efficiency should be decreased by deuteration of the inhibitors. It is noteworthy that isotope effects of a considerable magnitude have been observed in the chain carrying step of air oxidation[1] and in the degradative chain transfer reaction in allyl acetate polymerization.[2] It has been found that N-D-N-methylaniline and N-D-diphenylamine give oxidation curves, with cumene and tetralin as substrates in chlorobenzene solution, which are congruent with those observed in the presence of the corresponding undeuterated compounds. An illustrative example is shown in Fig. 1.

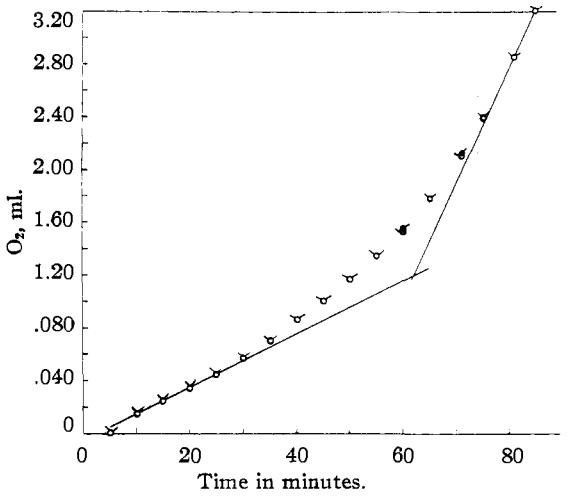
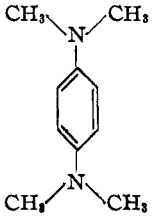


Fig. 1.—Oxidation of cumene (2.4*M*) in chlorobenzene at 62.5° inhibited by $C_6H_5NHCH_3$ (O) and $C_6H_5NDCH_3$ (O), initiated by $1.01 \times 10^{-2}M$ azoisobutyronitrile; inhibitor concentrations, 3.33×10^{-3} *M*.

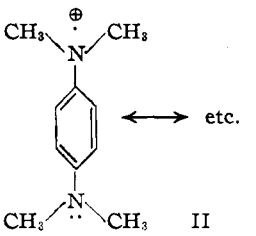
As a specific test for complex formation we have studied the action of tetramethyl-*p*-phenylenediamine (I).



I

Despite the fact that this compound contains no "labile" hydrogen it is a powerful inhibitor and stops two oxidation chains[3] in both nitromethane and chlorobenzene solution with tetralin as a substrate. Furthermore, in nitromethane the purple color of the Wurster cation II is developed during the early part of the inhibition period and is subsequently dissipated.

In an especially dramatic experiment water was included as a second phase in a repetition of the



II

oxidation in chlorobenzene. During the early part of the reaction *the color of the Wurster cation appeared in the aqueous phase.* The ion was positively characterized by measurement of its visible absorption spectrum. The inhibition continued but with a very low efficiency and eventually the dye disappeared from the solution. These observations indicate that hydrolysis of the complex occurs according to equation 4 and the result represents

$$In\text{-}RO_2\cdot + H_2O \rightleftharpoons In\cdot^+ + RO_2H + OH^- \quad (4)$$

the detection of gross amounts of an intermediate species which has neither undergone hydrogen abstraction nor attachment of $RO_2\cdot$ to a specific carbon atom of the inhibitor.

It is very evident that these formulations permit wide extension in the field of radical chemistry in solution. Examples too numerous to cite present themselves. A few especially significant related problems or observations are, (1) the postulated complex formation in the chain transfer reaction of growing polystyrene radicals in bromobenzene solution,[5] (2) the stereospecific free radical addition of hydrogen bromide to 1-cyclohexenyl bromide,[6] and (3) the general problem of the mechanism of aromatic substitution by free radicals.[7]

Acknowledgment.—We are highly indebted to the B. F. Goodrich Company for their generous support of this study.

(1) R. A. Max and F. E. Deatherage, *J. Am. Oil Chem. Soc.*, **28**, 110 (1951).

(2) P. D. Bartlett and F. A. Tate, THIS JOURNAL, **75**, 91 (1953).

(3) The stoichiometric assignment is made on the basis of the length of the induction period. This is made possible by the use of appropriate efficiency factors for the production of radicals from azo-bis-isobutyronitrile by two independent methods which will be reported at an early date.[4]

(4) G. S. Hammond, C. E. Boozer, J. N. Sen and C. E. Hamilton unpublished observations.

(5) F. R. Mayo, THIS JOURNAL, **75**, 6133 (1953).

(6) H. L. Goering, P. I. Abell and B. F. Aycock, *ibid.*, **74**, 3588 (1952).

(7) See D. I. Relyea and D. F. DeTar, *ibid.*, **76**, 1202 (1954), and previous papers in the series and G. S. Hammond, J. T. Rudesill and F. J. Modic, *ibid.*, **73**, 3929 (1951), for examples of discussions pertinent to the subject.

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RECEIVED JUNE 4, 1954

THE CONVERSION OF CHOLESTEROL TO PREGNENOLONE IN BOVINE ADRENAL HOMOGENATES[1]

Sir:

The transformation of cholesterol to 17-hydroxycorticosterone and corticosterone has been demonstrated in the isolated perfused bovine adrenal.[2] Based upon *in vitro* perfusion studies, the sequence of reactions shown in Fig. 1 was postulated to account for cholesterol conversion to corticoids.[3]

(1) Aided in part by the Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-255.

(2) O. Hechter, *et al.*, *Recent Progress in Hormone Research*, **6**, 215 (1951).

(3) O. Hechter, Ciba Foundation, "Colloquia on Endocrinology," **7**, 161 (1953). Edited by G. E. Wolstenholme, Little, Brown, Boston, Mass.

All of the reactions illustrated, with the single exception of the conversion of cholesterol to pregnenolone, have now been achieved in adrenal homogenates.[3] The lack of the experimental evidence for the cholesterol → pregnenolone reaction and apparent absence of pregnenolone in adrenal tissue constitute the major weakness of the reaction sequence shown in Fig. 1.

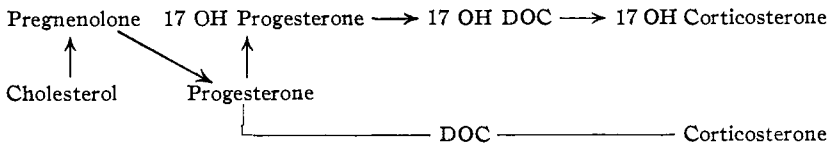

Fig. 1.

To obtain information on this point, C^{14} labeled cholesterol was incubated with adrenal homogenates and the resulting products fractionated by systematic paper chromatography. Under our conditions, we have regularly observed radioactive products similar to pregnenolone and progesterone in terms of paper chromatographic behavior and color reactions; corticosteroid production by these adrenal homogenates is more variable. In this communication we wish to describe results of an experiment wherein pregnenolone has been identified as one of the products of cholesterol metabolism.

Cow adrenal glands (60 g.) obtained fresh at the slaughter house were homogenized in a Waring blendor for 90 seconds with 120 ml. of ice-cold phosphate buffer of the composition: $M/150$ phosphate buffer pH 7.4, diphosphopyridine nucleotide (5×10^{-4} M), adenosine triphosphate, nicotinamide, magnesium sulfate, and sodium fumarate (all at a concentration of 5×10^{-3} M). To the homogenate 200 μg. of cholesterol-4C^{14} (5×10^{6} ct./min./mg.) in 0.2 ml. of propylene glycol was added and the mixture incubated for 3.5 hours at 37°, using 100% oxygen as gas phase. The C^{14}-cholesterol sample was homogeneous in the ligroin–propylene glycol[4] and heptane–phenyl cellosolve[5] paper chromatography systems. At the end of incubation, the homogenate was extracted exhaustively with acetone, ethyl acetate and chloroform; the total extracts were combined and distilled to dryness *in vacuo*. The crude lipoid residue was first fractionated by silica gel chromatography. This separated the radioactive fractions from the bulk of non-labeled materials which were eluted first from the column by hexane and benzene. Most of the cholesterol was then eluted with a benzene–ethyl acetate mixture (9:1), and the remaining products of greater polarity stripped from the column with ethyl acetate. All of the radioactivity in the (9:1) benzene–ethyl acetate eluate from the silica gel column was found to be associated with the cholesterol zone using the heptane–phenyl cellosolve system. Upon subsequent purification through the digitonide and recrystallization, the specific activity of the cholesterol isolated from the homogenate at the end of the experiment was found to be 4.5×10^{3} ct./min./mg. using a flow gas counter, counting for 10-minute intervals.

The ethyl acetate eluate from the column was chromatographed on paper using the ligroin–propylene glycol system for 4 hours. Three radioactive zones were detected on the paper with the following properties: zone (A) had mobility similar to progesterone in that it traveled 13–20 cm. from the starting line, and exhibited a Δ^4-3 ketone grouping as revealed by ultraviolet scanning, and an orange dinitrophenylhydrazine reaction. Zone (B) was similar to pregnenolone in that it travelled 4–7 cm. down the paper and showed the antimony trichloride reaction characteristic of hydroxysteroids.[6] Zone (C) remained at the origin and gave color reactions characteristic for corticosteroids.

Zone (B) was studied further as follows: after elution from paper, the residue was rechromatographed using the ligroin–propylene glycol system for 16 hours, and the radioactive zone of polarity equivalent to pregnenolone containing a total of 14,000 ct. per min. was then eluted and chromatographed on a 1 g. (ht. 10 cm.) silica gel column. Following percolation of hexane and benzene through the column, about 1–2 mg. of a crystalline material containing 12,000 ct./min. was eluted with benzene–ethyl acetate (9:1). The infrared spectrum of this solid dissolved in carbon disulfide was identical to that of an authentic sample of pregnenolone. Upon acetylation of 50% of the total sample, the radioactive material now had mobility similar to that of pregnenolone acetate in the ligroin–propylene glycol system. The absorption spectrum over the range 230 to 600 millimicrons, of the sulfuric acid chromogen of the acetate eluted from the paper was identical to that of an authentic sample of pregnenolone acetate. The total radioactivity of the eluted acetate, determined in duplicate using 2% of the total acetate per determination, was found to be 5,600 ct. per min. using flow-gas counter. By comparison of the sulfuric acid chromogen peaks (325 and 412 millimicrons) of two concentrations of authentic pregnenolone acetate and of the isolated material, the total amount of pregnenolone acetate in the sample was estimated to be 0.5 mg. The calculated specific activity of the free pregnenolone is 12.7×10^{3} ct./min. mg. This value is based upon the assumption that the intensity of absorption at the peak studied is a direct function of the concentration. The estimated conversion of cholesterol to pregnenolone on the basis of counts added is about 1%. Consideration of the data indicates that: (1) the bulk of the cholesterol added as C^{14} labeled substrate remained unchanged but is considerably diluted by cholesterol already present in the gland; (2) the pregnenolone isolated as a transformation product appears to have a specific activity higher than cholesterol isolated at the end of the experiment. This result is consistent with the view that cholesterol in the adrenal gland cannot be considered as a homogeneous metabolic

(4) K. Savard, *J. Biol. Chem.*, **202**, 457 (1953).
(5) R. Neher and A. Wettstein, *Helv. Chim. Acta*, **35**, 276 (1952).
(6) H. Rosenkrantz, *Arch. Biochem. Biophys.*, **44**, 1 (1953).

pool from which transformation products are formed.

The metabolism of cholesterol and its conversion to pregnenolone by adrenal homogenates have been consistently demonstrated in three additional experiments in approximately the same yield. The full details of these studies will be published subsequently. We should like to thank Dr. Harris Rosenkranz and Mr. Paul Skogstrom for the analysis and interpretations of the infrared spectra.

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RECEIVED MAY 18, 1954

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MASS SPECTRUM OF OCTABORANE

Sir:

From recent mass spectrographic studies[1] of various boron hydrides we have observed the mass spectrum of an octaborane, the existence of which was first postulated by Burg and Schlesinger[2] from vapor tension measurements.

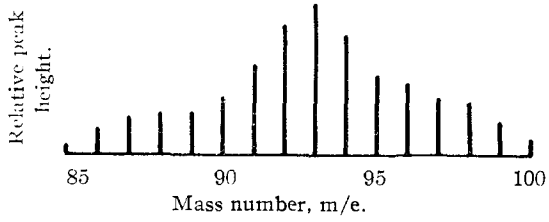

Fig. 1.—Partial mass spectrum of octaborane.

The mass spectrum of octaborane from mass numbers 85 to 100 is given in Fig. 1. The dominant peak occurs at mass number 93, and double ionization peaks are found in the region of mass numbers 44–48. The sharp cut-off in peak heights at mass number 100 suggests that the composition of the octaborane is B_8H_{12}, thus indicating that this compound belongs to the group of the (more) stable boron hydrides.[3]

In addition to the above findings we have been able to confirm Norton's finding of nonaborane.[4]

(1) A Consolidated Engineering Model 21-103 Mass Spectrometer operating at 70 volts was used in these studies.

(2) A. B. Burg and H. I. Schlesinger, THIS JOURNAL, **55**, 4009 (1933).

(3) E. Wiberg, *Ber.*, **69B**, 2816 (1936).

(4) F. J. Norton, THIS JOURNAL, **72**, 1849 (1950).

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RECEIVED JUNE 17, 1954

BIOSYNTHESIS OF OROTIC ACID FROM CITRULLINE

Sir:

Orotic acid, a pyrimidine precursor in bacteria[1] and in the rat,[2] is believed to arise from aspartate via ureidosuccinate and dihydroörotate. Ureidosuccinate has been pictured as arising from argininosuccinate, based on the finding that the ureide carbon of citrulline[3] as well as carbon dioxide[4] contribute to position-2 of tissue pyrimidines in the pigeon. In the rate and in rat liver homogenates, no such incorporation from citrulline could be demonstrated,[3,5] and this failure was attributed to active degradation of citrulline to urea in mammalian liver. In the present study, pigeon and rat liver slices have been compared in regard to incorporation into orotic acid of the ureide carbon of citrulline and the amidine carbon of arginine.

L(+)-Citrulline was prepared from urea-C^{14} by the method of Kurtz.[6] L(+)-Arginine·HCl was synthesized from cyanogen bromide-C^{14} via O-methylisouronium chloride.[6] Both materials had specific activities of 2.84×10^6 c.p.m. per mmole. Incubation was carried out essentially according to Reichard[7] using 15–18 g. of pigeon or rat liver slices, 50 ml. of Krebs–Henseleit bicarbonate medium supplemented with 90 mg. of glucose, 50 mg. of sodium ATP and 15 mg. of carrier orotic acid. Each bath also contained either 0.1 mmole of L-citrulline-C^{14} + 0.5 mmole of L-aspartate or 0.1 mmole of L-arginine·HCl-C^{14} + 0.5 mmole of fumarate. After 4 hours of incubation at 37°, orotic acid was recovered from each deproteinized medium as described by Reichard.[7] The product was characterized by ultraviolet absorption spectrum and m.p. (341–343°) after recrystallization from water. All samples were combusted, and counted as barium carbonate under a thin-window Geiger–Mueller counter.

TABLE I

RADIOACTIVITY OF ISOLATED OROTIC ACID FROM LIVER SLICE STUDIES

Substrate	Rat liver, c.p.m. per milliatom C	Pigeon liver, c.p.m. per milliatom C
L(+)-Citrulline-C^{14}	3,390	0
L(+)-Citrulline-C^{14}	3,040	0
L(+)-Arginine-C^{14}	0[a]	0
L(+)-Arginine-C^{14}		0

[a] Zero means <3 c.p.m. above background of *ca.* 25 c.p.m.

In contrast to expectations based upon the work of others cited above, significant incorporation of ureide carbon of citrulline into orotic acid was observed with rat but not with pigeon liver slices (Table I). An apparent paradox, as yet unresolved, arises from the demonstration that in the rat citrulline → orotic acid (present study), orotic acid → pyrimidines,[8] yet citrulline failed to contribute specifically to pyrimidines.[3,5] In the pigeon no contribution from citrulline to orotic acid was detected (present study) yet citrulline has been shown to contribute to pyrimidines.[3] The present findings complement the earlier ob-

(1) L. D. Wright, C. S. Miller, H. R. Skeggs, J. W. Huff, L. L. Weed and D. W. Wilson, THIS JOURNAL, **73**, 1898 (1951).

(2) H. Arvidson, N. A. Eliasson, E. Hammarsten, P. Reichard, H. von Ubich and S. Bergstrom, *J. Biol. Chem.*, **179**, 169 (1949).

(3) M. P. Shulman and S. J. Badger, *Federation Proc.*, **13**, 292 (1954).

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

(5) C. Cooper and D. W. Wilson, *Federation Proc.*, **13**, 194 (1954).

(6) A. C. Kurtz, *J. Biol. Chem.*, **122**, 477 (1937–38), and **180**, 1253 (1949).

(7) P. Reichard, *J. Biol. Chem.*, **197**, 391 (1952).

(8) L. L. Weed and D. W. Wilson, *J. Biol. Chem.*, **189**, 435 (1951).