dose of 60γ. (*Anal.* Calcd. for $C_{43}H_{66}N_{12}O_{11}S_2$: C, 52.1; H, 6.71; N, 17.0; mol.wt., 991. Found: C, 52.1; H, 6.83; N, 16.9; mol. wt.[14] 940).

From these data we can conclude that the phenolic hydroxyl group of oxytocin contributes strongly to the activity of the hormone but is not essential for biological activity.[15,16]

Acknowledgements.—The authors wish to thank Mr. Joseph Albert for the microanalyses, Mr. David De Peter for the determination of the molecular weight, Miss Dade Tull for the biological assays, and Mr. David N. Reifsnyder for technical assistance.

(14) E. V. Baldes, *Biodynamica* **No. 46**, 1 (1939).

(15) As the experiments reported here were completed, we learned at a lecture delivered by Dr. R. A. Boissonnas that the same analog of oxytocin was prepared and studied in his laboratory simultaneously but independently from our work.

(16) After this communication was submitted for publication, Professor H. B. van Dyke found that 2-phenylalanine oxytocin shows milk ejecting activity of about 60 units per mg.

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RECEIVED JANUARY 12, 1959

EVIDENCE OF A 1,4-METHYL MIGRATION DURING A FISCHER REACTION

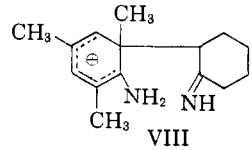
Sir:

An investigation of the structure of a compound isolated some years ago[1] as its picrate from the product of the action of boiling acetic acid on cyclohexanone mesitylhydrazone (I) has now disclosed that the compound is not "1,2,3,4-tetrahydro-6,8,12-trimethylisocarbazole" (III),[1] but is instead 6,7,8-trimethyl-1,2,3,4-tetrahydrocarbazole (II).

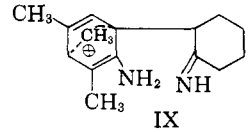
Mesitylhydrazine, m.p. 60–61° (N_2), was prepared by a new synthetic method based on the addition of mesitylene to ethyl azodicarboxylate.[2] The adduct, m.p. 159–160° (Found: C, 61.67; H, 7.61; N, 9.57[3]) was converted to mesitylhydrazine by boiling ethanolic potassium hydroxide. I, m.p. 45–47° (N_2), from mesitylhydrazine and cyclohexanone in the absence of solvent, was too unstable to analyze. Under nitrogen, boiling acetic acid converted I to II, isolated as its picrate, m.p. 171–172° (d.), as reported.[1] (Found: C, 57.61; H, 5.00; N, 12.87.) The tetrahydrocarbazole, from the picrate and sodium hydroxide, was extremely air-sensitive, m.p. 92–98° (N_2), insufficiently stable to analyze. Chloranil in xylene under nitrogen converted II to 1,2,3-trimethylcarbazole, m.p. 127.5–128.5°. (Found: C, 85.32; H, 7.37; N, 6.85); infrared and ultraviolet curves very similar to those of carbazole. Exposure in ether solution of II to air produced 11-hydroperoxy-6,7,8-trimethyl-1,2,3,4-tetrahydrocarbazolenine IV, m.p. 134° (d.). (Found: C, 72.46; H, 7.81; N, 5.77), which isomerized in ethanol solution to 9,10,11-trimethyl-1-benzazonidine-2,7-dione V, m.p. 171–172°. (Found: C, 73.08; H, 7.90; N, 5.55). The latter was converted by acid hydrolysis to δ-(2-amino-3,4,5-trimethylbenzoyl)-valeric acid VI, m.p. 146-149°. (Found: C, 68.56; H, 7.84; N, 5.50), and by alkali to 2,3-trimethylene-6,7,8-trimethyl-4-quinolone VII, darkens above 300°. (Found: C, 80.90; H, 7.64; N, 5.86). These transformations of II through IV and V to VI or VII parallel a precisely analogous series of reactions starting with tetrahydrocarbazole.[4] The ultraviolet and infrared spectra of V–VII are notably similar to those of the lower homologs derived from tetrahydrocarbazole.

The location of the methyl groups in the structure II and those of its derivatives was proven by synthesis of II from hemimellitene. The latter afforded an adduct with ethyl azodicarboxylate, m.p. 151–152° (Found: C, 60.10; H, 7.62; N, 9.60), which was converted to 2,3,4-trimethylphenylhydrazone, m.p. 105–106° (N_2), too unstable to analyze. The structure of this compound was demonstrated by its hydrogenation over Raney nickel to 2,3,4-trimethylaniline, characterized as its N-acetyl derivative. Cyclohexanone 2,3,4-trimethylphenylhydrazone, oily solid, too unstable to analyze, when boiled in acetic acid under nitrogen, afforded II, isolated as its picrate, m.p. and mixed m.p. 171–172°(d.). From this picrate II itself and from the latter 1,2,3-trimethylcarbazole and IV, V, VI and VII were prepared. Their m.pts. mixed m.pts. and spectroscopic properties identified them with samples obtained from I as starting material.

Although it is possible to rationalize the formation of II from I by means of a series of three consecutive 1,2 methyl shifts, along with the required accompanying reactions, a single 1,4 shift of methyl, although to our knowledge unprecedented, seems to us to offer a superior explanation. In either instance the shift would start with an intermediate VIII, a homolog of one for which evidence has been offered previously.[5] A single 1,4 shift of methyl, through transition state IX, would yield a second intermediate from which II could be formed by previously suggested routes.[6]



VIII



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(4) B. Witkop and J. B. Patrick, THIS JOURNAL, **73**, 2188, 2198 (1951).

(5) R. B. Carlin and D. P. Carlson, *ibid.*, **79**, 3605 (1957).

(6) See R. B. Carlin, W. O. Henley, Jr. and D. P. Carlson, *ibid.*, **79**, 5712 (1957).

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RECEIVED DECEMBER 24, 1958

AMINO ACID INCORPORATION INTO LIPOIDAL MATERIAL BY CELL-FREE LIVER PREPARATIONS

Sir:

The incorporation of amino acids into cellular constituents via adenosine triphosphate–amino acid activation has been studied by many workers,

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).
(8) W. Stepka, in Corcoran, "Methods in Medical Research," The Year Book Publishers, Chicago, 1952, Vol. 5, p. 25.

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.

Support of this work by a grant from the National Science Foundation (G-5685) is gratefully acknowledged.

JOURNAL PAPER NO. 1371 FROM THE AGRICULTURAL EXPERIMENT STATION DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY WEST LAFAYETTE, INDIANA

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RECEIVED DECEMBER 15, 1958

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

(1) I. E. Bush and A. A. Sandberg, *J. Biol. Chem.*, **205**, 783 (1953).
(2) P. K. Bondy, D. Abelson, J. Scheuer, T. K. L. Tseu and V. Upton, *ibid.*, **224**, 47 (1957).
(3) I. E. Bush, *J. Endocrinol.*, **9**, 95 (1953).
(4) P. K. Bondy, G. V. Upton and G. E. Pickford, *Nature*, **179**, 1354 (1957).
(5) G. Pincus and E. B. Romanoff, "Ciba Colloquia on Endocrinol.," J. & A. Churchill, Ltd., London, **8**, 97 (1955).
(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).