particularly Zamecnik and his collaborators.[1] That additional reactions may be involved in amino acid incorporation has been suggested by Hendler[2] upon noting a substantial incorporation of labeled amino acid by minced hen oviduct into lipoidal material similar to "proteolipids."[3]

We have examined the incorporation of labeled amino acids into lipid soluble material by cell-free preparations of rat liver. Phenylalanine was found to be incorporated readily into such material in both microsomes and microsome-free soluble cytoplasmic supernate alone or together (Table I).

TABLE I

Rat liver homogenates prepared according to Zamecnik and Keller[4] with medium A[5] were centrifuged at 10,000 × *g* for 10 min. to yield the whole supernate, 3.5 ml. of which was incubated at 37° for 2 hr. in N_2:CO_2 (95:5) with 5 μmoles of ATP, 50 μmoles of 3-phosphoglycerate and 1.15 μmoles (5 μc.) of DL-phenylalanine-3-C^{14}; total volume, 5 ml. Microsomes and soluble supernate were prepared before or after incubation by centrifuging the whole supernate at 105,000 × *g*. Both the microsomes obtained after 30 min. centrifugation, suspended in cold medium A, and the first supernate were centrifuged an additional 60 min. Protein and lipid were prepared from the trichloroacetate-precipitated material essentially as described by Siekevitz.[6]

	C.p.m./mg.[a]			
	Microsomal		Soluble supernate	
Fraction incubated	Protein	Lipid	Protein	Lipid
Whole supernate	390	218	36	334
	(14,200)[b]	(3860)	(5130)	(1002)
Microsomes	136	714	..	..
Soluble supernate	..	..	39	567

[a] Net incorporation. Blanks containing trichloroacetic acid initially and incubated for zero and 2 hours were less than 10% as radioactive. [b] Figures in parentheses indicate total c.p.m. in each fraction.

A major portion of the radioactive lipid-soluble material was separated from other lipids by silicic acid chromatography.[7] This fraction, which was free of phosphorus, yielded on hydrolysis (6 *N* HCl, 110°, 18 hr.) a number of amino acids including radioactive phenylalanine, which was identified by co-chromatography on paper (butanol–acetic acid–water[8]) and radioautography with authentic phenylalanine. Milder conditions (2 *N* HCl, 100°, 2 hr.) released negligible amounts of phenylalanine. A mixture of the labeled lipoidal fraction with C^{14}-phenylalanine was resolved by paper chromatography. The radioactivity of the lipoidal substance was not diminished by extensive washing with a solution of non-radioactive phenylalanine.

That the incorporation of the amino acid into the lipid did not occur via the "conventional" pathway of incorporation into protein was proven by the following: Microsomes were not required, as noted above. Conditions which interfered with the "conventional" pathway did not curtail incorporation into the lipid-soluble material (Table II).

(1) P. C. Zamecnik, M. L. Stephenson and L. I. Hecht, *Proc. Natl. Acad. Sci., U. S.*, **44**, 73 (1958).
(2) R. W. Hendler, *Science*, **128**, 143 (1958).
(3) J. Folch and M. Lees, *J. Biol. Chem.*, **191**, 807 (1951).
(4) P. C. Zamecnik and E. B. Keller, *ibid.*, **209**, 337 (1954).
(5) E. B. Keller and P. C. Zamecnik, *ibid.*, **221**, 45 (1956).
(6) P. Siekevitz, *ibid.*, **195**, 549 (1952).
(7) J. Hirsch and E. H. Ahrens, Jr., *ibid.*, **233**, 311 (1958).
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TABLE II

Treatment	Relative specific activity in microsomal Protein	Lipid
None (control)	100[b]	100[b]
Ribonuclease (0.1 mg./ml.)	42	107
ATP and 3-phosphoglycerate omitted	21	115
p-Chloromercuribenzoate (10^{-3} *M*)	15	86
Crotoxin[c] (20 μg./ml.)	13	393[d]

[a] Conditions as in Table I. Microsomes were analyzed after incubation of whole supernate. [b] Measured specific activity of the two fractions separately set equal to 100. [c] Kindly furnished by Dr. H. Fraenkel-Conrat. [d] This stimulation has been verified repeatedly.

Tryptophan and leucine were also incorporated into lipid while glycine, lysine and valine were incorporated to a much smaller degree.

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ISOLATION OF CORTISONE AND CORTISOL FROM THE PLASMA OF PACIFIC SALMON (*ONCORHYNCHUS NERKA*)

Sir:

Cortisol (I) has been identified as the principal adrenal-cortical hormone in the peripheral plasma of the normal human[1] where it occurs at a concentration of approximately 10 μg. per 100 ml.[2] It is also the principal steroid in the blood of several animals[3] and has been tentatively identified in carp plasma.[4] Attempts to find cortisone (II) in adrenal vein[5] and peripheral human blood[6] have been unsuccessful. Occasional blood samples have been reported to contain small amounts of a "cortisone-like" substance,[1,7] but the evidence is inadequate to establish identity.

We wish to report the isolation of I and II from Fraser River sockeye salmon (*O. nerka*) just prior to their arrival on the Adams River spawning grounds. Blood was obtained from (*ca*). 100 fish by severing the caudal artery. Ethyl acetate extractable steroids were obtained from 1760 ml. of plasma by the usual procedures. Chromatography on purified Whatman no. 1 paper was performed in three solvent systems known to resolve I and II from closelyre lated steroids; ($CHCl_3$:$C_6H_6$1:1–formamide (III), toluene–propylene glycol (IV) and butyl acetate:ethylene glycol:H_2O 20:1:1. The corresponding U.S.P. reference standards and I and II were located on chromatograms both by

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(2) P. K. Bondy, D. Abelson, J. Scheuer, T. K. L. Tseu and V. Upton, *ibid.*, **224**, 47 (1957).
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(6) J. Tamm, I. Beckmann and K. D. Voigt, *Acta Endocrinol.*, **27**, 403 (1958).
(7) C. J. O. R. Morris and D. C. Williams, "Ciba Colloquia on Endocrinol.," **8**, 157 (1955).

alkali fluorescence and the reduction of blue tetrazoleum. When substance I was treated with sulfuric acid at 22° for 2 hr. it exhibited the same spectra as U.S.P. I with $\lambda_{max.}$ at 237, 282, 395 and 478 mμ and O.D. ratios of 0.98:1:0.47:0.55 and II gave the same spectra as cortisone with $\lambda_{max.}$ at 284, 343 and 420 and O.D. ratios of 1:0.38:0.31. The concentration of I and II was determined by the quantitative Porter–Silber[8] test for the dihydroxyacetone side-chain and each gave typical spectra with $\lambda_{max.}$ at 410 mμ. Substance I gave ϵ242 mμ 15.8 $\times$ 10^3 (MeOH) and II gave ϵ237 mμ 15.8 $\times$ 10^3.

The dihydroxyacetone side chain of I and II was oxidatively removed with $NaBiO_3$.[1] The ring oxidation products of I and II were inseparable from authentic 11β-hydroxy-Δ^4-androstene-3,17-dione and Δ^4-androstene-3,11,17-trione respectively in both benzene–hexane:methanol:water[1] and heptane:benzene 1:1–formamide, each of which gives excellent resolution from closely related 17-keto-steroids. The yield of both oxidation products was comparable to that obtained from U.S.P. steroids and both gave positive tests for the Δ^4-3 ketone and 17-keto groups.

Homogeneous II was obtained after a single chromatogram in solvent III, whereas I was resolved further in solvent IV. The recovery of cortisol (I) was 17 μg. and cortisone (II) 37 μg./100 ml. of plasma, with an over-all recovery of *ca.* 60–70%.

At full sexual maturity Pacific salmon show extensive degeneration of the pituitary and a marked hyperphasia of the adrenal gland, and this has been postulated to play a dominant role in the degenerative changes and death of the fish after spawning.[9] The high levels of I and II lend support to the suggested high secretory activity of the gland. The plasma levels of I and II at various stages of sexual maturity will be reported elsewhere.

Compounds I and II exhibited infrared spectra identical with cortisol and cortisone, respectively.

Acknowledgment.—We are grateful to Beckman Instruments, Inc., for recording the infrared spectra of the micro size samples.

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ELECTROPHILIC ALIPHATIC SUBSTITUTION. I. ELECTROPHILIC SUBSTITUTION STUDIES WITH *cis*- AND *trans*-4-METHYLCYCLOHEXYLMERCURIC BROMIDES[1]

Sir:

We wish to report the synthesis of a pair of simple isomeric organo-mercury compounds, *cis*- and *trans*-4-methylcyclohexylmercuric bromides, which are suitable for studying the stereochemistry of electrophilic substitution at a saturated carbon atom. Furthermore the bromine cleavage of the *cis*- and *trans*-4-methylcyclohexylmercuric bromides has been investigated and the results clearly indicate that this electrophilic reaction proceeds stereospecifically with retention of configuration. This example indicates that the steric course of electrophilic cleavage of organometallic compounds is retention even in the absence of structural features which might alter the course of, or participate in, the reactions.[2]

A mixture of the isomeric 4-methylcyclohexylmercuric bromides was prepared by treating 4-methylcyclohexylmagnesium bromide with mercuric bromide. The less soluble *trans*-isomer was purified by recrystallization, m.p. 159.1–159.6°. *Anal.* Calcd. for $C_7H_{13}HgBr$: C, 22.26; H, 3.47; Br, 21.16. Found: C, 22.10; H, 3.65; Br, 20.99. The *cis*-isomer was purified by chromatography, m.p. 130.8–131.2°. *Anal.* Found: C, 22.14; H, 3.34.

The tentative assignment of the configurations of the *cis*- and *trans*-4-methylcyclohexylmercuric bromides based on melting points, relative stability, and product distribution from the Grignard reaction, was confirmed by preparing the corresponding *cis*- and *trans*-1-deutero-compounds. The C–D stretching frequencies for equatorial and axial deuterium atoms in cyclohexane systems have been determined previously.[3] *trans*-1-Deutero-4-methylcyclohexylmercuric benzoate was prepared from the corresponding 1-deutero-alkylmercuric bromide, m.p. 99.4–99.8°; *Anal.* Calcd. for $C_{14}H_{17}DO_2Hg$: C, 40.04; H, 4.56; Hg, 47.78. Found: C, 40.12; H, 4.38; Hg, 47.65. The infrared spectrum showed characteristic C–D stretching absorptions at 2127 cm.$^{-1}$ (weak), 2148 cm.$^{-1}$ (strong) and 2168 cm.$^{-1}$ (medium); therefore the deuterium is predominantly axial. *cis*-1-Deutero-4-methylcyclohexylmercuric benzoate was prepared similarly, m.p. 44.2–44.9°; *Anal.* Found: C, 40.48; H, 4.38; Hg, 47.60. The infrared spectrum showed characteristic C–D stretching absorptions at 2141 cm.$^{-1}$ (medium), 2166 cm.$^{-1}$ (strong), and 2191 cm.$^{-1}$ (shoulder); therefore the deuterium is predominantly equatorial. Our results indicate that the benzoxymercuri group, as compared to the methyl group, has a relatively small preference for an equatorial over an axial conformation.

The isomeric 4-methylcyclohexylmercuric bromides were cleaved with a variety of brominating agents in several solvents. The competing free radical and polar processes, which were earlier noted in the iodine cleavage of 4-camphylmercuric iodide,[2c] were encountered here. By varying the reaction conditions, either process could be made to predominate. When either isomeric organo-mercury compound was cleaved by bromine in carbon tetrachloride solution under nitrogen, the free radical cleavage predominated and the product distribution was 47.5% *cis*- and 52.5% *trans*-4-methylcyclohexyl bromides. When more polar solvents, or hypobromous acid and other sources of

(1) This work was supported in part by a grant from the Research Corporation.

(2) For examples using stereochemically complex compounds see (a) A. N. Nesmeyanov, O. A. Rustov, and S. S. Poddubnaya, *Izvest. Akad. Nauk, S.S.S.R. Otel, Khim. Nauk*, 649 (1953); and (b) S. Winstein, T. G. Traylor and C. S. Garner, THIS JOURNAL, **77**, 3741 (1955); (c) S. Winstein and T. G. Traylor, *ibid.*, **78**, 2597 (1956).

(3) E. J. Corey, M. G. Howell and A. Boston, *ibid.*, **78**, 5036 (1956).