pathway of the biochemical conversion of cholesterol to hydrocortisone, cholesterol-21-^{14}C was prepared[4] and gave, on incubation with bovine adrenal gland tissue, hydrocortisone-^{14}C.[5] A part of the isolated hydrocortisone-^{14}C (5.15 mg.; specific activity, 159 d.p.m./mg.) was diluted with an equal amount of non-labeled hydrocortisone, and then degraded by periodate oxidation according to a procedure previously employed for similar reactions.[6] From the oxidation mixture, 7.5 mg. of neutral material was recovered and was found to contain no hydrocortisone after paper chromatographic analysis. From the acidic fraction (9.7 mg.), 11β,17α-dihydroxy-3-oxo-4-etiocholenic acid[7] was isolated and identified by comparison with an authentic sample, by mixed paper chromatography,[8] and by its ultraviolet absorption spectrum in concentrated sulfuric acid.[9] This etio acid was found to contain no radioactivity.[10] Carbon atom 21 of the hydocortisone-^{14}C was obtained as formaldehyde which was isolated as its dimedone derivative, 4.6 mg., m.p. 191.5–192°. A mixture of this derivative and an authentic reference sample (m.p. 192-193°) melted at 191.5-192° (melting points not corrected). The dimedone derivative had a radioactivity[10] of 26 d.p.m./mg.[11]

This evidence demonstrates that carbon atom 21 of cholesterol is maintained during the biochemical conversion of cholesterol to hydrocortisone and that the biosynthesis of hydrocortisone from cholesterol in the adrenal cortex involves the elimination of carbon atoms 22–27 of the original cholesterol side chain.

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(5) F. M. Ganis, P. Kurath and M. Radakovich, *Federation Proc.*, **16**, 357 (1957).

(6) S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **37**, 1200 (1954).

(7) J. v. Euw and T. Reichstein, *ibid.*, **25**, 988 (1942); H. L. Mason, W. M. Hoehn and E. C. Kendall, *J. Biol. Chem.*, **124**, 459 (1938).

(8) A. Zaffaroni and R. B. Burton, *ibid.*, **193**, 749 (1951).

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

(10) Radioactivity determinations by New England Nuclear Corporation, Boston, Massachusetts.

(11) The amount of the dimedone derivative obtained was in excess of the expected amount and the radioactivity of the sample was lower than the calculated value of 98 d.p.m./mg. However, it was found that the elution of two filter paper blanks yielded 23.2 and 34.3 mg. of residue which gave upon analogous periodate oxidation[6] and reaction with dimedone, 2.4 and 3.5 mg. respectively of the dimedone derivative of formaldehyde. This would account for the lower radioactivity obtained in the experimental sample.

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A DIFFERENTIAL ULTRACENTRIFUGE TECHNIQUE FOR MEASURING SMALL CHANGES IN SEDIMENTATION COEFFICIENTS[1]

Sir:

With the recent adaptation of the Rayleigh interferometer to the ultracentrifuge,[2] it has become possible to perform differential experiments whereby very small changes in sedimentation coefficients can be measured accurately. This communication deals with such experiments and some potential applications.

The technique involves a comparison, at conjugate levels, of the refractive indices of two solutions contained in separate compartments of a double-sector ultracentrifuge cell. If both solutions are identical the Rayleigh pattern consists of a series of parallel, straight interference fringes. For the fringes to be straight even in the presence of concentration gradients, the two boundaries must migrate and spread at the same rate so that the refractive index difference at conjugate levels throughout the cell is constant. When one boundary moves faster than the other, the fringe pattern at the boundaries is warped to produce curved fringes whose shape resembles the tracings produced by schlieren optical systems.[3] Depending upon which compartment contains the faster moving species, the pattern will show a maximum or a minimum. With transport equations the difference in sedimentation coefficients can be expressed directly in terms of the change with time of the first moment of the area defined by the curved fringes.

To test the method small amounts of D_2O were added to different solutions of bushy stunt virus, and the reduction in sedimentation coefficient of the virus was measured by the differential technique. For decreases of 0.82% and 1.65% (determined by interpolation of data from conventional ultracentrifuge gives 0.78% and 1.61%, respectively. Comparable precision is realized in experiments with proteins.

The sensitivity and accuracy of the method even at this early stage of development are sufficient to commend it for many experiments involving small differences in sedimentation coefficients. These changes may result from reduction in the buoyancy term as with D_2O or from a change in the molecular weight or frictional coefficient of the macromolecule. The latter is illustrated by preliminary results from a study of the binding of small ions to serum albumin, in which the sedimentation coefficient decreased by about 0.5% despite an increase of 4% in molecular weight. The technique also provides valuable data for the analysis of equilibrium systems involving rapid reactions between a small ion, A, and a protein, P, to form complexes, PA_i, with i varying from 0 to n. For such systems equation (1) gives the equilibrium concentration of free A component

$$[A] = \frac{[\bar{A}]\{\bar{s}_P - \bar{s}_A - (1/n)(\bar{s}_P - s_P)\} + [\bar{P}](\bar{s}_P - s_P)}{\bar{s}_P - s_A - (1/n)(\bar{s}_P - s_P)} \qquad (1)^{5}$$

$[\bar{A}]$ and $[\bar{P}]$ are the total concentrations and s_A and s_P are the sedimentation coefficients of the pure components, and $\bar{s}_A$ and $\bar{s}_P$ are the constituent

(1) This work has been supported in part by a grant from the National Science Foundation.

(2) This optical system is available commercially from the Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

(3) Svensson[4] obtained similar fringe patterns with the Rayleigh interferometer during examination of two identical diffusing boundaries which were slightly displaced from one another.

(4) H. Svensson, *Acta Chem. Scand.*, **3**, 1170 (1949).

(5) H. K. Schachman, in preparation.

sedimentation coefficients $\left(\bar{s} = \sum_{i=0}^{n}\alpha_i s_i \text{ where } \alpha_i\right.$ is the weight fraction appearing as the ith species$\left.\right)$. Different methods can be used to evaluate $\bar{s}_A$.[5] The term $(\bar{s}_P - s_P)$ which may be very small, about 0.05 svedbergs, can be measured accurately by the differential technique. The same optical principles may be useful in the direct measurement of small changes in molecular weights and diffusion coefficients.

(6) du Pont Fellow in Biochemistry. This work is submitted in partial fulfillment of the requirements for the Ph.D. degree in Biochemistry at the University of California.

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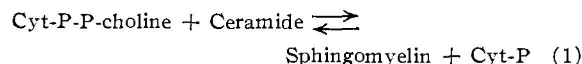
THE ENZYMATIC SYNTHESIS OF SPHINGOMYELIN[1]

Sir:

It has been suggested[2] that the enzymatic synthesis of sphingomyelin may take place by the transfer of the phosphorylcholine moiety of cytidine diphosphate choline (CDP-choline) to the primary hydroxyl group of an N-acylsphingosine (ceramide) in a reaction fundamentally similar to the enzymatic synthesis of lecithin[3]



$$\text{Cyt-P-P-choline} + \text{Ceramide} \rightleftharpoons \text{Sphingomyelin} + \text{Cyt-P} \quad (1)$$

An enzyme (phosphorylcholine-ceramide transferase) has now been found in chicken liver which catalyzes an extensive net synthesis of sphingomyelin from C^{14}-labeled CDP-choline and an "active ceramide." When the enzyme incubation was run on a large scale (100 ml.) under the conditions described in Table I, 28.5 mg. of enzymatically synthesized phospholipide was isolated by chromatography on silicic acid and identified as sphingomyelin by the following properties: stability toward mild alkaline hydrolysis; insolubility in ether and acetone; N/P ratio of 2.08; and an infrared spectrum closely resembling that of purified sphingomyelin. The purity of the isolated product, based on comparison of its specific radioactivity with that of the CDP-choline used, was at least 90%.

The enzyme is highly specific, both for CDP-choline and "active ceramide." Ceramides with short-chain fatty acids in amide linkage are much more active than long-chain fatty acid amides, presumably because the short chain compounds are more soluble and penetrate more readily to the enzyme surface. N-Acetyldihydrosphingosine and N-acetylphytosphingosine are inactive (Table I). Acetylation of *crude* sphingosine (obtained by acid hydrolysis of cerebrosides) with acetic anhydride in the presence of alkali[4] leads to the formation of "active ceramide." In contrast, N-acetylsphingosine prepared by treatment of recrystallized triacetylsphingosine with alkali shows little activity, but it is converted to "active ceramide" by heating with acetic–sulfuric acid. It is concluded that the sphingosine portion of "active ceramide" does not possess the D-erythro-*trans*-sphingosine structure which has been definitely established for the triacetylsphingosine described by Carter[4,5] and the N-acetylsphingosine derived from it. This result is unexpected in view of the evidence[5] that naturally occurring sphingolipides are also of the D-erythro-*trans* structure.

Two possibilities appeared most likely. The "active ceramide" might either have the *cis* rather than the *trans* configuration at the double bond, or the *threo* rather than the *erythro* relationship of the amino group to the hydroxyl on C-3. Generous

TABLE I
REQUIREMENT FOR "ACTIVE CERAMIDE" IN SPHINGOMYELIN SYNTHESIS

	Additions	Sphingomyelin synthesized, millimicromoles
1	Sphingosine	2
2	N-Acetyldihydrosphingosine	1
3	N-Acetylphytosphingosine[6]	1
4	"Active ceramide" (mixture of N-acetylsphingosine isomers)	85
5	N-Acetylsphingosine derived from recrystallized triacetylsphingosine[4]	17
6	N-Acetylsphingosine after treatment with acetic–sulfuric acid	155

Each tube contained 50 μmoles of Tris buffer, *p*H 7.4, 20 μmoles of cysteine, 4 μmoles of $MnCl_2$, 0.8 μmole of CDP-choline labeled with choline-1,2-C^{14}, 5 mg. of Tween-20 (polyoxyethylene sorbitan monolaurate), and 0.25 ml. of a suspension of particles obtained from 0.25 *M* sucrose homogenates of chicken liver by a method already described.[3] Four μmoles of substances to be tested were added as indicated. The final volume of the system was 1.0 ml., and the incubation was for 2 hours at 37°, after which the lipides were extracted repeatedly with hot methanol. The lipide extract was hydrolyzed in 0.4 *N* methanolic potassium hydroxide at 37° for two hours, transferred to chloroform and thoroughly washed.[3] Aliquots of the chloroform solution were then plated and counted.

TABLE II
ACTIVITY OF ISOMERS OF N-ACETYLSPHINGOSINE AS ENZYMATIC PRECURSORS OF SPHINGOMYELIN

Additions	Sphingomyelin synthesized millimicromoles
Experiment 1	
N-Acetyl-DL-erythro-*trans*-sphingosine[6]	4
N-Acetyl-DL-threo-*trans*-sphingosine[6]	105
Experiment 2	
N-Acetyl-DL-erythro-*trans*-sphingosine[7]	5
N-Acetyl-DL-threo-*trans*-sphingosine[7]	83
N-Acetyl-DL-erythro-*cis*-sphingosine[7]	1
N-Acetyl-DL-threo-*cis*-sphingosine[7]	1

The conditions of the experiment were the same as shown in Table I. Four μmoles of the N-acetyl derivatives of the sphingosine isomers was added as shown.

(1) Supported by grants from the Nutrition Foundation, the Life Insurance Medical Research Fund and the United States Public Health Service (B-1199). The authors are indebted to Dr. H. E. Carter for the infrared spectral analyses and for helpful discussions.

(2) M. Sribney and E. P. Kennedy, *Federation Proc.*, **16**, 253 (1957).

(3) E. P. Kennedy and S. B. Weiss, *J. Biol. Chem.*, **222**, 193 (1956).

(4) H. E. Carter, W. P. Norris, F. J. Glick, G. E. Phillips and R. Harris, *ibid.*, **170**, 269 (1947).

(5) H. E. Carter, D. S. Galanos and Y. Fujino, *Can. J. Biochem. Physiol.*, **34**, 320 (1956).