many instances, to profound alterations of the reflexes for several minutes, they did not cause any reflexes by themselves. However, they acted, in general, as was to be expected if Sherrington's rules could be applied to nociceptive stimuli although the latter did not evoke any reflexes. Since ipsilateral nociceptive stimuli elicit flexor- and inhibit extensor-reflexes whereas contralateral stimuli act in opposite manners ipsilateral nociceptive stimuli may be expected to increase flexor- and decrease extensor- (knee jerk) reflexes, and opposite effects should be produced by contralateral nociceptive stimuli. Our results show that this is, in general, the case. However, it is worthy of mention that, in several instances, contralateral stimulation of pain fibers by means of hypertonic NaCl solution markedly diminished the flexor reflex. The prolonged and sometimes considerable increase in pupillary diameter which accompanied this inhibitory effect (*cf.* Fig. 4) makes one suspect that this deviation from the rule occurred particularly when excessive pain was induced.

It was shown earlier (Gellhorn and Thompson, 1944) that the effects of electrical stimulation of the motor cortex were greatly increased by means of painful stimuli applied to the muscles of the limbs. In contradistinction to the influence of "muscle pain" on spinal centers described in this paper it was found that its action on the effect of cortical stimulation was independent of the "laterality" of the pain stimulus. Thus cortical stimulation of the left motor area lead to an intensification of the movement of the right foreleg after injection of hypertonic NaCl solution in either the right or the left hindleg. Apparently the alteration of the effects of cortical stimulation as a result of "muscle pain" is not simply the result of the action of these painful stimuli on spinal centers, although these centers are likewise profoundly influenced.

Summary. Stimuli such as injection of hypertonic NaCl solution into striated muscle or faradization of muscles which cause "muscle pain" in man, and pupillary dilatation and vocalization in the anesthetized animal modify spinal reflexes in the latter. It is shown that in general ipsilateral "painful" stimuli increase the response of a flexor reflex and diminish the response of the knee jerk to a standard stimulus. On the other hand, contralateral "pain" stimulation causes an increase in the knee jerk and a decrease in the flexor reflex. If excessive stimuli are used the knee jerk may be inhibited not only from the ipsi- but also from the contralateral side. The fact that "muscle pain" greatly enhances the effect of electrical stimulation of the motor cortex is not due to the alteration of spinal centers but suggests the involvement of supraspinal mechanisms.

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Effect of Tobacco Virus, Some Decomposition Products of Nucleoproteins, and Related Compounds on Acetylcholine Synthesis.*

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In the presence of serum,[1,2] spinal fluid,[3] and thymus[4] from patients with myasthenia gravis less acetylcholine was synthesized *in vitro* than in the presence of similar tissues

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from subjects without myasthenia gravis. Patients with myasthenia gravis may have an enlarged thymus and an increase of lymphoid tissue, tissues known to be important for the metabolism of nucleoproteins in the body,[5,6,7] and these patients are sometimes benefited by removal or irradiation of the thymus. Some extracts of the thymus decrease the synthesis of acetylcholine.[8] Irradiation of tissues or degenerative changes of the thymus lead to disturbances in the metabolism of nucleoproteins.[9,10]

On the other hand, some of the decomposition products and related substances of nucleoproteins may sensitize the effector cells to acetylcholine,[11] may cause vasodilatation,[12-22] and it is possible that adenosinetriphosphate supplies the energy required for the synthesis of acetylcholine.[23] In the following it was ascertained whether tobacco virus, some of the decomposition products of nucleoproteins, and some related compounds modify the synthesis of acetylcholine.

Method. The synthesis of acetylcholine was studied by the method of Quastel, Tennenbaum, and Wheatley[24] with minor modifications.[2] Varying amounts of the substances were added to mixtures containing 100 mg minced fresh frog brain, 3 mg physostigmine salicylate, 4.8 mg glucose, and 3 cc Ringer's solution. The pH of the mixtures was adjusted to 7.4. Identical mixtures without the substances served as controls. The mixtures were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amounts of free and total acetylcholine synthesized were assayed biologically on the sensitized rectus abdominis muscle of the frog. The amount of acetylcholine synthesized was calculated by subtracting from the acetylcholine content of the incubated mixtures the acetylcholine content of identical non-incubated mixtures. By adding the substances in varying concentrations to incubated control mixtures after incubation it was ascertained whether the substances modified the sensitivity of the rectus abdominis muscle to the acetylcholine content of the mixtures during the 2 minutes of immersion for the biological assay. If so the changes were taken in account by the calculation.

Results. The amounts of acetylcholine synthesized in the presence of the substances used are given in Table I. Within the range of concentrations used tobacco virus and the methylxanthines increased the amount of acetylcholine synthesized, whereas many of the decomposition products of nucleoproteins decreased the synthesis of acetylcholine. These results suggest the possibility that some of the decomposition products of nucleoproteins occurring in the body exert an unfavorable effect on the synthesis of acetylcholine (*e. g.* muscle inosinic acid, an usual constituent of the mammalian striated muscle, a product

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TABLE I.
Effect of the Substances on the Synthesis of Acetylcholine.

Substance	No. of exp.	Amt of acetylcholine synthesized in % of control*: Free acetylcholine; Amts of substances in mg added to 100 mg frog brain: 10	3	0.3	0.03	0.003	Total acetylcholine; 10	3	0.3	0.03	0.003
Tobacco virus	4			135	124				147	127	
Nucleic acid (yeast)	10		30	85	87			26	79	83	
Adenylic acid	10		127	119	108	91		122	115	110	100
Inosinic acid (muscle)	8			68	75	80			70	72	76
Adenosine	10		73	90	117			77	84	103	
Adenine sulfate	10	33	57	68	90	95	35	45	63	86	99
" acetate	8		40	65	80	86		39	60	80	86
Guanine	10		27	75	103			48	77	96	
Xanthine	10	85	82	84	81		72	82	90	92	
Uracil	10	80	102	97	106		85	105	100	107	
Thiouracil	10	76	75	81	104		75	83	87	100	
Uric acid	12	53	58	74	74		43	55	66	88	
Caffeine	10		164	150	130	101		170	142	126	93
Theobromine	10		170	148	135	110		178	157	130	112
Theofilline	10		165	150	130	105		177	156	120	100
Pyridine	12	43	77	83	103		66	83	81	90	
2-methyl-5-ethoxy-methyl-6-amino pyrimidine	8		60	68	75	82		61	66	78	84
4-methyl-5-β-mydroxyethyl thiazole	6		30	48	55	81		20	43	51	80
Pyrol	10	57	75	85	94	105		77	83	94	100
Piperidine	8		11	156	107	95		42	155	119	105
Alloxan	10		45	57	77	90		40	59	80	88
Methylguanidine	10		60	96	96	117		58	103	100	118
Urea	10	96	139	112	115		101	133	110	110	
Thiourea	10	96	97	99	107		106	104	105	116	

* The S.E. of the mean for each value was less than $\pm 5\%$. The amounts of acetylcholine synthesized in μg per 100 mg frog brain, followed by the S.E. of the mean were: Free acetylcholine, 0.70 ± 0.023; total acetylcholine, 1.50 ± 0.044.

formed also during muscle activity by deamination of muscle adenylic acid).

Summary and Conclusions. 1. The effect of tobacco virus, some of the decomposition products of nucleoproteins, and some related substances on the synthesis of acetylcholine was investigated. 2. Tobacco virus, methylxanthines, adenylic acid, and urea increased the synthesis of acetylcholine. 3. The synthesis was not modified by thiourea. 4. Piperidine increased the synthesis in lower concentrations and decreased it in higher ones. 5. Methylguanidine and uracil did not modify the synthesis in low concentrations and decreased it in higher ones. 6. The synthesis of acetylcholine was decreased in the presence of nucleic acid, inosinic acid, adenine, guanine, xanthine, adenosine, thiouracil, uric acid, pyridine, 2-methyl-5-ethoxymethyl-6-amino pyrimidine, 4-methyl-5-β-hydroxyethyl thiazole, pyrol, and alloxan.

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