the experiments lasted about fourteen days. The average number of animals was fifteen to twenty in each group. It was found that the *p*-nitrobenzalmalononitrile produced a retardation of growth of about 50% of the tumors C3H-S, and Eo771 and about 40% of S-37, while the 5-nitrofuranalmalononitrile showed 70% retardation of growth of C3H-S.

This work is being continued and the compounds that are found active are being followed up as to their fate and mechanism of action both *in vivo* and *in vitro*. Detailed results of this work will be reported elsewhere.

DIVISION OF BIOCHEMISTRY EMERY M. GAL
SCHOOL OF MEDICINE DAVID M. GREENBERG
UNIVERSITY OF CALIFORNIA, BERKELEY

RECEIVED NOVEMBER 20, 1950

STIMULATION BY THE ADENYLIC ACID SYSTEM OF AMINO ACID INCORPORATION INTO PROTEIN OF LIVER GRANULES

Sirs:

In view of the endergonic nature of peptide bond synthesis, and the key role of the adenosine phosphates in the energy transfer in biological oxidations, the adenylic acid system would be expected to participate at some stage in the over-all process of amino acid incorporation into protein. Up to the present the only evidence to support this expectation is: (1) Oxygen was found necessary for glycine uptake, while azide was inhibitory.[1] (2) The inhibitory effect of dinitrophenol on the incorporation of alanine into liver slice protein has been attributed to an interference with the synthesis of energy-rich phosphate bonds.[2]

On the other hand, very substantial evidence has been obtained for the participation of the adenylic acid system in the synthesis of peptide models: *p*-aminohippuric acid,[3] glutathione[4] and the acetylation of sulfanilamide.[5]

We have found that a liver granule fraction, nearly freed of soluble factors by draining, has relatively low activity with respect to amino acid incorporation. This system can be activated by the addition of magnesium ion, adenosine triphosphate, citrate and a mixture of L-amino acids. The activation occurred aerobically and not anaerobically.

A 1:5 homogenate of rat liver with ice-cold 0.9% KCl–0.4% $KHCO_3$ (saturated with 95% oxygen –5% CO_2 gas) was centrifuged for five minutes at 2,000 r.p.m. (680 × *g*) (International Refrigerated Centrifuge PR 1). The supernatant was centrifuged for thirty minutes at 4,000 r.p.m. (2700 × *g*). The supernatant liquid was decanted from the sedimented particles, and the inner walls of the centrifuge tubes blotted with filter paper. The particles were then suspended in an equal volume of KCl–$KHCO_3$ solution; 0.30-ml. aliquots of this preparation (containing 10 to 12 mg. of protein) were incubated with 0.03 ml. of each of the C^{14}-labeled amino acids in tubes containing substances dried *in vacuo* from aqueous solutions. The techniques of incubation and separation of radioactive protein have been described.[6]

Representative data for serine, glycine, leucine and phenylalanine are given in the accompanying table.

ACTIVATION OF RADIO-AMINO ACID UPTAKE BY ADENYLIC ACID SYSTEM

Activators	Micrograms[a] Labeled Carbon Per Gram Protein				
	Serine-β-C^{14} 0.9 mM.	Phenylalanine-β-C^{14} 0.26 mM.	Leucine-α-C^{14} 2.4 mM.	Glycine-α-C^{14} 1.2 mM. Initial	After SH[b]
None	0.30	0.10	0.40	0.34	0.30
	0.28	0.10	0.46	0.30	0.20
3 mM. ATP	0.33	0.10	0.43	0.33	0.20
	0.33	0.11	0.37	0.30	0.24
3 mM. ATP + 10 mM. $MgCl_2$	0.40	0.19	0.49	0.73	0.41
	0.41	0.20	0.59	0.76	0.43
3 mM. ATP + 10 mM. $MgCl_2$ + 6 mM. Citrate	0.75	0.37	0.99	2.48	0.82
	0.78		0.93	2.33	0.82
3 mM. ATP + 10 mM. $MgCl_2$ + 10 mM. MAGAPA[c]	0.56	0.42	1.08	3.69	1.32
	0.53		1.08	3.40	1.20
3 mM. ATP + 10 mM. $MgCl_2$ + 6 mM. Citrate + 10 mM. MAGAPA	1.50	0.80	1.79	5.24	1.86
	1.49(19)[d]	0.78(47)[d]	1.76(6)[d]	4.90(34)[d]	1.77(12)[d]
Above, omitting ATP	0.16	0.13	0.49	0.27	0.22
	0.16		0.56		

[a] Micrograms C*/g. protein = $\dfrac{\text{counts/min./g. protein}}{\dfrac{\text{M.w. of amino acid}}{12} \times \dfrac{\text{Specific activity of amino acid}}{1000}}$ [b] Mercaptoethanol treatment. [c] MAGAPA = 1.6 mM. L-methionine, 2.0 mM. L-aspartic acid, 3.0 mM. L-glutamic acid, 1.2 mM. L-arginine, 1.0 mM. L-proline and 2.1 mM. L-alanine. [d] Counts/min./mg.

With homogenates but not with tissue slices the radioactive protein obtained with radio-glycine

(1) T. Winnick, F. Friedberg and D. M. Greenberg, *J. Biol. Chem.*, **175**, 117 (1948).

(2) I. D. Frantz, P. C. Zamecnik, J. W. Reese and M. L. Stephenson, *ibid.*, **174**, 773 (1948).

(3) P. P. Cohen and R. W. McGilvery, *ibid.*, **169**, 119 (1947); **171**, 121 (1947).

(4) R. B. Johnston and K. Bloch, *ibid.*, **179**, 493 (1949).

(5) F. Lipmann, *ibid.*, **160**, 173 (1945).

(6) T. Winnick, E. A. Peterson and D. M. Greenberg, *Arch. Biochem.*, **21**, 235 (1949).

loses a considerable portion of its label when treated with mercaptoethanol. This effect is shown in the last column of the table.

DIVISION OF BIOCHEMISTRY ELBERT A. PETERSON
UNIVERSITY OF CALIFORNIA THEODORE WINNICK
SCHOOL OF MEDICINE, BERKELEY DAVID M. GREENBERG

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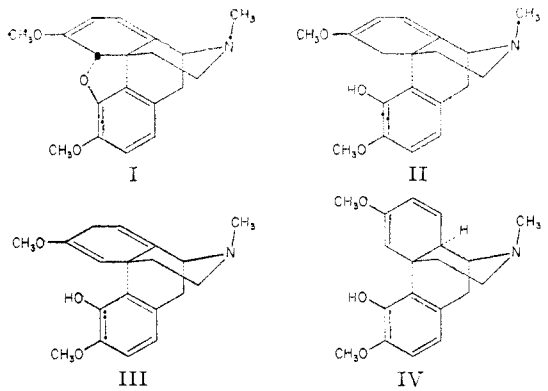
THE STRUCTURE OF PHENOLIC DIHYDROTHEBAINE AND OF β-DIHYDROTHEBAINE

Sir:

Thebaine (I) gives with sodium and alcohol,[1,2] as well as with sodium and liquid ammonia,[3] a phenolic dihydro compound, called phenolic dihydrothebaine, to which structure II has been assigned. This formulation is not compatible with the ultraviolet absorption spectrum of the compound which shows the low extinction coefficient associated with the guaiacol system ($\lambda_{max.}$ 282 mμ, log ε 3.3) without the additional presence of a conjugated alkoxydiene (compare thebaine hydrochloride, $\lambda_{max.}$ 283 mμ, log ε 3.8). The formation of a conjugated diene in good yield by a sodium and alcohol reduction is also not in accord with expectations.

The infrared absorption spectrum of phenolic dihydrothebaine has now been recorded and allows the assignment to the substance of structure III. This spectrum shows two sharp very characteristic bands at 5.9 and 6.0 μ which have been shown in this Laboratory to be characteristic of the unconjugated dihydroanisole system, while there is no band associated with the 1-alkoxy-1,3-diene between 6.1 and 6.2 μ. The new structure obviously fits all the accumulated data on the chemistry of the compound but requires interchange of the structures assigned[2] to "$\Delta^{5,6}$-dihydrothebainone methyl enolate" and "$\Delta^{6,7}$-dihydrothebainone enol methyl ether."

Recently, Schmid and Karrer[4] have proposed that the lithium aluminum hydride reduction product of thebaine, also a phenolic dihydro compound, which they called β-dihydrothebaine has structure IV with the unnatural configuration at C_{14}.



I II III IV

This view is difficult to reconcile with the stereochemistry of thebaine or the formation of dihydrothebainol-6-methyl ether on further catalytic hydrogenation. It is indeed apparent that Schmid and Karrer's substance has the structure formerly assigned to phenolic dihydrothebaine and is II, a fact in accord with the ultraviolet spectrum of the compound ($\lambda_{max.}$ 284 mμ, log ε 4.05). In agreement with this view it has now been found that the substance shows the same infrared spectrum as thebaine in the relevant region.

(1) M. Freund and C. Holtoff, *Ber.*, **32**, 168 (1899).
(2) L. Small and G. L. Browning, *J. Org. Chem.*, **3**, 618 (1939).
(3) K. W. Bentley and R. Robinson, *Experientia*, **6**, 363 (1950).
(4) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **33**, 863 (1950).

CHEMICAL LABORATORIES
HARVARD UNIVERSITY
CAMBRIDGE 38, MASSACHUSETTS GILBERT STORK

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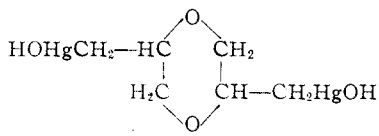
A DIMER OF HUMAN SERUM ALBUMIN WITH A BIFUNCTIONAL MERCURY COMPOUND

Sir:

A bifunctional organic mercurial[1] of the formula



has been successfully employed to link together two molecules of mercaptalbumin, a protein first isolated as a mercury dimer by reaction with mercuric chloride.[2,3] For brevity we denote the protein as AlbSH and the mercurial as HoHg-RHgOH. By light scattering measurements[4] and ultracentrifugal analysis,[5] evidence has been obtained that the reaction proceeds by the following scheme:

$$\text{AlbSH} + \text{HOHgRHgOH} \rightleftharpoons \text{AlbSHgRHgOH} + \text{H}_2\text{O} \quad (1)$$

$$\text{AlbSHgRHgOH} + \text{AlbSH} \rightleftharpoons \text{AlbSHgRHgSAlb} + \text{H}_2\text{O} \quad (2)$$

Turbidity measurements of a 1% solution of mercaptalbumin at *p*H 4.75 and Γ/2 0.05 showed a rapid increase to 1.8–1.9 times the initial value, within three minutes after the addition of 0.5 mole of the mercurial per mole of mercaptalbumin. This indicates that the total reaction described by steps 1 and 2 proceeds much more rapidly than the corresponding reaction of AlbSH with $HgCl_2$, which requires many hours to reach equilibrium.[6] Subsequent ultracentrifugal analysis showed a single boundary sedimenting faster than normal serum albumin (s = 4.6 S) and comparable to the analogous mercury dimer (s = 6.5 S).[3] The reaction proceeded more slowly at *p*H 6. Dimer formation was reversed, in part or completely, by reagents competing for the mercurial, such as sul-

(1) For preparation and proof of structure see: E. Biilmann, *Ber.*, **33**, 1641 (1900); *ibid.*, **35**, 2587 (1902); and J. Sand, *ibid.*, **34**, 1385 (1901).
(2) W. L. Hughes, Jr., THIS JOURNAL, **69**, 1838 (1947).
(3) W. L. Hughes, Jr., "Protein Mercaptides," *Cold Spring Harbor Symposia on Quantitative Biology*, **XIV**, 79 (1950).
(4) For method see: J. T. Edsall, H. Edelhoch, R. Lontie, and P. R. Morrison, THIS JOURNAL, **72**, 4641 (1950).
(5) Ultracentrifugal analyses were carried out by C. Gordon, and computed by Miss V. Gossard, under the supervision of Dr. J. L. Oncley.
(6) W. L. Hughes, Jr., R. Straessle, H. Edelhoch and J. T. Edsall, Abstracts of Papers, 117th Meeting of the American Chemical Society, 1950, 51C.