support, crystals with m.p. 151–153° were isolated from the reaction mixture, which contained 69.4% of bromine and could have been the bromination product of the acrylic amide, CF_2Br–$CHBr$–$CONHBr$, 69.8% bromine. Perfluorobutyramide, $C_3F_7CONH_2$, gave a 77% yield of a crude compound boiling up to 25°, a 21% recovery of free acid and 19% of NH_4Cl; this crude compound was, at the time, assumed to be C_3F_7NCO, but according to reference 2 it is C_3F_7Br.

The Schmidt-Curtius Reaction.—The relation between the two reactions is known,[9] and we found it best to use a combination of both, *i.e.*, form the azide in the absence of an inorganic acid, separate it, then rearrange it in the presence of a mineral acid. This procedure avoids the formation of ammonium salts from an excess of NaN_3 and from decomposing HN_3; free HN_3 reacts very slowly with fluorinated acids and is preferentially decomposed in the presence of an inorganic acid.

The acyl halide (0.1 mole) was heated with NaN_3 (0.12 mole) in 100 ml. of dry benzene for 24 hours at 55–65°. After cooling, crystalline NaCl and NaN_3 excess was filtered off. The filtrate was treated with 10 ml. of concentrated sulfuric acid added dropwise at 55–65°, then refluxed about eight hours until no more gas was evolved. After pouring over ice and decanting the benzene, the aqueous layer was made alkaline and the amines so liberated were distilled into 6 *N* HCl, from which their hydrochloride was obtained by evaporation. The benzene layer was examined for recovery of any isocyanate, and the aqueous layer for recovery of any organic acid.

$CF_3(CH_2)_2COCl$ gave 81% $CF_3(CH_2)_2NH_2\cdot HCl$; CF_3CH_2COCl gave 25% $CF_3CH_2NH_2\cdot HCl$, which is ten times the yield of the Hofmann degradation; C_3F_7COCl gave, in addition to the expected 75% of isocyanate,[3] a 10% yield of $C_2F_5CONH_2$, m. p. 95°, no depression with an authentic sample, which is the amide of the next lower acid.

Dissociation.—The measurements were made by titration of 0.004 *N* solutions of amine with 0.05 *N* HCl, by means of a model H_2 glass electrode Beckman *p*H meter. The calculations were conventional.[10]

PHYSICAL CONSTANTS AND ANALYSES

	B.p. or m.p. °C.	Mm.	n^t_D	*t*, °C.	d^t_4	Analyses. % Found	Calcd.
CF_3CH_2COCl	70.3	745	1.3382	29.5	1.422	Cl, 24.1	24.2
$CF_3CH_2CH_2COCl$	103	745	1.3610	24	1.361	Cl, 22.1	22.1
$CF_3CH_2CONH_2$	M. 108.8					F, 43.8	44.8
$CF_3CH_2CH_2CONH_2$	M. 136.4					N, 9.9	9.9
$CF_3CH_2CH_2NH_2$	67.8	744	1.3332	30	1.162		
Hydrochloride	M. 222–225					Cl, 23.7	23.7
$CF_3CH_2NH_2$[11]	36	744	1.295	30	1.245		
Hydrochloride	Sublimes					Cl, 26.3	26.2

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The Synthesis of Purines and Thymine from Methionine in the Rat[1]

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Formic acid has been shown to serve as a biological precursor of carbons 2 and 8 of the purines[2] and of the methyl group of thymine.[3] The degree to which the labile methyl group of methionine might serve as a source of these carbon atoms has been studied only to a limited extent, in spite of the suggested metabolic relationship between formic acid and methionine.[4] Brown has indicated that methionine was rather ineffective as a thymine precursor in the rat,[5] but no experimental data were provided. Sime and Johnson have recently demonstrated[6] that in the bird the methionine methyl group was readily converted to uric acid carbons 2 and 8.

The experiments to be described were performed to obtain further information on the use of methionine for the synthesis of various nucleic acid components. It was also thought desirable to obtain data which would provide a comparison of the relative utilization of formic acid and the methyl group of methionine for these processes. To attain these ends a study was made of the incorporation under comparable conditions of sodium formate-C^{14} and methionine-methyl-C^{14} into the nitrogenous bases of the deoxyribonucleic acid (DNA) of the rat.

As is illustrated in Table I, appreciable amounts of isotopic carbon appeared in the adenine, guanine and thymine of the rat DNA after injection of either labeled formate or methionine. No localization of the radioactivity in the purines was attempted. In each case, however, the methyl group of the thymine was converted to iodoform[7] which appeared to contain most of the isotopic carbon of the original thymine. Accurate measurement of the activity of the iodoform was not possible in an internal flow counter because of a quenching action of this compound on the counting rate, nor was sufficient material available for measurements with an end-window counter.

Previous work with formic acid has demonstrated that almost all of the observed activity of the purines would be found in carbons 2 and 8,[2] and that the activity of the thymine molecule would be found in the methyl group.[3] It has been assumed that the distribution of activity resulting with methionine as the precursor is similar to that obtained with formate. The low level of radioactivity found in the cytosine in all cases lends considerable support to this assumption. Certainly no non-specific precursor derived from the methionine contributed appreciable amounts of isotopic carbon to the synthesis of the various compounds.

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TABLE I

INCORPORATION OF FORMIC ACID-C^{14} AND METHIONINE-METHYL-C^{14} INTO RAT DNA COMPONENTS

Precursor	Expt. no.	Compound isolated: Adenine, c.p.m./μM	Adenine, diln.[a]	Guanine, c.p.m./μM	Guanine, diln.[a]	Thymine, c.p.m./μM	Thymine, diln.[a]	Cytosine, c.p.m./μM
Formic acid-C^{14}	1	10700	106	9000	126	1300	870	<100
	2	13100	86	10300	110	3000	378	<100
	Av.	11900	96	9650	118	2150	624	...
Methionine-methyl-C^{14}	3	380	777	310	952	220	1340	<10
	4	290	1020	430	687	260	1140	<10
	Av.	335	898	370	819	240	1240	...

[a] Dilution = specific activity of precursor/specific activity of compound isolated.

If the dilution of the isotope observed in each case is taken as a measure of the utilization of the two precursors, it can be seen that in the present work formate was used for purine synthesis to about ten times the extent to which the methyl group of methionine was used. On the other hand, when these two compounds are compared with respect to incorporation into thymine, formate was used to only twice the extent of methionine.

The obvious conclusion must be that methionine was used for thymine synthesis by some route not involving free formic acid. This is in agreement with the work of Elwyn and Sprinson[7] who have demonstrated that the β-carbon of serine is converted readily to the methyl group of thymine by a mechanism allowing the retention of both hydrogen atoms originally bound to this carbon. It can be calculated from the data of these workers that the incorporation of isotopic carbon from the β-carbon of serine into adenine was about 1.5 times the incorporation into thymine. In the present work with methionine about 1.4 times as much activity appeared in adenine as compared to thymine. With formate, on the other hand, a ratio of about 5 to 1 was obtained, a result similar to that found by Totter, *et al.*[3]

From the similarity of the data for the methyl group of methionine and the β-carbon of serine it would seem likely that both compounds may first be converted to a common intermediate which then may act either as a source of a formyl compound for purine synthesis or as a source of the methyl group of thymine. This common intermediate may be a hydroxymethyl derivative of a sulfhydryl compound[4] or, perhaps more likely, of tetrahydrofolic acid.[8,9]

While it is possible to consider that methionine might donate an intact methyl group directly to a thymine precursor, such a transmethylation reaction, if it occurs at all, cannot be the major route of thymine synthesis. The greater utilization of formate as compared with the methyl group of methionine is not compatible with the possibility that formate incorporation takes place through methionine as an intermediate.

The present results indicate that the methyl group of methionine, in addition to formate and the β-carbon of serine, can serve as a precursor of the ureide carbons of the purines and of the methyl group of thymine, presumably after conversion to "active one-carbon units" at the oxidation levels of formate and of formaldehyde. It is difficult to determine the extent to which each of these and possibly other compounds actually contribute to the production of these active units under normal conditions. It must be remembered that the sizes of the metabolic pools of the various precursors are unknown and that quite possibly they are of different magnitudes. A comparison of the amounts of isotopic carbon found in a given product after use of different labelled precursors must therefore be a dubious measure of the importance of each compound in the normal synthetic processes leading to the compound studied.

Subject to these reservations, it would appear from the present results that the methyl group of methionine is a relatively minor source of the ureide carbons of the purines of rat DNA. The data for thymine, however, indicate that the role of methionine as a source of the methyl group of the pyrimidine may approach the importance previously assigned to formate for this process.

Experimental

Materials.—Male albino rats weighing about 160 g. were injected intraperitoneally with one ml. of a water solution containing 0.1 mc. of the particular radioactive compound. To achieve this amount of isotopic carbon, 20 mg. was required for the methionine-methyl-C^{14} and 2.61 mg. for the sodium formate-C^{14}. The methionine was prepared by the method of du Vigneaud, Dyer and Harmon.[10] The sodium formate was a commercial sample. One rat was used in each experiment. Two complete experiments were carried out separately with each compound as an aid in determining the reproducibility of the data.

DNA Isolation.—The rats were killed by decapitation 24 hours after injection. The viscera were removed at once, homogenized in a Waring Blendor and extracted with ethanol and an ether–ethanol (1:3) mixture. The polynucleotides were removed from the residual material by extraction with 10% NaCl and were precipitated as sodium salts by the addition of 2.5 volumes of ethanol. The DNA was isolated from the mixed sodium nucleates by the method of Hammarsten.[11]

Isolation of Purine and Pyrimidine Bases.—The DNA was heated with 7.5 N $HClO_4$ at 100° for one hour, liberating the nitrogenous bases. Most of the perchlorate ion was precipitated by the addition of KOH until the pH was about 10. The precipitate of $KClO_4$ was removed by centrifugation and the alkaline solution was chromatographed on Dowex-1 by the procedure of Cohn,[12] using ammonium formate buffers as eluting agents. The purines and cytosine fractions were rechromatographed on Dowex-50 using HCl as the eluting agent by a method similar to that of Cohn.[13]

Isotope Measurement.—Aliquots of each chromatographic fraction of each base were placed in platinum dishes

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and the solvent was removed by evaporation. The activity was measured in a Tracerlab internal flow Geiger counter. As the concentration of nitrogenous base in each aliquot was known from optical density measurements, specific activity could be calculated.

Purity Criteria.—The fractions of each base were found to be satisfactorily pure as judged from the ratio of the optical densities at two selected wave lengths and by the ratio of radioactivity to optical density.

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Chemical Studies with 11-Oxygenated Steroids. V. 3α,20β-Dihydroxypregnan-11-one 11-Ethylene Ketal[1]

BY BARNEY J. MAGERLEIN AND ROBERT H. LEVIN

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It is well established that the 11-keto function frequently found in steroids fails to react with the usual carbonyl reagents.[2] Catalytic or chemical reduction to the 11-hydroxyl function[2,3] and participation in the Wolff–Kishner reduction[4] are the only recorded reactions of the 11-keto moiety.

Various investigators have reported the selective preparation of cyclic ketals at other positions in the molecule leaving the 11-keto function intact.[5] It was found in these laboratories that prolonged boiling of 3α,20β-dihydroxypregnan-11-one with ethylene glycol in benzene solution in the presence of *p*-toluenesulfonic acid gave the 11-ketal in 50% yield. This compound shows strong hydroxyl absorption and complete lack of carbonyl absorption in its infrared absorption spectrum. Acidic hydrolysis gave 85% yield of the starting 3α,20β-dihydroxypregnan-11-one which was identified by mixed melting point and infrared data.

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Experimental[6]

3α,20β-Dihydroxypregnan-11-one 11-Ethylene Ketal.—A mixture of 4.0 g. of 3α,20β-dihydroxypregnan-11-one,[7] 12 ml. of ethylene glycol, 100 mg. of *p*-toluenesulfonic acid monohydrate and 150 ml. of benzene was stirred and heated under reflux for 72 hours. The water formed in the reaction was codistilled with the benzene and removed in a water trap. The cooled solution was filtered to give, after washing with methylene dichloride, 2.27 g. (50.2% yield) of crystals, m.p. 250–255°. The benzene–ethylene glycol solution, after washing with water and concentrating, gave an additional 1.51 g. of crystals, m.p. 208–216°. The 2.27 g. of ketal was recrystallized from methanol to give 1.58 g., m.p. 257–259°. The infrared absorption spectrum of this material showed no carbonyl absorption in the 6 μ region.

Anal. Calcd. for $C_{23}H_{38}O_4$: C, 72.97; H, 10.12. Found: C, 72.92, 73.00; H, 10.24, 10.22.

Hydrolysis of 3α,20β-Dihydroxypregnan-11-one 11-Ethylene Ketal.—A solution of 200 mg. of the 11-ketal in 20 ml. of acetone and 2 ml. of water containing 2 drops of concentrated sulfuric acid was heated under reflux for 2 hours. The acetone was distilled under vacuum, water added and the product filtered. It weighed 150 mg. (84.6% yield), m.p. 231–235°. After recrystallization from benzene–methanol there was obtained 130 mg., m.p. 235–237°. This material gave no melting point depression with 3α,20β-dihydroxypregnan-11-one. Its infrared absorption spectrum was identical with the starting ketone.

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Deuterium-Hydrogen Exchange in the *para* Position of Phenyl Alkyl Ethers[1]

BY W. M. LAUER AND J. T. DAY[2]

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The influence of groups in the aromatic nucleus on chemical reactivity at a particular position has been the subject of many experimental and theoretical studies. The present study constitutes an investigation of the rates of deuterium–hydrogen exchange occurring in the *ortho* and the *para* positions of anisole and in the *para* positions of phenetole, phenyl *n*-propyl ether and phenyl isopropyl ether. These exchange reactions were conducted in acid solution at a temperature of 100°.

The deuterium labeled aromatic ethers were prepared from the corresponding bromo ethers by first converting these compounds to their lithium derivatives and then replacing the metal by deuterium with heavy water. These labeled aromatic ethers after dilution with unlabeled material were dissolved in glacial acetic acid containing sulfuric acid and the time required for one-half of the deuterium to be replaced by hydrogen was determined. The labeled aromatic ethers, held at constant temperature for definite time intervals, were isolated from solution by treatment with aqueous sodium hydroxide. These samples were then burned in a stream of oxygen and the water of combustion, after purification, was analyzed for deuterium. This analysis was accomplished by the falling drop procedure.[3]

The half-life of deuterium in the case of *para*-labeled anisole at 100° in a solution containing the aromatic ether (0.01 mole), glacial acetic acid (0.05 mole) and sulfuric acid (0.001 mole) was found to be 39 minutes ($k = 3.0 \times 10^{-4}$ sec.$^{-1}$), and the half-life of deuterium in the case of *ortho*-labeled anisole under similar conditions was found to be 75 minutes ($k = 1.5 \times 10^{-4}$ sec.$^{-1}$). A comparison of the exchange rates of *o*-deuteroanisole with the

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