphatic hydrocarbons in radioactive form in high yield and high specific activity.

A 5 mole per cent. solution of aniline in normal pentane, 20 cc. of which was enclosed in a quartz tube and irradiated for one week in the CP-3′ pile at a flux of 10^{11} neutrons per cm.^{2} per second, proved to yield about 25% of the radiocarbon in the form of radioactive normal pentane, with less than 1% as iso- or neo-pentane; about 15% in the form of radioactive hexane, which apparently is about two-thirds normal hexane; and the remainder in heavier hydrocarbons. The distribution is given in Table I.

(1) This research was supported by the United States Air Force through the Office of Scientific Research of the Air Research and Development Command.

TABLE I

COMPOSITION OF THE RADIOACTIVE HYDROCARBONS FORMED BY THE IRRADIATION OF A 5 MOLE PER CENT. SOLUTION OF ANILINE IN NORMAL PENTANE

5% of total C^{14} was extractable into 12 *N* HCl

Chemical form	Per cent. of the total radiocarbon
Gases (Boiling up to room temperature)	12
n-Pentane	25
i-Pentane	1
n-Hexane	12
Other hexanes	6
Heptane and heavier hydrocarbons, boiling according to following ranges, °C.	
95–125[8]	8
125–155[8]	5
155–175[8]	6
175–215[8]	5
215–245[8]	3
245–290[8]	3
Residue	9

It is clear from these preliminary results, which were duplicated by a second run in which ethylamine was substituted for aniline, that a high velocity carbon-14 on colliding with the liquid aliphatic hydrocarbon has a very good chance of entering the chain. The reasons for this may be debatable, but the facts seem to be clear. It should be realized that the hexane and heavier hydrocarbons produced in the above-described bombardment were essentially carrier free except for any which may have been produced by gamma and fast neutron radiation. It would seem therefore that the process described can produce radioactive hydrocarbons of high specific activity. It is further clear that as far as neutron economy is concerned, this process could well compete with any organic synthesis, for the sole labor involved is the purification of the original chemical, the preparation of the samples for irradiation, and the subsequent distillation and separation. There is reason to believe that the procedure outlined would also serve to introduce radiocarbon into heavy lubricating oils.

DEPARTMENT OF CHEMISTRY AND INSTITUTE FOR NUCLEAR STUDIES
UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS
ARIEL G. SCHRODT
W. F. LIBBY

RECEIVED MAY 10, 1954

ENZYMATIC REDUCTION OF CORTISONE[1]

Sir:

Previous *in vivo* and *in vitro* studies reveal that the major pathway of cortisone metabolism is the reduction of the Δ4-3 ketone group to the saturated 3-alcohol by the liver.[2,3,4] We should like to report the presence of an enzyme system in rat liver

(1) Abbreviations as used in this communication are: cortisone (Δ4-pregnene-17α,21-diol-3,11,20-trione), dihydrocortisone (pregnane-17α,21-diol-3,11,20-trione), tetrahydrocortisone (pregnane-3α,17α,21-triol-11,20-dione), TPNH and TPN (reduced and oxidized triphosphopyridine nucleotide, respectively), DPNH and DPN (reduced and oxidized diphosphopyridine nucleotide, respectively).
(2) J. J. Schneider, *J. Biol. Chem.*, **194**, 337 (1952).
(3) J. J. Schneider and P. M. Horstmann, *ibid.*, **196**, 629 (1952).
(4) E. Y. Caspi, H. Levy and O. M. Hechter, *Arch. Biochem.*, **45**, 169 (1953).

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).

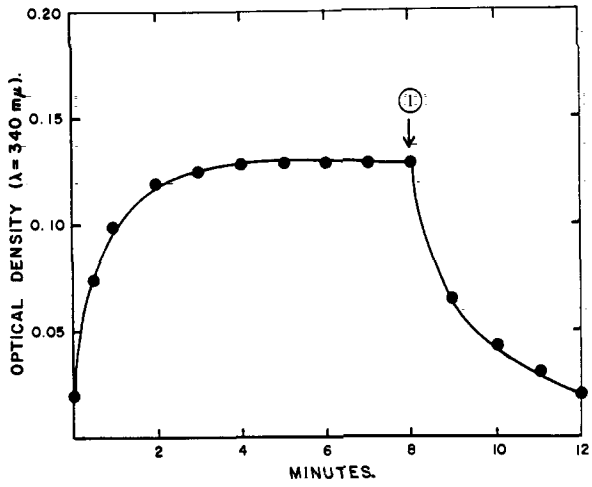

Fig. 1.—Ascending curve represents formation of TPNH as a result of the oxidation of tetrahydrocortisone on adding 1 μM. TPN, 1 μM. tetrahydrocortisone and 15 mg. protein of ammonium sulfate fraction at *p*H 7.4. Reversibility is demonstrated at (1) when 0.15 μM dihydrocortisone is added, causing decrease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

NATIONAL HEART INSTITUTE, AND
NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA 14, MARYLAND
G. TOMKINS
K. J. ISSELBACHER

RECEIVED MAY 10, 1954

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).