4. Gregersen, M. I., Am. J. Physiol., 1932, v102, 344.
5. Stricker, E. M., ibid., 1966, v211, 232.
6. Stricker, E. M., Wolf, G., Physiol. Behav., 1967, v2, 33.
7. Epstein, A. N., Spector, D., Samman, A., Goldblum, C., Nature, 1964, v201, 1342.
8. Vance, W. B., Psychol. Monogr., 1965, v79, Whole No. 598.
9. Hainsworth, F. R., Epstein, A. N., Science, 1966, v153, 1255.
10. Andersson, B., McCann, S. M., Acta Physiol. Scand., 1956, v35, 312.
11. Stevenson, J. A. F., Welt, L. G., Orloff, J., Am. J. Physiol., 1950, v161, 35.

Received November 9, 1966. P.S.E.B.M., 1967, v124.

Anesthesia LXXI. Pharmacologic and Physicochemical Comparison of Flurothyl and Its Isomer.* (31861)

JOHN C. KRANTZ, JR., FRIEDA G. RUDO, AND CLAIRE K. LOECHER
Huntingdon Research Center and Department of Pharmacology, School of Medicine, University of Maryland, Baltimore, Md.

In our studies with fluorinated ethers the anesthetic, fluroxene(1) ($CF_3 \cdot CH_2\text{-}O\text{-}CH{:}CH_2$) and the convulsive flurothyl ($CF_3 \cdot CH_2\text{-}O\text{-}CH_2 \cdot CF_3$)(2) were developed and later used clinically. Recently we obtained the isomer of flurothyl, hexafluoroisopropyl methyl ether (ISO) $\begin{matrix}CF_3\\CF_3\end{matrix}\!>\!CH\text{-}O\text{-}CH_3$ and observed it to possess anethetic activity comparable to ether in several species of animals.

We considered the two compounds ideally suited for detection of a physicochemical clue that might shed light on their respective activities. Pharmacologically, the responses evoked by these isomers are at the opposite ends of the spectrum of normal central system activity, namely, anesthesia and convulsions.

Materials and methods. The flurothyl was supplied by the Ohio Chemical and Surgical Equipment Co., Madison, Wisc. The isomer was supplied by Dr. Louise Speers, Air Reduction Co., Murray Hill, N. J. ISO is a clear, colorless liquid with an ethereal odor; B.P. 50° and Sp.G. 1.36.

The antagonism of the two isomers was studied in mice by the inhalation method of Cascorbi and Rudo(3).

The physicochemical methods were the well known standard procedures.

* Supported by a Grant from Life Sciences Division, Army Research Office.

TABLE I. Effect of ISO on Convulsive Properties of Flurothyl in Mice.*

Vol % ISO†	Vol % Flurothyl	No. convulsions	Time of onset of convulsions (sec)	No. dead
0	.25	8	30	1
.63	.25	1	300	0
1.25	.25	0	—	0
0	.50	10	5	9
.63	.50	9	40	0
1.25	.50	2	45	0
2.50	.50	0	—	0

* 10 mice in each group exposed for 10 min.
† Anesthetic concentration is 5.0 vol %.

Results. Table I shows that the addition of ISO in subanesthetic doses protects mice from flurothyl convulsions at 0.25 vol % and enables them to survive the lethal dose of 0.50 vol %. Further, it was observed that mice exposed to a mixture of 1.25 vol % ISO and 0.25 vol % flurothyl for 10 minutes did not exhibit any behavior characteristics that could be differentiated from normal mice (balanced state). The antagonism of the two isomers as shown in mice prevailed in rabbits, rats, and monkeys also.

An examination of the physical measurements in Table II reveals a difference in the dipole moments of ISO and flurothyl which are considered to have relevance in the potency of anesthetics. These do not appear to be capable of significant interpretation in view of the following data taken from the literature(4). For example, the dipole moments of other compounds are: $CHCl_3$ (anes-

TABLE II. Physical Measurements.

	Flurothyl	ISO
Liquid molar vol 37° ml/mole	1.32	1.37
ΔH_v 37° k cal/mole	6.75	6.49
Dipole moment	2.4	2.9
Viscosity	.66	.51 @ 25° ($H_2O = 1$)
Surface tension	19.2 dynes/cm @ 26°	18.5 dynes/cm @ 27.5°
Interfacial tension	36.5 " @ 27.5°	37.0 " @ "
Surface tension of saturated aqueous sol	72 " @ 25°	72 " @ 25°
Human plasma* saturated with the isomers	53 " @ 23°	49 " @ 23°
Solubility in water	.2 vol %	.08 to .1 vol %
Oil/water coefficient	66 ± 5	200 ± 5

* Surface tension of human plasma is 53 dynes/cm @ 23°.

thetic) 1.02; CHF_3 (inert) 1.60; CCl_4 (anesthetic) 0; and $(C_2H_5)_2O$ (anesthetic) 1.15.

The solubilities of the isomers in water and their oil/water coefficients are quite dissimilar. Although ISO enjoys a much higher oil/water coefficient than does flurothyl, this, like the differences in dipole moments, cannot be considered as a significant difference between the convulsant and the anesthetic. Many reasonably potent anesthetics have a low oil/water coefficient; that of ether lies between 4 and 6. In former experiments we showed that anesthetic potency and oil/water coefficients varied directly, and, furthermore, that anesthetic potency varied directly with the reciprocal of the solubility of the agent in water(5) (Richet's rule)(6). This relationship was established among the non-halogenated ethers, known to be anesthetics. It appears that fluorination vitiates this relationship.

Surface phenomena of flurothyl. In our studies of the water solubility of flurothyl, we observed that the ether exhibited surfactant properties which were not shared by ISO. We pursued the study of the foaming characteristic of flurothyl with water in an effort to determine if this striking physical property was in any way associated with the pharmacologic properties of the ether.

The foam that adhered so tenaciously to the sides of the glass cylinder was enmeshed air bubbles, since the foam could not be produced in a vacuum. The foam did not form in plastic containers. It, therefore, appeared to be associated with the adhesive force of flurothyl with the surface of glass, a property not enjoyed by ISO. The contact angles with glass were measured. As each isomer wetted glass, the contact angle of each was zero.

Surfactant properties and pharmacologic action. Considering that the foaming activity of flurothyl might be associated with its convulsive pharmacologic response, the foaming capacity was destroyed by adding Silain. Silain (simethicone) is a silicon derivative used in gastroenterology to release gas from bubbles in whch it is entrapped.

A saturated solution of flurothyl (0.2% V/V—0.4 ml) was injected intravenously into 5 mice. Marked convulsive seizures occurred in each animal. A saturated solution of Silain at the same dosage level evoked no response. The flurothyl solution was saturated with Silain which lowered its surface tension and destroyed its surfactant activity. Six mice were injected with this solution at the previous dosage level. All animals convulsed. Therefore, when this unusual foaming property of flurothyl was destroyed the convulsive effect evoked in the mouse persisted.

Not all fluorinated ethers, which evoked convulsions in mice, displayed this surfactant property peculiar to fluorthyl. Further, not any of the fluorinated or non-fluorinated anesthetics available elicited this surfactant phenomenon.

Discussion and summary. The pharmacologic antagonism between the isomers is striking. It appears that each isomer is competing for its respective receptor site, namely, the convulsive or anesthetic site. In the "balanced state" the action of one is nullified by the other, and an apparently normal animal results.

Not any of the physicochemical properties or measurements conducted on the isomers,

flurothyl and ISO, revealed a marked difference which might be considered responsible for their respective pharmacologic responses. The surfactant phenomenon was not a constant finding with all fluorinated, convulsive ethers. We were unable to establish any relationship between this physical property and pharmacologic response; however, it was sufficiently unusual and interesting to warrant exploration.

1. Krantz, J. C., Jr., Carr, C. J., Lu, G. G., Bell, F. K., J. Pharmacol. Exp. Therap., 1953, v108, 488.

2. Krantz, J. C., Jr., Truitt, E. B., Speers, L., Ling, A. S. C., Science, 1957, v126, 353.

3. Cascorbi, H. F., Rudo, F. G., Anes. & Analg., 1964, v43, 163.

4. Weast, R. C., Handbook of Chemistry and Physics, 46th Ed. Chemical Rubber Co., Cleveland, 1965.

5. Krantz, J. C., Jr., Carr, C. J., Iwamoto, H. K., Sauerwald, M. J., Anesthesiology, 1947, v8, 497.

6. Richet, C., Compt. rend. Soc. de biol., 1893, v45, 775.

Received November 16, 1966. P.S.E.B.M., 1967, v124.

A Vascular Permeability Defect in Experimental Cholera. (31862)

GERALD T. KEUSCH,* PONGSOM ATTHASAMPUNNA, AND RICHARD A. FINKELSTEIN
Department of Clinical Research and Department of Bacteriology and Mycology, USA Medical Component, SEATO Medical Research Laboratory, Rajavithi Road, Bangkok, Thailand†

The nature of the pathophysiologic lesion in cholera has remained undefined following the demonstration by Gangarosa *et al*(1) that the intestinal epithelial lining remained intact in the human disease. The introduction of an experimental model (the suckling rabbit) faithfully reproducing almost all facets of cholera in man(2,3), and the discovery of a cell-free vibrio product, choleragen, which causes cholera in this animal model(4) as well as in the human(5) have allowed laboratory investigation of the two major hypotheses of pathogenesis: *1*) inhibition of sodium pump activity(6), and *2*) increased vascular permeability(7).

Choleragen has been shown to have no significant effect upon ion transport systems in the short circuited frog skin (Neptune, E. M., personal communication), or the everted rabbit ileal loop (Field, M., Schultz, S. G., personal communication). Intact sodium pump activity has recently been demonstrated in adjacent control and choleragen treated ileal loops of adult rabbits (Norris, H. T., Schultz, S. G., Curran, P. F., Finkelstein, R. A., submitted) showing that enterosorption of fluid in the choleraic loop proceeds without inhibition of pump activity. In contrast, choleragen is an exceedingly potent permeability factor, causing a "delayed-prolonged" response in rabbit skin when injected in sub-microgram amounts(8). The present communication adds to the evidence that experimental cholera involves an alteration of vascular permeability by the demonstration of such an effect in the intestinal villi of infant rabbits made choleraic with choleragen.

Methods.‡ The technique of "vascular labelling"(9) was employed. This involves intravenous injection of a visible colloidal marker, for example India ink, which is trapped by the leaking vessel, thereby identifying it. In the present study labelling with carbon black could not be demonstrated. Instead a commercial iron-dextran complex (Imferon, Lakeside Laboratories) was successfully utilized. This material contains iron coupled to dextran and appears to be of high molecular weight, exceeding 150,000 and approximately 3-4 mμ in size(10). Purified choleragen was prepared and fed *via* stomach tube to infant rabbits pretreated by gastric lavage as previously described(7). At intervals following administration of 50 μg of choleragen, a single dose of iron-dextran was injected intravenously in a volume of 0.1 cc through a

* Associate, International Research Career Development Program, USPHS, Nat. Inst. Health. Current address: New England Center Hospital, Boston, Mass.

† U.S. Address: USA Medical Component, SEATO APO San Francisco, 96346.

‡The principles of laboratory animal care as promulgated by Nat. Soc. for Med. Res. were observed.