support these findings since the ability of clonidine to block the pressor response to arecholine at the highest dose is most likely due to blockade of ganglionic transmission. Clonidine, by acting at α-adrenergic receptors located on peripheral cholinergic nerve terminals can block the release of ACh[8,9]. Alternatively, clonidine could also block ganglionic transmission by occupying pre-ganglionic nicotinic receptors. The lack of effect of clonidine on the pressor response to DMPP, however, rules out this possibility. Also, the fact that the 10 µg/kg dose of clonidine blocked the pressor response to physostigmine but not that to arecholine indicates that central cholinergic neurons are more susceptible to the inhibitory effects of clonidine than are peripheral cholinergic neurons. This difference may be related to the actual concentration of clonidine present at central or peripheral sites following injection i.v. of the drug and/or the density of α-adrenergic receptors located on the nerve terminals of these cholinergic neurons.

The induction of hypertension by a central cholinergic mechanism offers another animal model to evaluate the mechanism of action of many antihypertensive drugs, and also provides another approach to the understanding of human essential hypertension.

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The role of hyperoxygenation in facilitating the induction of pulmonary histiocytosis by low doses of chlorphentermine[1]

S. Kacew[2] and R. Narbaitz[3]

Departments of Pharmacology and Anatomy, Faculty of Health Sciences, School of Medicine, University of Ottawa, Ottawa (Ontario, Canada KIN 9A9), 14 September 1979

Summary. Hyperoxygenation modified the susceptibility of neonatal rat lung to respond to chlorphentermine with an accumulation of hypertrophic macrophages, a morphologic change undetected when an anorectic drug was given alone. Implications of this observation for pediatrics are discussed.

In adult rats, treatment with 20 mg/kg chlorphentermine for 8 days produced an accumulation of masses of foam cells (hypertrophic, phospholipid-rich histiocytes) in the alveoli and bronchi of the lungs[4]. In contrast, Kacew et al.[5] found that the daily administration of 20 mg/kg chlorphentermine for 1 week failed to produce any apparent morphologic alteration in neonatal rat lung. However, an increase in anorectic drug dose to 40 or 60 mg/kg resulted in an accumulation of hypertrophic macrophages in newborn pulmonary alveoli, suggesting that neonates may be less responsive to chlorphentermine than adults. In view of the fact that neonates are also less susceptible to the toxic actions of high concentrations of oxygen[6] and oxygen is known to modify drug-induced lung changes[7] it was of interest to examine the interaction between chlorphentermine and hyperoxygenation on pulmonary morphology.

Methods. Female rats of the Sprague-Dawley strain with 1 day-old litters were used. Chlorphentermine (20 mg/kg) was administered daily by gastric intubation. Corresponding controls received an equal volume (50 µl) of physiological saline. For hyperoxia experiments, animals were maintained for 3 days in a spherical airtight plexiglass chamber filled with 95% O_2 at a rate of 3 l/min and gas was monitored with a Beckman model OM-11 gas analyzer. In sequential experiments, pups which had previously received 20 mg/kg/day chlorphentermine for 6 days were subsequently exposed to 95% O_2 for 72 h, or newborns which were initially exposed to 95% O_2 for 3 days were given the drug for 6 days. In addition, groups of newborns were simultaneously exposed to 95% O_2 and given chlorphentermine for either 1, 2, 3 or 5 days. From each animal, portions of one lung were fixed and processed for electron microscopy as described previously[8], and the contralateral lung was fixed intact in Bouin's fixative and processed for light microscopy. Although few alveolar macrophages were found both in control and experimental animals, only the hypertrophic, vacuolized macrophages ('foam cells') were counted. Quantitation of foam cells (FC) in alveoli was conducted using an arbitrary scale as follows: (+ + +), 1-4 FC in many peripheral alveoli of all experimental animals; (+ +), 1-2 FC in few peripheral alveoli of all experimental animals; (+), 1-2 FC in few peripheral alveoli of at least half the experimental animals; (−), no FC observed.

Results. The lung and body weights of control rats used in this study were approximately 200 mg and 7 g, respectively. In all treated groups lung and body weights did not differ significantly from those of the respective controls throughout the experimental period. In cases where animals failed

Effect of hyperoxygenation and chlorphentermine on pulmonary foam cell reaction in newborn rats

Series number	Number of animals	Treatment schedule	Foam cell quantitation*
1	4	Control	−
2	4	O_2 alone for 3 days	−
3	10	Drug alone for 6 days	−
4	8	O_2 for 3 days followed by drug for 6 days	+ + +
5	8	Drug for 6 days followed by O_2 for 3 days	+
6	4	Simultaneous drug and O_2 for 1 day	+
7	4	Simultaneous drug and O_2 for 2 days	+ +
8	4	Simultaneous drug and O_2 for 3 days	+ + +
9	4	Simultaneous drug and O_2 for 5 days	+ +

* See materials and methods.

to grow, the neonates were discarded. It is of interest that Yam et al.[12] also found no significant difference in lung to body weight ratio between control and 5-day hyperoxia exposed rats. The 1st series of experiments (series 3, table) confirmed previous observations[5] in the sense that no alveolar foam cells appeared in the neonatal rat after the administration of 20 mg/kg chlorphentermine. The combined treatments of hyperoxygenation and chlorphentermine (series 4–9) however, always produced histiocytosis. In all cases, the foam cells were mainly restricted to peripheral, sub-pleural alveoli (figure). Ultrastructural characteristics were identical to those described previously[5]. The number of foam cells was greater when hyperoxygenation preceded drug administration (series 4) than when chlorphentermine followed O_2 (series 5). When both agents were administered concurrently, the number of foam cells reached its peak on the 3rd day (series 8). The electron microscopical observation of the alveolar walls demonstrated the presence of intense interstitial edema after 3 days of hyperoxygenation (figure 2). The intensity of this edema appeared to be similar whether hyperoxygenation was preceded or not by chlorphentermine treatment. In the cases in which chlorphentermine treatment followed hyperoxygenation, interstitial edema was less notable. No characteristic cytoplasmic alterations were found in pneumocytes type 1 or 2 following hyperoxygenation, although the continuity of the alveolar epithelium was interrupted in a few places probably as a result of the interstitial edema.

Discussion. The present results indicate that a short treatment using a 95% O_2 atmosphere can facilitate the production of pulmonary histiocytosis by a dose of chlorphentermine which would not otherwise produce this morphologic change. The mechanisms by which hyperoxia facilitates these effects are not clear. The most notable effect of hyperoxygenation on the lungs of neonatal mice is known to be the production of lesions in vascular endothelium and alveolar epithelium resulting in interstitial edema and fluid accumulation in the alveolar lumen[6,9]. Our present experiments show that interstitial edema is also a characteristic response of neonatal rats submitted to hyperoxygenation.

Alveolar histiocytes are believed to originate from blood monocytes after a short sojourn in the interstitial tissue of the lung[10]. If this is accepted, then it can be suggested that in our combined experiments the increased permeability produced by hyperoxia would facilitate the migration of histiocytes towards the alveolar lumen. It could be argued, of course, that the concomitant flooding of the lumen could also produce the reverse effect of reducing the number of alveolar histiocytes by hastening their elimination through the bronchial pathways. The number of alveolar histiocytes present can be considered to result from the balance between the rate of migration towards the alveolus and the rate of elimination through bronchi. A 20 mg/kg chlorphentermine dose may produce few hypertrophic histiocytes; however, these were not detected[5] due to a rapid

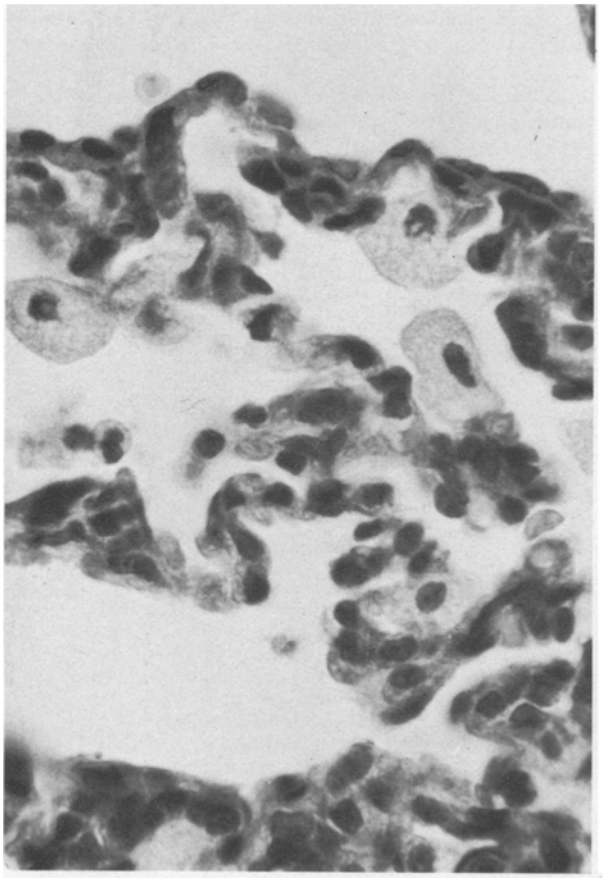

Fig. 1. Section of the lung from a rat treated simultaneously with chlorphentermine and hyperoxygenation for 3 days. Note the presence of 'foam cells' in the lumen of sub-pleural alveoli. H. and E. × 875.

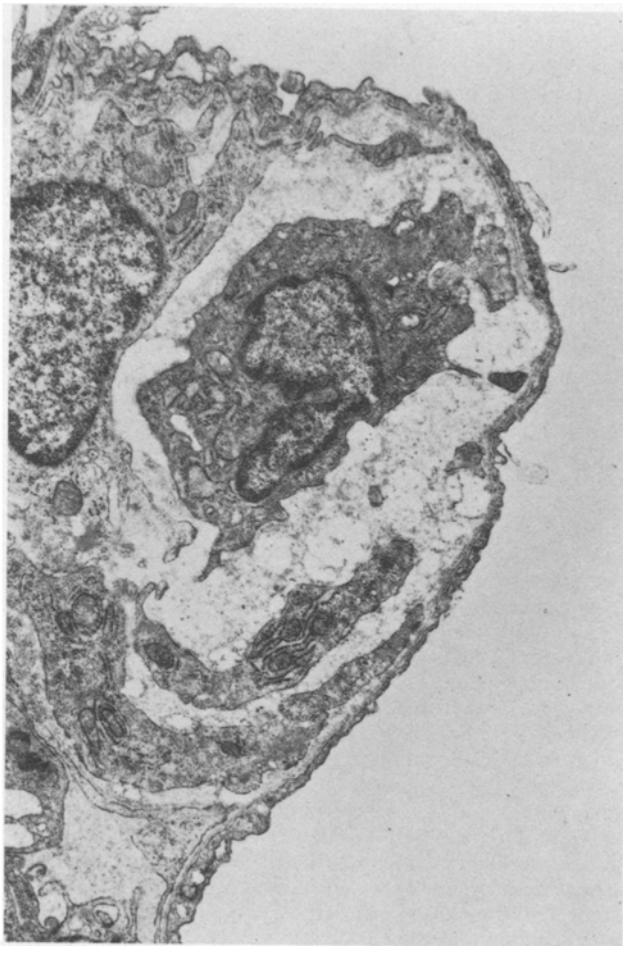

Fig. 2. Electron-micrograph of a lung from a neonatal rat treated for 3 days with hyperoxygenation. Note interstitial edema and the normal characteristics of cytoplasm of pneumocyte I. × 11,150.

elimination. Hyperoxygenation, by altering the balance between production and elimination rates, may permit the accumulation of hypertrophic histiocytes in some of the alveoli, enabling their detection. The fact that in our experiments most of the hypertrophic histiocytes are found in peripherally located alveoli, which probably evacuate their contents with more difficulty, adds substance to this interpretation. It must be admitted, however, that alternative interpretations are possible. Thus the facilitating action of hyperoxygenation could be related to completely different mechanisms, such as an interference with liver metabolism[11] resulting in diminished capacity to catabolize the drug. Although the mechanism responsible for the observed pulmonary histiocytosis is still unknown, the fact that it does exist is of great interest to investigators of neonatal physiology, since successive or concurrent hyperoxygenation and treatment with amphiphilic drugs are possible occurrences in pediatric practice.

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2 To whom reprint requests should be sent.
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Isoelectric focusing of mosquito esterases in the presence of Triton X-100[1]

E.J. Houk and J.L. Hardy

Naval Biosciences Laboratory, University of California, School of Public Health, Oakland (CA 94625, USA), 20 August 1979

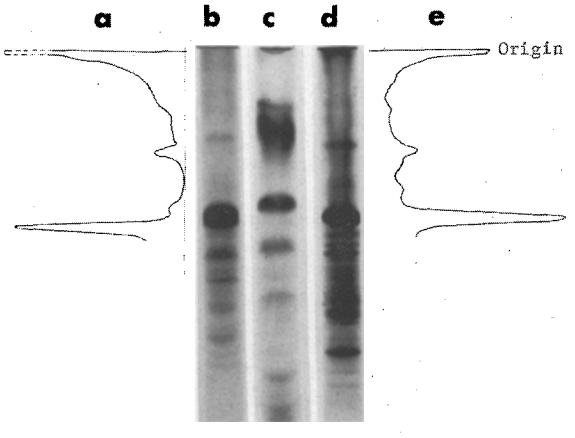
Summary. Triton X-100 improves nonspecific esterase solubilization from mosquito samples and also leads to increased resolution in an isoelectric focusing electrophoresis.

Houk et al.[2,3] observed that most electrophoretic studies of insect nonspecific esterases had dealt with H_2O and buffer soluble species. A single investigator had ventured to add a mild detergent, sodium deoxycholate, to aid in releasing membrane bound esterolytic material[4]. Deoxycholate does solubilize a greater amount of esterolytic activity into the 10,000 × g - 20 min supernatant as measured spectrophotometrically[5]. Subsequent electrophoresis revealed that new isozymic species were detected and the relative activities of some previously detected species were enhanced. However, substantial esterolytic activity remained at the origin and did not enter into the isoelectric focusing system[3]. In an attempt to release more nonspecific esterolytic material into the supernatant fraction, Triton X-100 was adopted as the solubilizing agent[6]. This report substantiates the increased solubilization and improved resolution of mosquito nonspecific esterase isozymes in a Triton X-100-isoelectric focused system.

Female mosquitoes, *Culex tarsalis,* origin and maintenance described elsewhere[2,3], were ground in Tris buffer (0.2 M; pH 6.7) containing either 0.05% (w/v) sodium deoxycholate or 0.5% (v/v) Triton X-100. The suspension was centrifuged at 10,000 × g - 20 min and the supernatant subjected to isoelectric focusing electrophoresis for either 24 h at a constant 200 V^2 or at constant power, 1/3 watt gel^{-1}, for 16 h. The Triton X-100 solubilization system was examined with and without additional Triton X-100 (0.1%; v/v) incorporated into the acrylamide gel matrix. The preparation of the acrylamide gels and subsequent histochemical detection of nonspecific esterases has been described[2,3].

A comparison of deoxycholate and Triton C-100 nonspecific esterase zymograms reveals an increase in the resolution and number of detectable isozymes in the mosquito, *C. tarsalis* (figure, b and d). In the deoxycholate system, substantial unsolubilized esterolytic activity at the origin is represented by a truncated peak (figure, a) whose actual height is considerably off scale. The Triton X-100 system (figure, e) is a complete representation of the apparent esterolytic activity at the origin, with a major extraneous optical contribution from the air-acrylamide gel interface.

Triton X-100 is requisite both in the sample and within the gel matrix for optimal results[6] (figure, c and d). The absence of Triton X-100 from the gel matrix leads to large scale aggregation of esterolytic material in the more alkaline regions (pH = 6.5) of the gel (figure, c).



Influence of solubilization medium and acrylamide gel composition on densitometric tracings and electropherograms of mosquito nonspecific esterases: *a* Deoxycholate solubilized samples with substantial residual activity at the origin, *b* deoxycholate solubilized electropherogram, *c* Triton X-100 electropherogram without Triton X-100 incorporated into the gel matrix, *d* electropherogram of Triton X-100 solubilized samples with Triton X-100 included in the gel and *e* Triton X-100 solubilized samples with a complete representation of residual activity at the origin.