ployed.

Summary. When a subcutaneous inflammation is induced in the mouse through mechanical injury, and when into such a traumatized site a fragment of experimental sarcoma is deposited, a striking edematous reaction ensues, accompanied by a conspicuous acceleration of tumor growth; these associated phenomena being well defined by the 4th to 6th day after implantation.

This effect is construed as a specific interaction between the neoplastic characteristics of the tumor fragment and the cellular and/or vascular sequences of subcutaneous inflammation. The effect does not occur when nonmalignant tissues are similarly implanted.

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Veratrinic Effects of Pentamethylenetetrazol (Metrazol) and 2,2-Bis (P-Chlorophenyl) 1,1,1 Trichloroethane (DDT) on Mammalian Neuromuscular Function.*

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An extraordinarily diverse group of agents produces in excitable tissues a stereotyped, veratrinic effect: a single brief stimulus evokes a burst of repetitive responses.[1,2] Incomplete evidence indicates that the appearance of the veratrinic response in nerve fiber always is accompanied by a similar alteration in muscle fiber, and suggests that repetitive response is a general phenomenon which occurs in all excitable tissues exposed to veratrinic agents.

To explore further this general phenomenon, the study reported here was made of two agents which produce altered states of excitability. *Pentamethylenetetrazol* (metrazol) was selected because of its predominant effect on central neural activity and investigated for its effect on the peripheral neuromuscular unit. *2,2-bis (p-chlorophenyl) 1,1,1 trichloroethane* (DDT) was studied in the mammalian preparation because recent extensive investigations have demonstrated its potent veratrinic effect on the peripheral nerve of both Hexapoda and Crustacea.[3,4]

Methods. Details of the preparation and recording technics have been described.[2] Briefly, adult rats under pentobarbital anesthesia were used throughout. Isometric myograms were made from the triceps surae by means of resistance-wire strain gauges and recorded by means of a cathode ray oscillograph. Electromyograms were recorded through needles placed in the belly of the muscle. Electroneurograms were recorded from the sciatic-tibial nerve which had been isolated centrally and peripherally, Square-wave supramaximal stimuli were delivered to the sciatic nerve or directly to the curarised muscle. Metrazol was injected intraperitoneally (i-p) in doses of 25 to 100 mg/kg. DDT† was suspended in peanut oil-saline homogenate by means of lecithin (Asolectin)‡[5] and injected i-p in doses

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1 Krayer, O., and Acheson, G. H., *Physiol. Rev.*, 1946, **26**, 383.

2 Eyzaguirre, C., Folk, B. P., Zierler, K. L., and Lilienthal, J. L., Jr., *Am. J. Physiol.*, 1948, **155**, 69.

3 Roeder, K. D., and Weiant, E. A., *Science*, 1946, **103**, 304.

4 Welsh, J. H., and Gordon, H. T., *J. Cell. Comp. Physiol.*, 1947, **30**, 147.

† A twice-crystallized sample was kindly provided by the Medical Division, Army Chemical Center, Edgewood, Md.

‡ Generously provided by Associated Concentrates, Inc., Woodside, Long Island, N. Y.

of 50 to 100 mg/kg. Neostigmine methylsulfate was injected i-p in doses of 50 μg/kg.

Measurements of refractory period of nerve were made by delivering pairs of supramaximal stimuli through the same electrodes at various short intervals and determining the

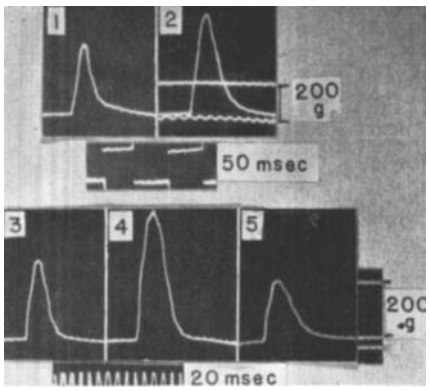

FIG. 1.
Myograms. 1. Control (indirect stimulation). 2. After 8 mg metrazol (resting tension 200 g). 3. Completely curarized muscle (direct stimulation). 4. After 20 mg DDT. 5. After 30 mg quinine diHCl (resting tension 35 g).

amplitude of the second response in relation to the first.

Results. Qualitatively, the effects of metrazol and of DDT were indistinguishable and are presented together. Both agents evoked in muscle a myotonic response which mimicked faithfully the functional and pharmacological characteristics of the myotonia occurring spontaneously in man and goat.[6] The myotonic response was seen as an increase in tension and duration of the twitch evoked by a single brief stimulus,[7] whether delivered indirectly through the sciatic nerve or directly into the curarised muscle (Fig. 1). The electrical basis of the augmented mechanical response was seen in the corresponding electromyograms, where the normal diphasic action potential was followed by a train of spikes (Fig. 2). Similar repetitive responses followed mechanical stimulation by tapping. Repeated stimulation at a rate of 12/min. produced a progressive waning of the myotonic response; the phenomenon of "warm-up". The isolated sciatic nerve studied *in situ* responded with a burst of spikes to a single stimulus following injection of either metrazol or DDT (Fig. 3).

Examples are given of the effect of agents which suppress the repetitive phenomenon: quinine (Fig. 1), Mg^{++} (Fig. 2) and Ca^{++} (Fig. 2 and 3). Accentuation of the myotonic effect by K^{+} is seen in Fig. 2.

The effect of an injection of neostigmine, which itself did not produce repetition at the

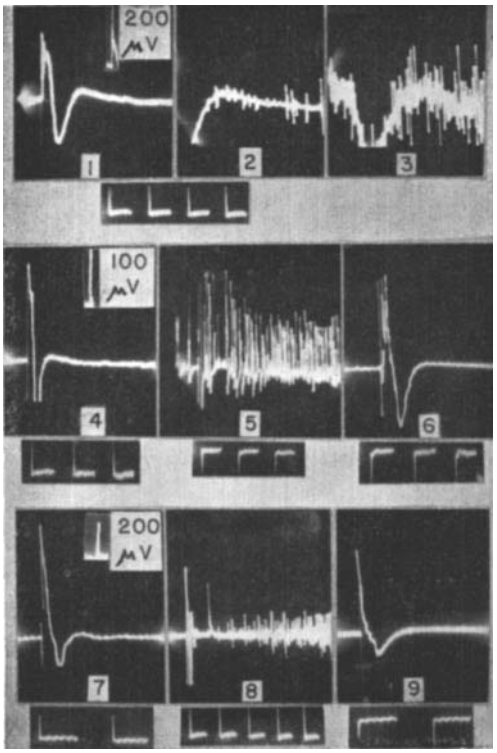

FIG. 2.
Electromyograms. 1. Control (indirect stimulation). 2. After 5 mg metrazol. 3. After 60 mg KCl. 4. Control (indirect stimulation). 5. After 20 mg DDT. 6. After 100 mg Ca gluconate. 7. Curarized control (direct stimulation). 8. After 20 mg DDT. 9. After 50 mg $MgSO_4$. All time scales = 100 msec.

neuromuscular junction, was related to the amount of metrazol which had been given. After a small dose of metrazol (25 mg/kg), which produced a few random spikes, neostig-

[5] Philips, F. S., and Gilman, A., *J. Pharmacol.*, 1946, **86**, 213.

[6] Brown, G. L., and Harvey, A. M., *Brain*, 1939, **62**, 341.

[7] Köllensperger, F. K., *Klin. Wchnschr.*, 1940, **19**, 128.

mine accentuated the repetitive response. Conversely, after a larger dose of metrazol (50 mg/kg), which evoked intense repetition, the injection of neostigmine suppresed the usual response.

As a gauge of one phase of excitability, measurements were made in nerve of the rate of recovery of responsiveness, the relatively

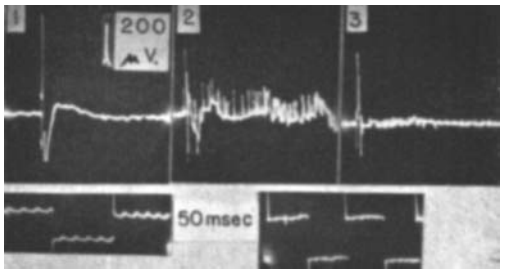

FIG. 3.
Electroneurograms. 1. Control. 2. After 10 mg DDT. 3. After 50 mg Ca gluconate.

refractory period, before and after treatment with metrazol. The results of 2 experiments have been grouped and are presented in Fig. 4. Concurrent with the appearance of repetition after administration of metrazol, the nerve recovered more rapidly and the relatively refractory state subsided more abruptly. In addition, a minimal but recognizable period of supernormality appeared.

Discussion. Evidence is accumulating slowly to support the hypothesis that agents which produce repetitive responses in one sort of excitable tissue induce generally the same altered state of excitability in other tissues of the same organism. An example of such a general effect is furnished by metrazol, which produces central rhythmic discharge and convulsions, and in the periphery evokes repetition in isolated nerve and striated muscle.

The same alteration of excitability resulting in repetition can be evoked in members of widely separated phyla. An example of this broad action is furnished by DDT which produces the same veratrinic response in the peripheral neuromuscular structures of both arthropods and mammals. DDT also fits into the group of substances which affect peripheral as well as several central structures.[8,9]

The changes which underlie the development of veratrinic repetition are obscure. The assumption that repetition occurs in a setting of exalted excitability is compatible with the demonstration of an accelerated rate of recovery demonstrated in nerve treated with metrazol or 2, 4-dichlorphenoxyacetate (2,4-D).[10] But the number of apparently unrelated agents which evoke repetition makes it difficult to construct an hypothesis encompassing all reported observations. A recent and attractive working hypothesis of repetition has emphasized the primary role of free and surface-bound calcium as a modulator of excitability in nerve.[11] Whether this engaging concept will explain repetition occurring in many

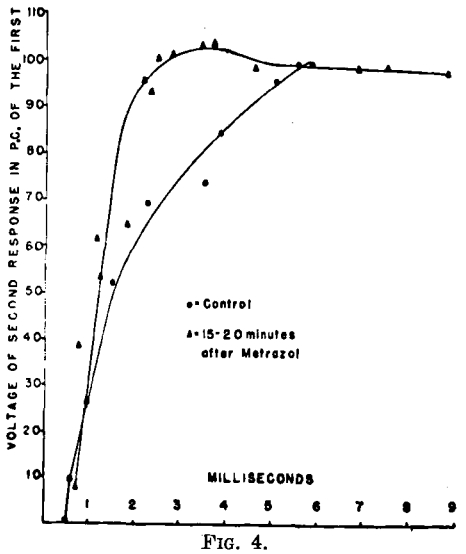

FIG. 4.
Relatively refractory period of nerve, measured before and after metrazol.

tissues and under many varying conditions is an unanswered question.

The development of repetition in the peripheral neuromuscular unit results in a functional disorder which is indistinguishable from

[8] Creseitelli, F., and Gilman, A., *Am. J. Physiol.*, 1946, **147**, 127.

[9] Bromiley, R. B., and Bard, P., *Bull. Johns Hopkins Hosp.*, in press.

[10] Eyzaguirre, C., Jarcho, L. W., and Lilienthal, J. L., Jr., in preparation.

[11] Gordon, H. T., and Welsh, J. H., *J. Cell. Comp. Physiol.*, 1948, **31**, 395.

the myotonia occurring spontaneously in man and goat. There is no evidence, however, to establish the identity of the induced and the spontaneous phenomena, similar though they appear. Nevertheless, the demonstrated effect of veratrinic agents on both nerve and muscle suggests that in spontaneous myotonia an as yet undetected repetition may occur in nerve as well as in muscle.

Summary. 1. Both metrazol and DDT evoke a veratrinic response in mammalian nerve and muscle.

2. The characteristics of this response are indistinguishable from those of myotonia occurring spontaneously.

3. The veratrinic effect of repetitive responses to single stimuli is accompanied by a shortening of the relatively refractory period.

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Comparison of Atropine and Tripelennamine in Treatment of Peptone Shock in Dogs.

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Dale and Laidlaw[1] first called attention to the close similarity existing between anaphylactic and peptone shock on the one hand and the type of shock produced by an injection of histamine on the other. Indeed, they postulated that histamine is the substance active in producing the classical signs of anaphylaxis. It was not until 1932, however, that their theory received substantiation, when Dragstedt[2] demonstrated the presence of a substance in blood and lymph in early stages of anaphylactic shock in dogs which resembled histamine chemically and physiologically. It is now generally accepted that histamine plays the major role both in peptone and in anaphylactic shock-like states.

Since the advent of potent anti-histaminic compounds, it has been demonstrated that all drugs which show a marked antagonism to histamine are capable of diminishing the severity of anaphylactic shock.[3] A number of observations, however, would indicate that some factor or factors other than histamine may be involved. For example, Went and Lissak[4] reported that the choline content of the isolated, sensitized heart of the guinea pig decreased following addition of antigen, and, moreover, that acetylation of the perfusion fluid evoked an acetylcholine-like reaction in leech muscle. Furthermore, the isolated, sensitized rodent heart slowed when antigen was added to the perfusing fluid. More recently, Farber and his co-workers[5] found that in 3 of 27 isolated, sensitized hearts (guinea pig) an acetylcholine-like substance was liberated to a perfusate containing physostigmine when the antigen was added. The *normal* heart failed to show the same response. Prior to this, Wenner and Buhrmester[6] reported the acetylcholine concentration in the blood of rabbits in anaphylactic shock to be 1:1,000,000 to 1:10,000,000, whereas measurable amounts were not detected in the blood of normal animals.

The relative importance of acetylcholine in the genesis of peptone and anaphylactic shock

* The data in this paper were submitted to Boston University Graduate School in partial fulfillment of the requirements for the degree of Master of Arts.

† Deceased June 28, 1948.

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2 Dragstedt, C. A., and Gebauer-Fuelnegg, E., *Am. J. Physiol.*, 1932, **102**, 512.

3 Loew, E. R., *Physiol. Rev.*, 1947, **27**, 542.

4 Went, S., and Lissak, K., *Arch. f. Exp. Path.*, 1936, **182**, 509.

5 Farber, S., Pope, A., and Landsteiner, E., Jr., *Arch. Path.*, 1944, **37**, 275.

6 Wenner, W. F., and Buhrmester, C. C., *J. Allergy*, 1937, **9**, 85.