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.

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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).

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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.

(Difco); the medium was sterilized at 110 °C for 20 min, then 1% glucose sterilized by filtration was added; the final pH was 7.0.

The protozoans were grown in test tubes containing 20 ml of medium, each tube being inoculated with 1.0 ml of culture and then incubated at 27 °C for 48 h. The cultures were centrifuged at 3000 rpm for 5 min, then washed twice with 0.42% sodium chloride solution at pH 7; the transmittance was adjusted to an extinction of 0.22 at 525 nm in test tubes with a diameter of 12 mm (spectrophotometer: Spectronic 20, Bausch & Lomb). The cells thus prepared were allowed to rest at room temperature for 2 h and so resume their original motility.

Then 0.5 ml portions of the protozoan suspension were mixed with 1.5 ml portions of various dilutions of the contrast media prepared to correspond to final concentrations of 12.5, 10.0, 8.0, 6.4, 5.1, and 4.0% of iodinized acid (respectively acetrizoic, diatrizoic, iothalamic, diiodopyridone-N-acetic acid and iodamide) salified with NaOH or methylglucamine.

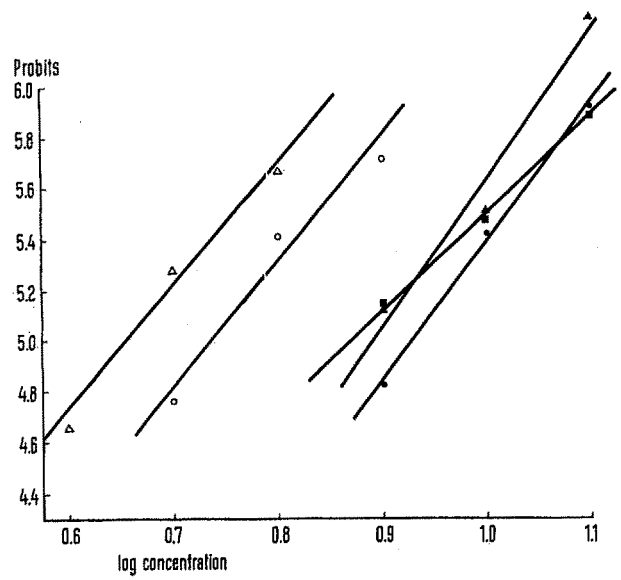
After 1 h at room temperature (approximately 20 °C) we counted the mobile and immobilized protozoans in a Thoma-Zeiss counting cell. With the results of this count, as obtained with each of the compounds tested, we calculated the regression straight line representing the concentration/immobilizing effect (dose response) by the use of probits (see Figure) and the median immobilizing concentration (IC_{50}) with the respective limits of confidence ($P = 0.05$). The values corresponding to the various compounds tested are shown in the Table.

Results. Our results are comparable with those obtained by MARK et al.[2], with due allowance for the fact that they expressed the concentrations of the solutions as salts, whereas in our tests we preferred to express them as acid, also in order to facilitate comparison between salts with different bases.

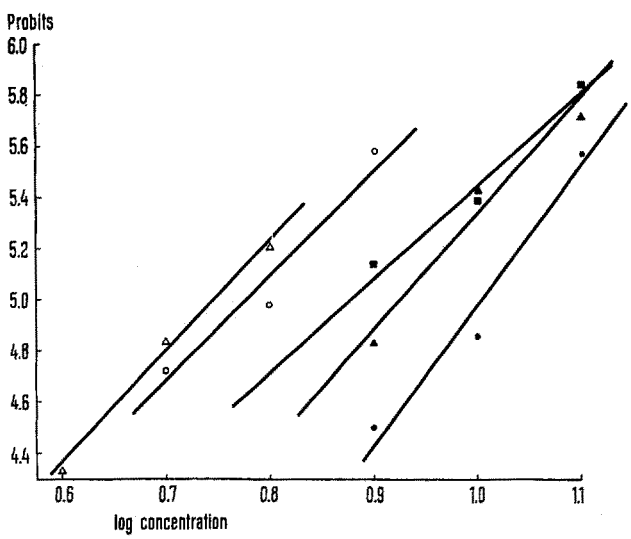
Once again acetrizoate, both sodium and methylglucamine, turned out to be the most toxic of the compounds used in these tests; diiodopyridone acetate was the next most toxic. Diatrizoate, iothalamate, and iodamide were better tolerated as a group; among these, however, iodamide was the best tolerated of all.

A comparison of IC_{50}s evinced a statistically significant difference ($P < 0.05$) between the IC_{50} of iodamide as a methylglucamine salt and that of all other compounds salified with the same base; the sodium salt of iodamide was invariably tolerated best, but the differences between it and sodium iothalamate and sodium diatrizoate were not significant by the criteria specified above. Conversely, the differences observed relative to the sodium salts of diiodopyridone and acetrizoic acid were statistically significant.

We also observed that the methylglucamine salts were slightly but consistently better tolerated than their sodium counterparts by Tetrahymena, and hence, if we accept the tempting hypothesis of MARK and his associates, by animal tissues. This held true for all the compounds used in our tests, with the exception of diatrizoic acid, in which case the methylglucamine and sodium salts were tolerated equally.



(A) Sodium salts



(B) Methylglucamine salts

Abscissae: logarithm of percentile content (w/v) as iodinized acid. Ordinates: % of organisms immobilized (probits). ● iodamide, ▲ iothalamate, ■ diatrizoate, △ acetrizoate, o diiodopyridone

	IC_{50}[a] ($P = 0.05$)	
	Methylglucamine salt	Sodium salt
Iodamide	10.05 (9.51–10.60)	8.56 (8.22–8.90)
Iothalamate	8.44 (7.79– 8.92)	7.79 (7.62–7.97)
Diatrizoate	7.50 (6.52– 8.62)	7.52 (6.64–8.52)
Diiodopyridone	5.98 (5.59– 6.38)	5.49 (5.12–5.87)
Acetrizoate	5.68 (5.24– 6.16)	4.61 (4.36–4.86)

[a] In % w/v, expressed as iodinized acids.

Zusammenfassung. Zur Auswertung der Gewebetoxizität der Kontrastmittel wurde der Immobilisierungstest der *Tetrahymena pyriformis* angewandt. Geprüft wurden: Acetrizoe-, Diatrizoe- Jotalamsäure, Dijodopyridon-N-essigsäure und Jodamid als Natrium- sowie als Methylglucaminsalz.

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