dimensions (up to 3 cm diameter) could be palpated in the subcutaneous tissues of most individuals. These decreased gradually in size and were still evident in roughly 40% of the cases 6 months after vaccination. In 2 cases abscesses were noted about 2 months after the injection, one of which had to be drained surgically. This number among 120 sites of injection is not too discouraging in the light of the early experience with alum precipitated toxoids.

15024 P

Effect of Amino Acids on Acetylcholine Synthesis.*

CLARA TORDA AND HAROLD G. WOLFF.

From the New York Hospital and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York City.

In the presence of serum and spinal fluid more acetylcholine is synthesized by the frog brain *in vitro* than in the absence of these body fluids.[1,2,3] In search for the agents responsible for the greater acetylcholine synthesis the effect of amino acids was investigated.

Experimental. The effect of the substances used on the acetylcholine synthesis was studied by the method described previously.[2] Mixtures containing varying amounts of the substances, 100 mg minced fresh frog brain, 3 mg physostigmine salicylate, 4.8 mg glucose, and 3 cc Ringer's solution were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amounts of acetylcholine synthesized were assayed biologically on the sensitized rectus abdominis muscle of the frog.

The amounts of acetylcholine synthesized are given in Table I. Most of the amino acids in the concentrations used (10^{-2} to 10^{-5} mol.) increased the amount of acetylcholine synthesized. The naturally occurring form of the amino acids seemed to increase the synthesis more than the naturally not occurring form.

Nachmansohn and John[4] under different experimental conditions (different enzyme preparation, different substrate, and anaerobically) found a similar increase of the amount of acetylcholine synthesized in the presence of *l* (+) glutamic acid, cysteine, alanine, phenylalanine, *l* (—) tryptophane, *l* (—) proline, and valine.

It was also found[5] that acetylcholine synthesis in the presence of serum from the working arm was 40% less than in the presence of serum from the immobilized arm. Since it is possible that during muscle work some transamination occurs[6] the effect of free ammonia on the synthesis of acetylcholine was also investigated. It was found that ammonia decreased the acetylcholine synthesis. Therefore, if ammonia accumulates during performance of muscle work it may depress the local acetylcholine synthesis, a synthesis known to occur during performance of prolonged work by cholinergic systems.[7,8] The accumulation of ammonia may thus contribute to the genesis of muscle fatigue.

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1 Torda, C., and Wolff, H. G., *Science*, 1943, **98**, 242.

2 Torda, C., and Wolff, H. G., *J. Clin. Invest.*, 1944, **23**, 649.

3 Torda, C., and Wolff, H. G., *Science*, 1944, **100**, 200.

4 Nachmansohn, D., and John, H. M., *J. Biol. Chem.*, 1945, **158**, 157.

5 Torda, C., and Wolff, H. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 13.

6 Cohen, *Wisconsin Symposium on Respiratory Enzymes*, 1942, p. 210.

7 Brown, G. L., and Feldberg, W., *J. Physiol.*, 1936, **88**, 265.

8 Kahlson, G., and MacIntosh, F. C., *J. Physiol.*, 1939, **96**, 277.

TABLE I.
Effect of Amino Acids on the Synthesis of Acetylcholine.

Substance*	Amt of acetylcholine synthesized in % of control†				
	Amt of substances (mg) added to 100 mg frog brain				
	30	3	0.3	0.03	0.003
I. Aliphatic Amino Acids					
(A) Monoamino monocarboxylic acids:					
glycine (SMACO)		98	103	101	102
dl-alanine (Fischer)		116	117	113	104
dl-serine (Dr. duVigneaud)		119	126	110	100
dl-threonine (SMACO)		117	115	116	112
l(—)leucine (Dr. duVigneaud)		106	104	105	103
d(+)leucine (Dr. duVigneaud)		111	111	105	98
(B) Diamino monocarboxylic acids:					
l(+)arginine (Pfanstiehl)		140	129	120	100
l(+)arginine (SMACO)		128	125	115	105
l(+)lysine monohydrochloride (SMACO)		123	112	107	103
l(+)lysine dihydrochloride (Eastman)		136	128	123	110
dl-lysine dihydrochloride (Eastman)		109	108	106	105
(C) Sulfur-containing amino acids:					
l(—)cystine (Fischer)		123	117	107	100
l(—)cystine (Dr. duVigneaud)		126	115	110	103
dl-methionine (SMACO)		102	99	103	99
l(—)methionine (Dr. duVigneaud)		145	129	113	103
l(—)cysteine (SMACO)		134	135	130	112
l(—)homocystine (Dr. duVigneaud)		120	110	114	98
II. Aromatic Amino Acids)					
dl-phenylalanine (SMACO)		110	114	102	105
l(—)tyrosine (Fischer)		112	110	111	104
III. Heterocyclic Amino Acids					
dl-histidine (Fischer)		107	106	108	104
d(+)histidine (Dr. duVigneaud)		134	120	108	102
l(—)histidine (Dr. duVigneaud)		142	125	115	103
l(—)tryptophane (Merck)		132	114	117	103
dl-tryptophane (Dr. duVigneaud)		123	116	114	94
dl-tryptophane (SMACO)		107	105	103	98
tyroxine (Squibb)			133	113	113
Dipeptide: carnosine (Dr. duVigneaud)		160	136	121	100
Tripeptide: glutathione (Fischer)			128	124	114
Decomposition Products:					
creatine		121	116	108	104
creatinine		103	97	101	98
ammonia	15	34	67	90	101

* The substances prepared by Dr. duVigneaud were pure amino acids, the other amino acids may have contained impurities.

† Each value represents the average of 8 separate experiments. The S.E. of the mean for each value was less than ±5%. The amount of acetylcholine synthesized in μg per 100 mg frog brain, followed by the S.E. of the mean, was: 1.50 ± 0.044.

Discussion. The increase of the synthesis of acetylcholine in the presence of amino acids is probably due to various factors: (1) the ability of amino acids to remove heavy metals (such as Cu); (2) to the property of some amino acids to re-establish the free –SH group, an active group of the enzyme involved in the synthesis of acetylcholine; and (3) other not yet identified mechanisms. The $-CH \cdot NH_2 \cdot COOH$ group is partly responsible for the increase of acetylcholine synthesis since greater increase was observed in the presence of substances containing 2 or more $-NH_2$ group (diaminomonocarboxylic acids, peptides) than in the presence of the amino acids containing only one $-NH_2$ group; and phenylalanine and tryptophane increased the synthesis while phenol and indol decrease it.[9]

[9] Torda, C., and Wolff, H. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 183.

It seems that the ketogenic amino acids increased the acetylcholine synthesis less than the non-ketogenic amino acids.

Summary. 1. The effect of amino acids, some polypeptides, and a few decomposition products on the synthesis of acetylcholine was investigated. 2. Most of the amino acids, carnosine, glutathione, and creatine increased the synthesis. 3. Glycine, leucine, and creatinine did not modify the synthesis. 4. Ammonia decreased the amount of acetylcholine synthesized.

15025 P

Effect of Some Isocyclic, Aromatic, and Heterocyclic Compounds on Acetylcholine Synthesis.*

CLARA TORDA AND HAROLD G. WOLFF.

From the New York Hospital and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York City.

In the presence of body fluids of patients with myasthenia gravis less acetylcholine is synthesized than in the presence of body fluids of healthy subjects.[1,2,3] In the following it was ascertained whether some isocyclic, aromatic hydrocarbons, and heterocyclic compounds modify the synthesis of acetylcholine. These substances were chosen because some of them occur in the animal organism as intermediate or end product of the metabolism, some are widely administered for their therapeutic effect, and some are known to modify the activity of various enzymes.

Experimental. The effect of the substances on the synthesis of acetylcholine was studied by the method described previously.[2] The amounts of acetylcholine synthesized are given in Table I. Isocyclic (cyclohexane, inositol) and aromatic hydrocarbons (benzol, toluol, benzoic acid, naphthalene) either did not modify the synthesis of acetylcholine or decreased it only in higher concentrations (10^{-2} mols). Hydroxyl derivatives (phenol, *p*-aminophenol, hydroquinone, salicylic acid, α-naphthol, β-naphthol), aldehydes (benzaldehyde), and ketones (camphor, penicillin) decreased the synthesis in concentrations from 10^{-5} mol. Conjugated products either did not modify the synthesis or decreased it slightly (potassium phenol sulfonate, phenacetine, diphenylamine, benzidine, acetylsalicylic acid, sulfonamides). From the heterocyclic compounds indol decreased the synthesis in concentrations from 10^{-5} mol. Substitution in β position decreased this property of indol: skatol was somewhat less active depressor, indol-3-acetic acid was nearly inactive, *dl*-tryptophane was inactive, and *l* (—) tryptophane, the naturally occurring amino acid, increased the ability of frog brain to synthesize acetylcholine. Quinoline decreased the synthesis in concentrations from 10^{-5} mol.; carbazole decreased it to a lesser degree.

Discussion. The substances used differ in their chemical structure and their ability to enter into various chemical reactions but are known to inactivate proteins. Therefore, it is possible that the decrease of acetylcholine synthesis was due to a partial inactivation of the protein component of the enzyme involved in the synthesis. Many of the substances used are readily combined in the animal organism forming less toxic compounds. Conjugated substances depressed the synthesis of acetylcholine to a lesser degree than did the corresponding acids.

A correlation between the decrease of synthesis of acetylcholine *in vitro* and the physio-

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1 Torda, C., and Wolff, H. G., *Science*, 1943, **98**, 242.

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3 Torda, C., and Wolff, H. G., *Science*, 1944, **100**, 200.