ther purified by use of a column of cation resin XE-97(3) to give fraction FSH49F8 which contained only 0.118 mg of protein per g eq. of dry pituitary tissue. The results of the assay of the crude 21A and the purified FSH49F8 fractions in hypophysectomized male rats indicate that a significant amount of LH activity was removed by the purification steps as indicated by the decrease in the ventral postate weight from 17.1 mg for the crude to 11.6 mg for FSH49F8.

The weights of the adrenals and thyroids from the hypophysectomized rats used for assay of the various FSH fractions were not significantly different from the weights of these glands obtained from control uninjected rats. This indicates that the FSH preparations were free of ACTH and TSH at the high dosage levels used for the assay of the FSH.

Summary. (1) The effect of salt concentration and pH during extraction of sheep pituitary tissue, and temperature and pH of fractionation of these extracts on the preparation of FSH were studied. The most highly purified FSH from the biological standpoint was obtained by extracting at pH 5 with saturated NaCl solution and fractionating at pH 2.5 and 30°C. (2) The FSH preparations made by extracting with 90% saturated and saturated NaCl at pH 5, 5.5 and 6 and fractionating at 30°C and pH 2.5 contained little LH. (3) These preparations were free of ACTH and TSH at the levels tested. (4) The FSH can be concentrated and purified by use of anion and cation exchange resins. (5) Preparations made from 80% saturated NaCl extracts and from extracts made at pH 8 and 9 with saturated NaCl contained significant amounts of LH.

1. McShan, W. H., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 295.

2. McShan, W. H., Meyer, R. K., and Kagawa, C. M., *ibid.*, 1954, v85, 393.

3. McShan, W. H., and Meyer, R. K., *ibid.*, 1955, v88, 278.

4. Greep, R. O., Van Dyke, H. B., and Chow, B. F., *ibid.*, 1941, v46, 644.

5. Greep, R. O., *Endocrinology*, 1942, v30, 635.

Received April 16, 1956. P.S.E.B.M., 1956, v92.

Antagonists to Neuromuscular Block Produced by Sarin. (22533)

A. M. KUNKEL, J. H. WILLS AND J. S. MONIER.
Directorate of Medical Research, Army Chemical Center, Md.

The action of cholinesterase inhibitors on the neuromuscular apparatus has been explained by Burns and Paton(1) as originating by increased spread from the end-plate of the membrane depolarization normally elicited by ACh release. After complete cholinesterase inactivation, the end-plate region is thought to become sufficiently depolarized through the action of the ACh released by a single nerve discharge to be responsive no longer to indirect stimulation. The present work was undertaken to determine whether or not the block of neuromuscular transmission induced by comparatively large doses of Sarin can be overcome by chemical means, and whether any light on the mechanism of action of Sarin can be derived from a knowledge of chemicals capable of overcoming the neuromuscular block.

Methods. Sciatic nerves of cats lightly anesthetized with sodium pentobarbital were stimulated electrically with square wave stimuli at a frequency of 1 every 2 seconds, a pulse duration of 0.1 millisecond, and a supramaximal voltage (0.2 to 0.7 volt). Contraction of the gastrocnemius-soleus muscle group was recorded by a partially isometric lever. After control muscle responses had been obtained, the cat was given 200 μg/kg of Sarin through one antebrachial vein. Artificial respiration (interrupted positive pressure) was administered when required to maintain life. Five minutes later the treatment agent, if any, was administered by the

same route. If the treatment used was successful in restoring the muscle response to 90% of its original value within 30 minutes, a second dose of 300 μg/kg of Sarin was injected intravenously to determine whether or not the treatment had prophylactic as well as therapeutic value. Drug effects were measured by the times required for restoration of the twitch height to 50 and to 85% of its magnitude before Sarin administration. The significance of differences between mean times for the control groups and for the experimental groups was judged by Student's "t-test."

Results. The twitch height in animals given no treatment other than positive pres-

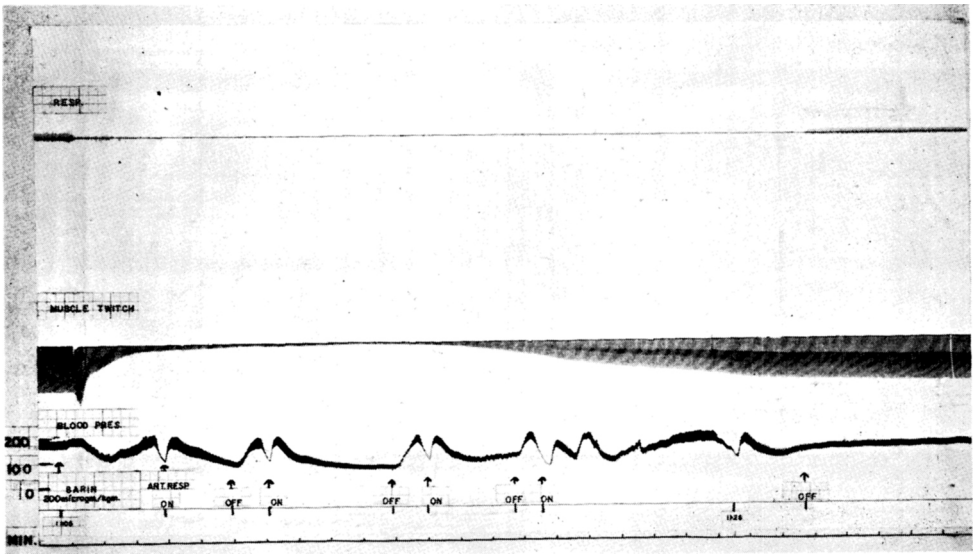

FIG. 1. Spontaneous return of twitch height to normal after Sarin, injected intravenously. Gastrocnemius-soleus muscle group of the cat, excited by just supramaximal electrical pulses. "Off" and "on" refer respectively to termination and to initiation of artificial respiration by a piston pump.

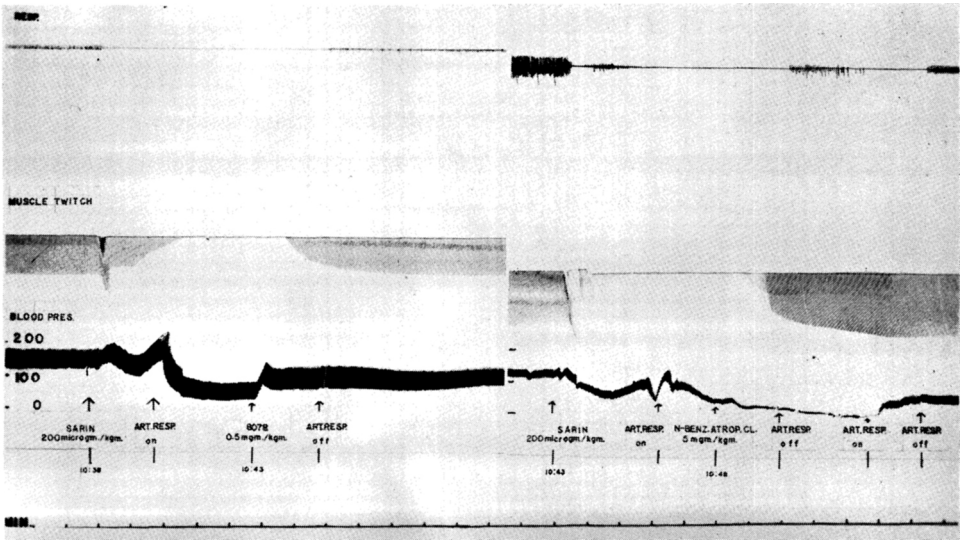

FIG. 2. Effect of WIN 8078 or of benzyl atropinium chloride on rate of return of twitch height to normal after Sarin. Conditions as for Fig. 1.

sure ventilation, as necessary, after Sarin administration returns slowly to normal (Fig. 1). Administration of a comparatively large dose of atropine (2 mg/kg, i. v.) 5 minutes after the poisoning with Sarin had no striking effect on the rate of return of the muscle twitch to normal magnitude. It was observed that quaternary derivatives of atropine, methyl atropinium nitrate and isopropyl atropinium bromide, had moderately potent, but temporary, effects in promoting return of the height of the muscle twitch toward normal. Two other quaternary derivatives of atropine, benzyl atropinium chloride and phenacyl atropinium bromide, were therefore made and tested. Both of these compounds produced a striking and lasting return of the twitch height toward normal (illustrated for benzyl atropinium chloride in Fig. 2). Other quaternary atropinemimetic compounds, like Lergigan methiodide and Dibutoline, also possessed this ability. None of these compounds conferred any significant protection against a second dose of Sarin given 30 minutes after the initial one.

Other compounds known to affect the neuromuscular junction (quaternary oxamide derivatives(2), d-tubocurarine, Flaxedil and decamethonium) were studied in the same way. D-tubocurarine and Flaxedil both increase very significantly the rate of return of the twitch height to 50% of its pre-poisoning value. The rate of return to 85% of the original contraction height was increased significantly by d-tubocurarine but not so significantly by Flaxedil. Decamethonium prolonged significantly the time for return to 50% of the original value and much less significantly the time for 85% recovery. Certain of the oxamides (WIN 8078, WIN 8626 and WIN 12306) had highly significant effects in shortening both times (Fig. 2), but a fourth one (WIN 8077) prolonged both times. Nicotine hydroxamic acid methiodide, a quaternary salt of one of the hydroxamic acids reported to be capable of reversing *in vitro* inhibition of cholinesterase by P-containing compounds(3), was found to enhance somewhat the recovery of twitch height when given intravenously in sufficiently large doses. Table I gives the mean values, for groups of 6 cats, of the times for recovery of 50 and 85% of the pre-poisoning twitch height and the corresponding "t" values for the differences between the control times and those for the animals given drugs. This table gives also information about the abilities of the various compounds to prevent the action on

TABLE I. Effectiveness of Various Compounds in Promoting Recovery of Twitch Response to Sciatic Nerve Stimulation following Injection of 200 μg/kg of Sarin. All treatment compounds injected intravenously 5 min. after Sarin.

Treatment compound	Dose, mg/kg	Minutes for 50% recovery	Minutes for 85% recovery	"t" drug *vs* control for 50% recovery	"t" drug *vs* control for 85% recovery
none	—	19.6	27.1		
atropine SO_4*	2.0	16.1	22.3	1.0	1.2
N-B A*	5.0	6.4	8.3	5.4	6.3
N-P A*	5.0	6.1	9.8	5.5	4.9
Dibutoline*	5.0	6.6	10.7	5.3	5.5
d-tubocurarine‡	.3	6.7	8.1	5.2	6.3
Flaxedil*	.25	7.1	16.9	5.0	2.1
decamethonium*	.01	25.4	30.0	2.4	1.0
WIN 8077†	.05	25.2	38.4	1.1	1.0
" 8078†	.5	6.5	9.0	5.4	6.0
" 8626†	.5	7.0	8.9	5.1	5.9
" 12306‡	1.0	6.4	8.0	5.4	6.4
NHAM*	35-70	14.0	20.7	1.8	2.1

N-B A = N-benzyl atropinium chloride. N-P A = N-phenacyl atropinium bromide. NHAM = nicotine hydroxamic acid methiodide.

* This compound protected none of 3 to 5 animals against a second dose of Sarin.
† " " " 1 or 2 out of 3 or 4 animals against a second dose of Sarin.
‡ " " " all of 4 animals against a second dose of Sarin.

twitch height of a second dose of Sarin. It is especially striking that the N-benzyl and N-phenacyl atropinium salts and Dibutoline had no prophylactic value against a second dose of Sarin although WIN 8078, WIN 8626 and WIN 12306, which were not strikingly more potent therapeutically, had definite, and in the last instance striking, ability to prevent the effect of a second dose of Sarin on the response to sciatic nerve stimulation.

Discussion. The foregoing findings may be applied to an examination of the idea of Cohen and Posthumus(4) that the P-containing anticholinesterases react primarily with an "esteratic" site of the receptor surface, thereby sensitizing an adjacent "anionic" site to acetylcholine. Compounds capable of being attracted to the "anionic" site by electrostatic charges, like those containing electropositive nitrogen atoms, are held to block the depressant effects of acetylcholine on the previously sensitized muscle by competitive occupation of the "anionic" site. This theory is fitted by all our findings except the one that decamethonium does not antagonize the Sarin-induced decrement in the response of the muscle to electrical excitation of the motor nerve. Cohen and Posthumus postulated that decamethonium reacts with the "esteratic" group, and this idea may be strengthened somewhat by the finding of Paton and Zaimis (5) that decamethonium is mildly inhibitory of cholinesterase. By reacting with the "esteratic" group rather than, or in addition to, the "anionic" group, decamethonium would enhance rather than antagonize the sensitization to acetylcholine induced by the P-containing anticholinesterases. It may be significant in the same regard that WIN 8077, which has been shown by Arnold *et al.*(2) and Lands *et al.*(6) to be both the most toxic compound and the most potent cholinesterase inhibitor of the 4 oxamides used here, is the only oxamide which prolonged the recovery times. The more weakly antiesteratic compounds may be assumed to be directed by their quaternized nitrogen atoms to the "anionic" site predominantly and WIN 8077 to be directed by its greater affinity for cholinesterase to the "esteratic" site predominantly. It is difficult for us to see how this mechanism could be in operation after administration of the large dose of Sarin used in our experiments, however; we have used 10-12 LD50's of Sarin intravenously, and find no demonstrable cholinesterase in the muscles. This leaves us, therefore, without explanation for the different behaviors of decamethonium and WIN 8077; one possibility would be that cholinesterase is not the sole point of attack of Sarin at the neuromuscular junction. Groblewski *et al.*(7) have found other evidence that the P-containing moieties of DFP and Sarin may have direct actions on the contractile mechanism in striated muscle without reference to cholinesterase.

Conclusions. 1. Certain compounds containing quaternary nitrogen atoms are able to overcome the decrease in twitch height, of the cat gastrocnemius-soleus muscle group excited by maximal electrical stimulation of the sciatic nerve at one shock per two seconds, induced by large doses of Sarin. 2. Compounds having significant anticholinesterase activity may enhance the Sarin-induced decrease in twitch height despite the abolition by Sarin of demonstrable cholinesterase activity in the muscle.

1. Burns, B. D., and Paton, W. D. M., *J. Physiol.*, 1951, v115, 41.

2. Arnold, A., Soria, A. E., and Kirchner, F. K., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 393.

3. Wilson, I. B., and Meislich, E., *J. Am. Chem. Soc.*, 1953, v75, 4628.

4. Cohen, J. A., and Posthumus,, C. H., *Acta Physiol. Pharmacol. Neerl.*, 1955, v4, 17.

5. Paton, W. D. M., and Zaimis. E. J., *Nature*, 1948, v162, 810.

6. Lands, A. M., Karczmar, A. G., Howard, J. W., and Arnold, A., *J. Pharmacol. Exp. Therap.*, 1955, v115, 185.

7. Groblewski, G. E., McNamara, B. P., and Wills, J. H., *ibid.*, in press.

Received April 30, 1956. P.S.E.B.M., 1956, v92.