siderations. We have no measure of the work done either by the chemical agent or the physical agent in eliciting a convulsion. The mechanism of stimulation by chemical agents must differ in some essential respect from that of stimulation by physical agents since the sum of the 2 effects, *i. e.,* excitation by a chemical agent first, and then by a physical agent, or conversely, excitation by a physical agent first and then by a chemical agent, differs so widely from the total effect of either chemical or physical excitation alone. From the numerical ratio one might say that one chemical excitation will do about as much damage as several physical excitations and that the end result of the processes set up by either form of excitation renders the cells more vulnerable to the end result of processes set up by the other.

6332

Buffer Influence Upon Response of Striated Muscle to Caffeine Stimulation in Fatigue Studies.

RALPH H. CHENEY. (Introduced by Eric Ponder.)

From the Department of Biology, College of Arts and Sciences, Long Island University, and Marine Biological Laboratory, Woods Hole, Mass.

The experiments cited here indicate the influence of the buffered solvent upon the response of the gastrocnemius muscle of *Rana pipiens* Schreber with respect to caffeine action. In recognition of the importance of the pH factor upon physiological phenomena, this environmental variant was controlled at a constant value. It was noted. however, in studying the effect upon neuro-muscular responses in fatigue studies, that in spite of the constant pH conditions as maintained at 7.3, which is the pH of frog blood and lymph, definitely variable results were obtained. Even the complete reversal of the characteristic responses was observed in accordance with the nature of the buffer employed as the particular injection medium for the substance concerned.

The influence of ion variation on the action of different concentrations of Ca and K in a Locke solution medium upon smooth muscle action, as shown by Salant and Parkins,[1] demonstrates that the effect of an alkaloid—in their experiments ergotamine was

[1] Salant, Wm., and Parkins, Wm. M., *J. Pharm. and Exp. Therap.*, 1932, **45**, 315.

used—varies with the pH and with the nature of the ion. There is also a definite relationship of the observed effect to the inter-relationship of both the factors concerned. In the experimental series here presented, the influence of the medium is noted to be effective also upon the striated muscle response, independent of the pH. The constant pH value with a variation in the nature of the ions employed for that purpose demonstrated the divergent effect of caffeine upon striated muscle in the presence of different buffer media.

Caffeine effects have been reported by several investigators. In low concentrations, Bock[2] found that caffeine increases the extent of the contraction record for striated frog muscle; but, in high concentrations, Hartree and Hill[3] noted contracture and rigor with an accompanying loss of excitability. Cheney[4] has demonstrated that caffeine improves the uniformity of the crest wave of fatigue curves of the gastrocnemius muscle—sciatic nerve preparation and has shown[5] that the caffeine treatment of pithed frogs by a dorsal lymph sac injection followed by a 25-minute absorption period prior to stimulation, increases slightly both the height of contraction and the time to fatigue. In addition, Cheney[6] determined the optimum caffeine dosage for the frog (*Rana pipiens* Schreber) to be 0.1 mg. per gm. of body weight.

The mechanical apparatus was essentially the same as described by Cheney[5] for the pithed frog, isotonic contractions. The dosage in all instances, however, was the optimum dosage referred to above. In cases in which the medium was injected without the caffeine, a volume was used equivalent to the quantity representing the amount of injection required to give the optimum dosage (0.1 mg./gm. body weight) if caffeine had been involved. The absorption time from the dorsal lymph sac was constant: a 30 minute period. Other constants included the strength and rate of stimulation (1 per 5 seconds), and the pH of the injection medium; *i. e.*, 7.3, except in the distilled water and aqueous caffeine, which was 6.7 as determined by the electrometric method. A uniform rate of rotation was maintained with respect to the recording drum on which 1 cm. of graph surface registered 22 contractions. The after-load in each instance was equal to the body weight of the particular animal utilized. Moreover, the gastrocnemius record of the untreated limb was always compared directly with the caffeine-treated gastrocnemius of

[2] Bock, J., *Heffter's Handb. d. exp. Pharm.*, 1920, **2**, 508.

[3] Hartree, W., and Hill, A. V., *J. Physiol.*, 1924, **58**, 441.

[4] Cheney, R. H., *J. Pharm. and Exp. Therap.*, 1931, **43**, 457.

[5] Cheney, R. H., *Arch. Intern. d. Pharm. et d. Therap.*, 1932, **42**, Fasc. II, 173.

[6] Cheney, R. H., *J. Pharm. and Exp. Therap.*, 1932, **45**, 389.

the *same* animal. One-half of the data was derived from the use of the right muscle as the non-treated record, and the remaining half employed the left gastrocnemius, in order to eliminate any possible difference in the paired muscles of the same animal.

The pH constant was maintained by various buffers which showed a difference in the effect on muscular fatigue in accordance with the buffer composition. The statistical basis of comparison for the different series was the Xy value (Time to one-half fatigue x Height of Contraction at that time) in frogs of miscellaneous body weights; and also, the percentage increment or decrement of B/A, where "B" represents the caffeine-treated muscle record of its Graph Area, and "A" refers to the non-treated muscle. Ten or more experiments were performed in each of the series listed below.

TABLE I.

Series	Preparation	Difference in XY value between A and B.	Aver. % Increment or Decrement of B/A GRAPH AREA.
I	Non-treated Right Gastrocnemius "A" Non-treated Left Gastrocnemius "B"	0.5	4
II	Non-treated Muscle "A" Distilled water-treated "B"	10.62	26
III	Non-treated Muscle "A" Aqueous caffeine-treated "B"	11.76	30
IV	Non-treated Muscle "A" Bicarbonate buffer Ringer-treated "B"	13.4	20
V	Non-treated Muscle "A" Bicarbonate buffer Ringer-caffeine "B"	15.55	33
VI	Non-treated Muscle "A" Phosphate buffer-treated "B"	11.32	26
VII	Non-treated Muscle "A" Phosphate buffer caffeine-treated "B"	—4.69	13
VIII	Non-treated Muscle "A" Phosphate buffer Ringer-treated "B"	7.43	13
IX	Non-treated Muscle "A" Phosphate buffer Ringer caffeine-treated "B"	—3.55	8

It will be noted that the Xy values of the right and left non-treated muscles are approximately equal regardless of which leg was fatigued first. A 50% alternation of the limb treated was carried out routinely to avoid any possible error. It is clear also that the medium alone so affects the osmotic conditions, even in the case of frog Ringer's solution, that some positively stimulatory effect is produced. Such an effect is, however, always less than the results recorded *after* the addition of the optimum dosage of caffeine. A reversal of the preceding values occurs for the caffeine effect in the presence of a phosphate buffer.

Such a reversal of the effect of a substance with a change in the buffered solvent, and especially with phosphate buffers, has been observed also in blood studies as well as in these muscle investigations. In some way the phosphate ion seems to prevent the caffeine from entering the tissue or of acting upon the tissue with its characteristic effect. It is highly improbable that there is any significant reaction between the caffeine and the phosphate since the caffeine is a very weak base. A reaction with the formation of such a compound as caffeine phosphate would not occur, at least until the pH was far more alkaline than 7.3, which is the figure with reference to frog blood and lymph, and therefore, this value was maintained throughout these experiments.

Summary. 1. The characteristic positive effect of caffeine in delaying the rapidity of fatigue in the gastrocnemius muscle is apparent when administered in an aqueous medium, or in a bicarbonate buffered-Ringer medium. 2. The effect of the medium containing the caffeine is greater than the effect of the medium alone. 3. A reversal of the usual caffeine effect upon the fatigue record of striated muscle occurs when the caffeine is introduced in a phosphate buffered medium, or in a phosphate buffered-Ringer medium. 4. Both phosphate-buffered caffeine solutions cause a negative effect to that of the medium alone. 5. The necessity of discretion in the choice of a buffer in the maintenance of the desired pH of a solvent medium is evident; and, the elimination of an aqueous or Ringer phosphate buffered solution as a solvent for caffeine, in studies of the effect of caffeine upon the fatigue record of the striated (gastrocnemius) muscle, is indicated specifically.

6333

Occurrence of Rough Pneumococci in Lungs of Patients with Lobar Pneumonia.

GERALD S. SHIBLEY AND EDWARD S. ROGERS.

From the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland, Ohio.

"S" and "R" forms of pneumococci are considered, in a general way, to represent respectively virulent and avirulent phases of the organism. It seemed reasonable to suspect that degradation from