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EFFECT OF SPINAL FLUID FROM PATIENTS WITH MYASTHENIA GRAVIS ON THE SYNTHESIS OF ACETYLCHOLINE IN VITRO[1, 2]

IT was found that less acetylcholine was synthesized in the presence of serum from patients with myasthenia gravis than serum from healthy persons or patients with diseases other than myasthenia gravis.[3, 4] Some of the factors modifying the synthesis of acetylcholine seem to be of relatively small molecular size, since they pass through a semipermeable Cellophane membrane.[4] To ascertain whether these factors are able to pass into the spinal fluid the effect of spinal fluid of patients with myasthenia gravis and of control subjects on the synthesis of acetylcholine *in vitro* was investigated.

METHOD

The amount of synthesis of acetylcholine was ascertained using an adaptation[4] of the method of Quastel, Tennenbaum and Wheatley.[5] The spinal fluid was assayed immediately after collection. The pH of the spinal fluids was adjusted to 7.4. One cc of spinal fluid was added to a mixture containing minced frog brain (100 mg), Ringer's solution (2 cc) at pH 7.4, physostigmine salicylate (3 mg) and glucose (4.8 mg). The mixtures were shaken and incubated aerobically at 37° C for 4 hours and the amount of free and total acetylcholine synthesized was assayed biologically, using the sensitized rectus abdominis muscle of frog.

RESULTS

The effects of spinal fluid of 3 patients with myasthenia gravis and 25 control subjects were studied. The clinical states of the patients with myasthenia gravis are summarized in Table 1. The control subjects were patients with convulsions, fits, displaced intervertebral disks, headaches, brain tumors or were suspected of having brain tumor.

TABLE 1

SHORT SUMMARY OF THE CLINICAL STATE OF THE PATIENTS WITH MYASTHENIA GRAVIS

Name	Sex	Age	Severity of disease	Duration (yrs)	X-ray treatment	Thymectomy	Symptomatology	Neostigmine (Prostigmine Bromide Hoffmann-LaRoche)	
								Dose mg/day	Achievement after medication
R	F	23	3+	9	yes	no	moderate lid ptosis, occasional diplopia, occasional difficulty in chewing and swallowing, moderate muscular fatiguability	90	Walks 1–2 blocks
Sa	F	32	3+	7	no	no	moderate lid ptosis, occasional diplopia, occasional difficulty in chewing, moderate muscular fatiguability	90–150	housework
P	M	36	2+	2	no	no	difficulty in chewing, moderate muscular fatiguability	45–75	Walks, but unable to work

The amounts of acetylcholine synthesized in the various mixtures are summarized in Table 2. In the presence of spinal fluid an average of 50 per cent. more acetylcholine was synthesized than in the presence of serum from the same subject. This observation suggests that at least some of the factors increasing the synthesis of acetylcholine pass into the spinal fluid. Less acetylcholine was synthesized in the presence of spinal fluid from patients with myasthenia gravis than with spinal fluid from the control subjects. The percentage defect in the synthesis of acetylcholine in the presence of spinal fluid from the patients with

TABLE 2

AMOUNTS OF ACETYLCHOLINE SYNTHESIZED IN THE PRESENCE OF SPINAL FLUID FROM PATIENTS WITH MYASTHENIA GRAVIS AND CONTROL SUBJECTS

Subject	Average of acetylcholine synthesized			
	Free acetylcholine in μg per 100 mg frog brain	Per cent. of control	Total acetylcholine in μg per 100 mg frog brain	Per cent. of control
Controls	2.11 ± 0.053		3.16 ± 0.049	
Patients with myasthenia gravis				
R	1.21 ± 0.025	57	1.87 ± 0.032	59
Sa	1.27 ± 0.025	60	2.00 ± 0.037	62
P	1.41 ± 0.029	67	2.20 ± 0.040	69

[1] This study was aided by a grant from the Josiah Macy Jr. Foundation.

[2] From the New York Hospital and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York, N. Y.

[3] C. Torda and H. G. Wolff, SCIENCE, 98: 224, 1943.

[4] C. Torda and H. G. Wolff, in press, *Jour. Clin. Invest.*, September, 1944.

[5] J. H. Quastel, M. Tennenbaum and A. H. M. Wheatley, *Bioch. Jour.*, 30: 1668, 1936.

myasthenia gravis was about the same as the percentage defect in the synthesis of acetylcholine in the presence of serum from the same patient.[4]

DISCUSSION

H. C. Stoerk and E. Morpeth,[6] using rat brain as a source of the enzyme, found the same amount of acetylcholine synthesized in the presence of serum from patients with myasthenia gravis as in the presence of serum from control subjects. Since they also were unable to demonstrate any difference in the amounts of acetylcholine synthesized in the presence of serum from control subjects as compared to Locke's solution, it would appear as though their adaptation of the method of Quastel, Tennenbaum and Wheatley, using rat brain, is not sensitive enough to demonstrate slight differences in the synthesis of acetylcholine due to the presence or absence of substances in the serum of patients with myasthenia gravis. This lack of sensitivity is probably due in the main to the greater lability and the relatively lower concentration of the enzyme and to the chemical properties of the substances contained in the rat brain.

SUMMARY

Human spinal fluid is a more favorable medium to further the synthesis of acetylcholine *in vitro,* using enzyme obtained from frog brain, than is serum. Also, since less acetylcholine was synthesized in the presence of spinal fluid from patients with myasthenia gravis than spinal fluid of control subjects, it is probable that at least some of the factors responsible for the decrease and increase of the synthesis of acetylcholine pass into the spinal fluid.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE MEASUREMENT OF "FOLIC ACID"

DURING the past few years we have been interested in measuring the "folic acid" activity of the liver concentrates which we have been using in animal experiments. Both the *Streptococcus lactis* R and *Lactobacillus casei* e methods have been used[1, 2, 3] and certain improvements have been made in each case.[4, 5] However, many difficulties are still encountered in any attempt to express the activity of a given preparation so that results in different laboratories may be compared. We have found Table 1 to be useful in comparing the results of different workers, and we hope it may be useful to others.

The columns in the table labeled "½ maximum" indicate the approximate number of micrograms of material which provides one half of the maximum growth (as measured by turbidity) or acid production (as measured by titration) per 10 ml of complete medium. Although rather large differences may occur between the turbidimetric and titrimetric methods, these val-

TABLE 1

Material	Source	Investigator	S. lactis R		L. casei e	
			½ maximum	Potency	½ maximum	Potency
			ug.		ug.	
1. Lederle crystals	Liver	Stokstad[6]	.0025	78,000	.00055	79,000
2. Lederle crystals	Yeast	Stokstad[6]	.005	38,000	.0005	75,000
3. Lederle crystals	?	Hutchings[7] *et al.*	.042	5,000	.00061	70,000
		Luckey[4]	.05	2,000	.0035	10,000
		Teply[5]	.1	3,500	.0012	13,000
4. Parke Davis crystals (Bc)	Liver	Pfiffner[8] *et al.*			.0005	
		Luckey	.0013	77,000	.0013	27,000
		Teply	.004	88,000	.0004	40,000
5. Merck crystals	?	Keresztesy[9] *et al.*		*		*
6. Texas preparation A	Spinach	Luckey	.004	25,000	.002	21,000
Texas preparation B	Spinach	Teply	.027	13,000	.0027	6,000
7. Thymine	Synthetic	Luckey	2	50	4	9
8. Solubilized liver	Pork	Luckey	100	1	35	1
		Teply	350	1	16	1
9. Liver fraction B	Pork	Texas group		1		1
		Stokstad (calculated)	200	1	40	1
		Luckey	90	1	70	.5

* Potency for this material is unpublished; however, calculations from footnote 9 indicate it to be 140,000 times as active for *Streptococcus lactis* R as for *Lactobacillus casei* e.

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