The improvement of wound healing by vitamin C is of special interest because collagen formation in wounds in man is reported to begin only 5 or 6 days after the incision[19]. Thus collagen formation either starts earlier in dogs or vitamin C also acts on other cell functions involved in wound healing. The vitamin C was useful in animals that presumably had normal tissue levels, inasmuch as dogs fed an adequate diet normally synthesize all their needed vitamin C[20]. Possible influence of the calcium in the preparation used cannot be excluded.

Fibrinogen's effectiveness may have been due to the fibrin matrix expediting fibroplastic proliferation. Alcohol's dramatic improvement suggests that its use in an incision would not only improve antisepsis, but would also improve the healing rate.

Zusammenfassung. Eine neue Methode wurde entwickelt, um die Wundheilung (Zugfestigkeit) nach Verabreichung von Medikamenten objektiv beurteilen zu können. Als Kriterium wird die Kraft bestimmt, die notwendig ist, um eine Wunde nach Entfernung der Nähte wieder aufzureissen.

W. R. KLEMM[21,22]

Department of Physiology and Pharmacology, College of Veterinary Medicine, Iowa State University, Ames (Iowa, USA), July 5, 1966.

[19] TH. GILLMAN, in *La Cicatrisation* (Centre National de la Recherche Scientifique, Paris 1965), p. 117.

[20] *Basic Guide to Canine Nutrition* (Gaines Dog Research Center, 1965), p. 37.

[21] The author is grateful for the laboratory assistance of Mr. ROBERT FANTON.

[22] Present address: Department of Biology, Texas A & M University, College Station, Texas, USA.

Effects of Etonitazene upon Respiratory Neurons

Administration of etonitazene[1,2] – a benzimidazole derivative, 1-(β-diethyl-aminoethyl)-2-(*p*-ethoxy benzyl-5-nitrobenzimidazole) – in suitable doses produces muscular paralysis in a wide variety of species, where the paralysis is of central origin since the stimulation of a motor nerve produces contraction of the innervated muscle(s). In preliminary experiments (unpublished), it was determined that the administration of successively higher doses of etonitazene to the squirrel monkey produced respiratory paralysis before the locomotor musculature was visibly affected. In contrast, in the dog, locomotor collapse appeared before respiratory paralysis. These observations suggested that the structures involved in the central control of the respiratory musculature, i.e. the respiratory centers, are highly sensitive to the effects of etonitazene in the monkey, and more resistant in the dog. The purpose of this study was to verify these preliminary findings with more systematic research and to determine the sensitivity of inspiratory and expiratory neurons of the 2 species to etonitazene.

Methods. Adult animals of either sex were used in these experiments. Squirrel monkeys (36) weighing 550–950 g were anesthetized with sodium pentobarbital (30 mg/kg), urethane (1.5 g/kg), or urethane-chloralose (800 and 35 mg/kg, respectively) administered i.p. Dogs (10) weighing 9.2–15 kg were anesthetized with sodium pentobarbital (25–30 mg/kg) i.v. A tracheotomy was performed and a jugular vein was cannulated. The animal was then placed in a stereotaxic instrument and the brain stem respiratory centers were approached, perpendicularly in the squirrel monkeys and obliquely backward at an angle of 55° with the horizontal in dogs, with a microelectrode through a small hole drilled in the top of the skull. The microelectrodes consisted of tungsten rods (1.1 mm in diameter), tapered in a reducing bath to measure less than 5 μ at the tip and subsequently coated with insulating material. The respiratory movements were recorded as either rate of

[1] A. HUNGER, J. KEDRLE, A. ROSSI, and K. HOFFMAN, Experientia *13*, 400 (1957).

[2] P. JANSEN, Anaesthesist *11*, 1 (1962).

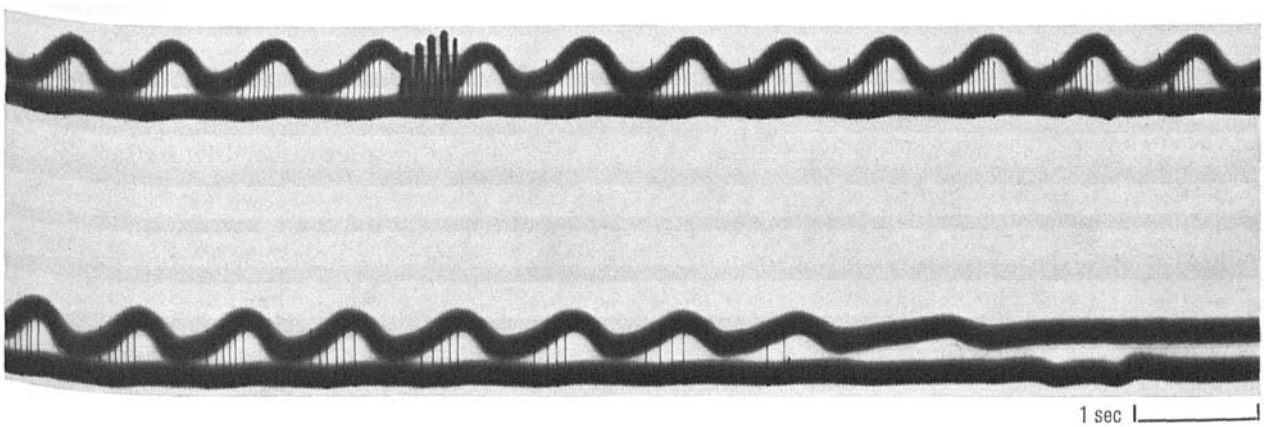

Fig. 1. Squirrel monkey. Continuous uninterrupted recording of respiration as temperature changes in the trachea (top tracing of each pair), with upward direction indicating inspiration, and the activity of an inspiratory unit (bottom). The oscillation in the top tracing indicates the time of i.v. injection of 3 μg/kg of etonitazene.

air flow through a flowmeter connected in series with the tracheal cannula, or as temperature changes recorded by a thermistor placed in the lumen of the trachea. The potentials were AC amplified and displayed on the screen of an oscilloscope simultaneously with the respiratory movements, and photographs were taken on moving film. At the end of the experiment the animals were sacrificed and the heads perfused with saline solution followed by 10% formalin. Frozen sections 50 μ thick were cut and stained with thionine. Every section was optically projected and the plane of section identified.

Results. All the units recorded in these experiments were found within the limits of the medulla oblongata. A typical experiment ran as follows. After a respiratory neuron was found, some time was allowed to elapse until the recording had stabilized. Then, as the activity displayed on the oscilloscope was being photographed, saline was administered i.v. to assure that injection would not disturb the recording of the respiratory unit. Soon afterward, a dose of etonitazene was given i.v. Whenever doses of 3 μg/kg in the monkey and 15–20 μg/kg in the dog, or higher, were used, respiratory paralysis occurred within 10–20 respiratory cycles after the injection. Doses of etonitazene lower than those indicated above produced either no effect on respiration or small changes in depth and frequency lasting only a few seconds to 1 min. In several experiments in which respiration was recorded spirometrically, the administration of etonitazene produced respiratory paralysis in mid-position, never in either inspiration or expiration.

In seven monkeys, a respiratory paralyzing dose of etonitazene (3 μg/kg) was administered i.v. while the activity of an inspiratory neuron was being continuously recorded. Inspiratory neurons were defined as units whose bursts of firing coincided with the inspiratory phase of the respiratory cycle, as recorded by the methods described above. In every case the inspiratory neuron under observation reacted to the drug by gradually decreasing the duration of its burst of firing and the number of spikes/burst until, within 10–20 respiratory cycles, the neuron stopped firing completely (Figure 1). In a few experiments, gallamine triethiodide was administered before etonitazene, and the respiratory movements stopped but the unit under study continued firing in bursts. The immediate administration of etonitazene produced the same results as without previous injection of gallamine triethiodide.

The effects of 20 μg/kg etonitazene on the activity of 5 inspiratory neurons in 5 dogs, were in all aspects similar to those obtained for the squirrel monkey. The effects of

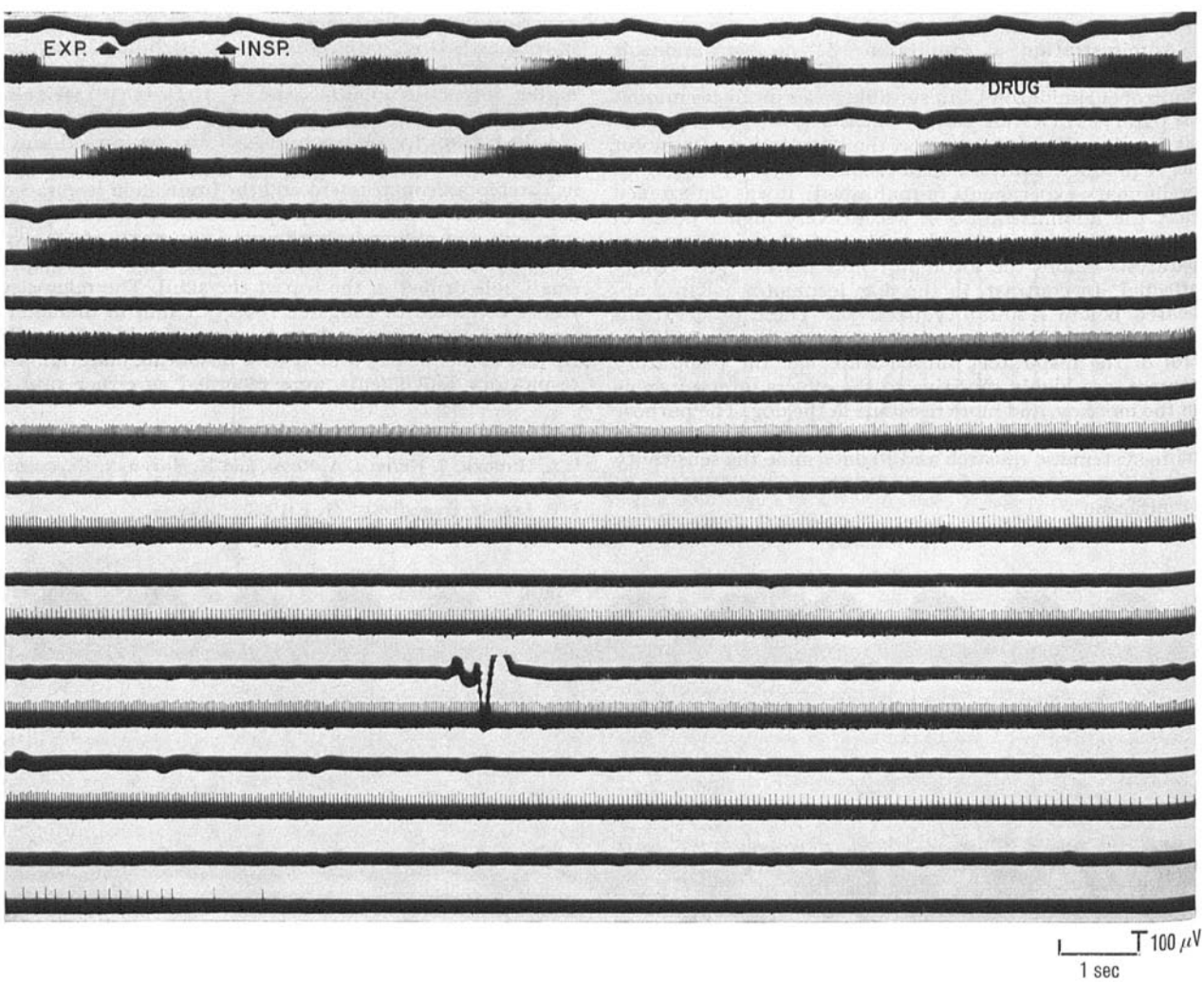

Fig. 2. Squirrel monkey. Continuous uninterrupted recording of respiration as rate of air flow (top tracing of each pair), and the activity of an expiratory unit (bottom). Drug line represents time and duration of i.v. injection of 3μg/kg of etonitazene.

a paralyzing dose of etonitazene on the activity of an expiratory neuron in 8 squirrel monkeys were also evaluated. Expiratory neurons were defined as units whose bursts of firing coincided with the expiratory phase of the respiratory cycle. The results were identical in every case. The duration of the bursts and the number of spikes/burst increased gradually, starting almost immediately after the injection. After 10–20 respiratory movements, respiratory paralysis occurred and the unit fired continuously at a very high rate. This high rate of firing was maintained for 1–2 min and then the frequency decreased gradually until, eventually, all activity disappeared (Figure 2). In a few experiments gallamine triethiodide was injected before etonitazene. In every case the results were the same, i.e. the gallamine triethiodide did not modify the effects of etonitazene.

In 2 dogs, a paralyzing dose of etonitazene (20 mg/kg) was injected while the activity of an expiratory neuron was being recorded. The results were similar to those described for the monkey. A number of respiratory units displayed discharge patterns that did not fall within the definitions of inspiratory or expiratory neurons used in this study. These units reacted to the administration of etonitazene in an unpredictable manner by either increasing or decreasing their rates of firing.

Discussion. The results indicate that, in both dog and squirrel monkey, etonitazene has a clear, definitive effect upon respiratory neurons whose bursts of firing coincide with either the inspiratory or the expiratory phase of the respiratory cycle. Etonitazene has, on the other hand, an unpredictable effect upon neurons that fire synchronously with respiration, but whose bursts of firing do not coincide with either phase of the cycle. The latter neurons are not recording artifacts because, following the injection of gallamine triethiodide or curare and sufficient locomotor and respiratory paralysis, the units continued their firing in bursts. In terms of their response to the administration of etonitazene, respiratory neurons could therefore be divided into 2 groups. Whether such dicotomy exists also from a functional point of view remains to be determined. It is interesting to note that, among the atypical neurons investigated, no obvious localization within any part of the medulla oblongata was observed, i.e. they were intermingled with the typical respiratory neurons.

The results also indicate that etonitazene has an excitatory effect upon neurons firing during the expiratory phase of the respiratory cycle, and an inhibitory effect upon neurons firing during the inspiratory phase of the cycle. It could not be determined, however, whether etonitazene acts directly upon the respiratory neurons or whether these effects are secondary. It is interesting to note that although etonitazene has a clear excitatory effect upon the expiratory component of the respiratory centers (or at least some of its individual cellular components), the respiratory arrest produced by the drug is not at expiration but at mid-position. We found no qualitative difference between the 2 species studied in regard to the response of individual respiratory neurons to the administration of etonitazene. Thus, the question of differential sensitivity of the 2 animal species to etonitazene remains unanswered.

The action of etonitazene upon respiratory neurons bears some similarities to that described for sodium pentobarbital by ROBSON et al.[3]. These investigators found that both inspiratory and expiratory neurons responded to the administration of sodium pentobarbital by either firing continuously or by total silence. However, we consistently found that inspiratory neurons stopped firing and that expiratory neurons fired continuously in response to the administration of etonitazene. Therefore, a different mechanism of action must be postulated. Tentatively, however, our results do not seem to be in disagreement with the concept that respiratory periodicity depends on the relative activity of the 2 mutually inhibitory networks[4], a notion supported by the results of ROBSON et al.[3,6].

Resumen. En este estudio se analizan los efectos de la administración de etonitazene sobre la actividad de las neuronas respiratorias en el perro y el mono. Las neuronas inspiratorias responden con cese total de su actividad y las neuronas expiratorias responden con una descarga de actividad contínua hasta la muerte del animal.

E. J. A. CARREGAL[5] and BEATRIZ WILLIAMS

Stanford Research Institute, Menlo Park (California, USA), August 2, 1966.

[3] J. G. ROBSON, M. A. HOUSELEY, and O. H. SOLIS-QUIROGA, Ann. N.Y. Acad. Sci. *109*, 494 (1963).

[4] G. C. SALMOIRAGHI, Ann. N.Y. Acad. Sci. *109*, 571 (1963).

[5] Present address: University of Southern California, School of Medicine, Los Angeles (California, USA).

[6] We thank Dr. LUCY BIRZIS of Stanford Research Institute, and Dr. J. HANCE of Stanford University, School of Medicine, for their help during the preliminary experiments and for reading the manuscript.

Tissue Toxicity of Radiologic Contrast Media Evaluated with *Tetrahymena pyriformis*

The use of certain protozoans in toxicologic tests has been developed to an appreciable extent only in recent years (HUTNER[1]). Recently the ciliated protozoan, *Tetrahymena pyriformis*, has been utilized for the evaluation of the tissue toxicity of radiologic contrast media (MARK et al.[2]). In these tests the % of individual protozoans immobilized turned out to be proportional to the concentration of contrast medium in the suspension fluid, and parallel to the damaging effects of the same media in the vascular endothelium of superior animals.

Following the development and experimentation (FELDER et al.[3] and BONATI et al.[4]) of a new contrast medium for use in urography (Iodamide), characterized by a very high tissue tolerance, we found it interesting to investigate the effect of this medium on *T. pyriformis* as compared to the other media already studied in this respect.

Method. We used *T. pyriformis* var. 1 type II[5] cultivated in a medium containing 1% proteose peptone

[1] S. H. HUTNER, Protozoology *11*, 1 (1964).

[2] M. F. MARK, A. M. IMPARATO, S. H. HUTNER, and H. BAKER, Angiology *14*, 383 (1963).

[3] E. FELDER, D. PITRÉ, and L. FUMAGALLI, Helv. chim. Acta *98*, 259 (1965).

[4] F. BONATI, G. F. ROSATI, and M. G. POLETTO, Arzneimittel-Forsch. *15*, 222 (1965).

[5] The strain of *T. pyriformis* was received from S. H. HUTNER of Haskins Laboratories, New York, N.Y., to whom our thanks are hereby extended.