

Application of Low-temperature Autoradiography to Studies of the Uptake and Metabolism of Volatile Anesthetics in the Mouse:

II. Diethyl Ether

Ellis N. Cohen, M.D.,* and Nancy Hood, B.S.†

The uptake and metabolism of ^{14}C -diethyl ether in the mouse were studied with low-temperature whole-body autoradiography. Precise concentrations of anesthetic within body tissues were established by impulse counting of biopsy specimens. Following administration of the anesthetic, increased relative concentrations of radioactivity developed in the liver and kidney, reflecting the accumulation of nonvolatile metabolites. At two hours the measured nonvolatile metabolites accounted for 3.6 per cent of the administered radioactivity. Thin-layer radiochromatography of an extract from the liver established the presence of four nonvolatile metabolites. Enzymatic treatment of these materials with β -glucuronidase suggests the major metabolite to be a glucuronide of ether.

DIETHYL ETHER has enjoyed wide clinical acceptance as an inhalation anesthetic since its introduction in 1846. Nonetheless, there remain a number of significant gaps in our knowledge of this agent. Relatively little information about the elimination or metabolism of diethyl ether is available, and only a limited number of *in-vivo* measurements define anesthetic uptake within the body. Blood/tissue partition coefficients have been determined for a few tissues: blood/gas = 12.0, brain/blood = 1.14, and fat/blood = 5.0, the latter value representing an equilibration with olive oil.¹

Until recently, diethyl ether was considered to be eliminated unchanged from the body, but studies by Van Dyke *et al.*² have provided direct evidence for its metabolism. In these investigations, ^{14}C -diethyl ether was injected intraperitoneally in rats, and approximately 4 per cent of the administered radioactivity was recovered as exhaled $^{14}\text{CO}_2$. During the same 24-hour period, an additional 2 per cent of radioactivity accumulated in the form of nonvolatile urinary metabolite(s).

A major difficulty limiting experimental study with diethyl ether relates to its extreme volatility (bp = 34.6 C), which creates significant problems in its quantitative analysis. Recent developments in the technique of low-temperature autoradiography afford a new approach to the *in-vivo* study of highly volatile anesthetic agents.³ The present investigation concerns the uptake and metabolism of ^{14}C -diethyl ether, combining low-temperature autoradiography and radioisotope analytic methods.

Procedure

$1\text{-}^{14}\text{C}$ -diethyl ether of high specific activity (1.7 mC/mM) was obtained from a commercial source.[§] Purity of the material was established by radio gas chromatography. The labeled anesthetic was diluted threefold with nonradioactive diethyl ether, and 35 μC of drug (equivalent to 8 per cent ether vol/vol oxygen) administered by inhalation for ten minutes to each of six N.M.R.I. white mice, averaging 20 gm in weight. Following inhalation of the anesthetic, the animals were trans-

* Professor of Anesthesia.

† Senior Research Assistant.

Received from the Department of Anesthesia, Stanford University Medical School, Stanford, California, and the Departments of Pharmacology and Medical Chemistry, Royal Veterinary College, Stockholm, Sweden. Accepted for publication March 17, 1969. Supported by National Institutes of Health Program Project Grant GM 12527.

§ New England Nuclear Corporation, Boston, Massachusetts.

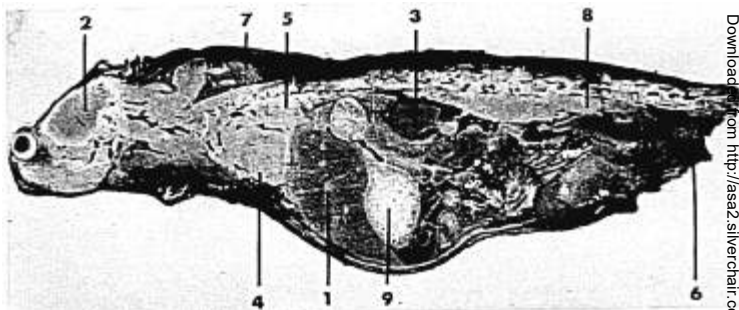


FIG. 1. Autoradiograph prepared from hemisection of mouse sacrificed immediately following ten-minute inhalation of ^{14}C -diethyl ether. 1, liver; 2, brain; 3, kidney; 4, heart; 5, lung; 6, fat; 7, brown fat; 8, muscle; 9, stomach.

ferred to room air and sacrificed at 0, 15, and 120 minutes by immersion in liquid nitrogen. The anesthetic inhalation chamber, technique employed for freezing and mounting the biologic specimens, collection of biopsy samples, and preparation of the autoradiograms, etc., have been described previously.³ Due to the extreme volatility of the diethyl ether (freezing point = -116 C), it proved necessary to store all biologic specimens and photographic films under liquid nitrogen during the period required for autoradiographic exposure (five days).

Following preparation of the whole-body autoradiograms from the frozen hemisections stored under liquid nitrogen, thin sections ($40\ \mu$) were cut from the same blocks and used as anatomic references and for studies of the nonvolatile metabolites. Presence of the latter was verified by first drying the thin sections at -15 C for 48 hours and then heating the sections to 60 C for one hour to remove all volatile radioactivity. Alternate frozen hemisections were used as a source of tissue biopsy material for quantitative analysis employing impulse-counting.

In other aspects of the study, a homogenate from the livers of the animals sacrificed at 120 minutes was prepared by grinding the tissue in dry ice. The nonvolatile radioactive materials were then extracted into ether for subsequent analysis by thin-layer and radio gas chromatography.

In an additional facet of the study, $25\ \mu\text{C}$ of ^{14}C -diethyl ether were injected into the tail veins of three male white mice. These animals were sacrificed 120 minutes after injection of the anesthetic, and the liver and entire intestinal tract were removed. After these tissues were weighed, biopsy specimens were obtained from the livers and from serial segments of intestine. These biopsy specimens were taken to dryness, treated, and counted in a similar fashion to the other biopsy materials.

Results

The autoradiographs in figures 1 and 2 demonstrate the uptake and elimination of ^{14}C -diethyl ether in the mouse. In figure 1, the animal was sacrificed immediately after a ten-minute inhalation of the anesthetic. The anesthetic appears to be rather uniformly distributed throughout the body, although the darkened areas indicate higher concentrations of radioactivity in the brain, kidney, liver, and brown fat. At 120 minutes (fig. 2), most of the radioactivity has left the body, but residual concentrations are found in the liver, kidney, intestines, and nasal mucous membranes.

Table 1 indicates the concentrations of radioactivity measured in tissue biopsies taken from the animals at various times of sacrifice. By assigning a relative value of 1.00 to the concentration of radioactivity present in the blood, we can compare this concentration with that found in each of the tissues. Following

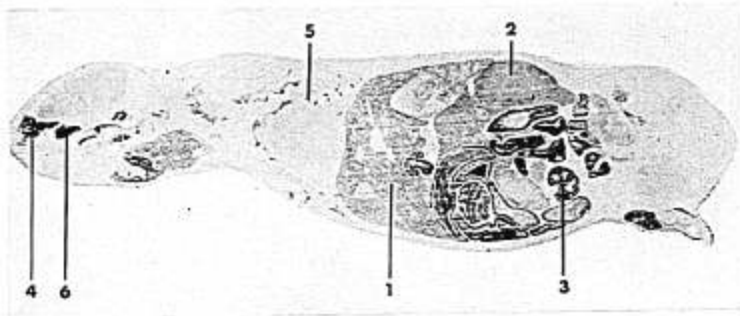


FIG. 2. Autoradiograph prepared from hemisection of mouse sacrificed 120 minutes following inhalation of ^{14}C -diethyl ether. 1, liver; 2, kidney; 3, intestine; 4, nasal mucous membrane; 5, bronchi; 6, Harder's gland.

ten minutes' inhalation of anesthesia, most body tissues approach a unit concentration with blood. However, concentrations of radioactivity in the brown fat, liver, kidney, and brain exceed unity, while that in general body fat remains low. By 15 minutes the radioactivity in brown fat has reached its peak relative concentration, and is threefold that in the blood. Relative concentrations of radioactivity in liver and kidney continue to increase: at the termination of the study (120 minutes), high residual concentrations of radioactivity were present in both liver and kidney. At this time, the liver contained approximately 13 times the amount of radioactivity present in the blood.

Table 2 compares the concentrations of volatile and nonvolatile radioactivity found in the liver. In mice sacrificed immediately after a ten-minute inhalation of anesthetic, the nonvolatile radioactivity represents 10.8 per cent of the total amount present. At 15 minutes, the relative concentration of nonvolatile radioactivity has increased to 49.2 per cent, and by 120 minutes essentially all radioactivity in the liver is nonvolatile.

The autoradiographs in figures 3 and 4 were prepared from thin whole-body sections ($40\ \mu$) from which all volatile radioactivity had been removed by evaporation and heating (see procedure). These autoradiographs provide visual

TABLE 1. Concentrations of Radioactivity (Diethyl Ether plus Metabolites) in Various Tissues*

Tissue	Total Radioactivity (counts/min/mg)			Tissue/Blood Ratio		
	0 Min	15 Min	120 Min	0 Min	15 Min	120 Min
Blood	421 \pm 41.9	169 \pm 24.2	24 \pm 4.3	1.00	1.00	1.00
Fat	239 \pm 25.1	159 \pm 27.5	38 \pm 4.9	0.57	0.94	1.58
Lung	405 \pm 33.8	185 \pm 30.1	47 \pm 8.3	0.96	1.09	1.96
Muscle	455 \pm 67.5	221 \pm 31.2	24 \pm 6.1	1.08	1.31	1.00
Brain	531 \pm 18.4	240 \pm 11.4	31 \pm 5.7	1.26	1.42	1.29
Brown fat	609 \pm 93.0	545 \pm 80.5	28 \pm 3.4	1.45	3.21	1.16
Kidney	615 \pm 33.2	251 \pm 18.1	72 \pm 11.0	1.46	1.48	3.00
Liver	741 \pm 42.4	315 \pm 36.4	312 \pm 29.7	1.76	1.87	12.90

* Mice sacrificed at 0, 15, and 120 minutes following ten-minute inhalation of ^{14}C -diethyl ether. Data represent duplicate determinations in two animals for each time sequence (\pm SE).

TABLE 2. Concentrations of Volatile and Nonvolatile Radioactivity in the Livers of Mice Sacrificed 0, 15, and 120 Minutes Following Ten-minute Inhalation of ^{14}C -diethyl Ether*

	0 Minutes	15 Minutes	120 Minutes
Total radioactivity†	741 \pm 42.4	315 \pm 36.4	312 \pm 29.7
Volatile radioactivity	661 \pm 37.6	160 \pm 19.6	0
Nonvolatile radioactivity	80 \pm 9.8	155 \pm 18.8	316 \pm 8.4

* Volatile radioactivity removed by drying tissue for 48 hours and heating for 1 hour at 60 C. Data represent duplicate determinations in two animals for each time sequence (\pm SE).

† Count/min./mg liver tissue.



FIG. 3. Autoradiograph (40- μ section) from mouse sacrificed immediately following ten-minute inhalation of ^{14}C -diethyl ether. Sections dried 48 hours at -15 C and heated for one hour at 60 C to remove volatile radioactivity. 1, liver; 2, intestine; 3, nasal mucous membrane.

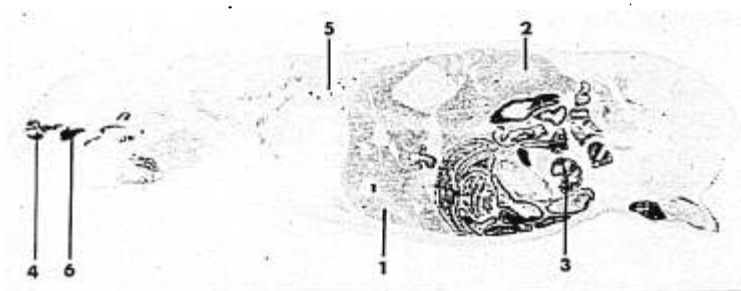


FIG. 4. Autoradiograph (40- μ section) from mouse sacrificed 120 minutes following inhalation of ^{14}C -diethyl ether. Sections dried 48 hours at -15 C and heated for one hour at 60 C to remove volatile radioactivity. Note close similarity to figure 3, indicating radioactivity is essentially nonvolatile. 1, liver; 2, kidney; 3, intestine; 4, nasal mucous membrane; 5, bronchi; 6, Harder's gland.

confirmation of the data in table 2. In figure 3 a small amount of nonvolatile radioactivity can be seen in the liver immediately following a ten-minute inhalation of anesthetic. By 120 minutes the absolute amount of nonvolatile radioactivity in the liver has increased four-fold (fig. 4). The passage of nonvolatile metabolites from the liver into the intestine may also be noted, as well as residual concentrations present in the bronchi and nasal mucous membranes.

Table 3 indicates the concentrations of nonvolatile radioactivity remaining in the liver and intestines of mice sacrificed 120 minutes after an intravenous injection of ^{14}C -diethyl ether. Calculation of the percentage of the injected diethyl ether converted to nonvolatile metabolites indicates that at two hours $3.61 (\pm 0.41)$ per cent of the administered dose is present in metabolized form in the liver and intestine.

A homogenate prepared from the livers of mice sacrificed 120 minutes after anesthesia was extracted into ether, and this extract examined by thin-layer and radio gas chromatography. Figure 5 (upper trace) demonstrates a separation into four separate components as defined in the thin-layer radiochromatogram. Peak 1 ($R_f = 0.08$) represents 9.1 per cent, peak 2 ($R_f = 0.21$) 3.5 per cent, peak 3 ($R_f = 0.43$) 18.0 per cent, and peak 4 ($R_f = 0.80$) 69.4 per cent of the extracted radioactivity. Further attempts were made to define the characteristics of the two major peaks.

Aliquots of peaks 3 and 4 were taken to dryness, dissolved in chloroform, and adjusted to pH 6.2 with phosphate buffer. Each aliquot was treated with 8,000 units of bacterial β -glucuronidase. Type II, for 24 hours in a constant-temperature bath at 37 C. The ether-

soluble materials were extracted in turn and subjected to thin-layer radiochromatography. Under these conditions, peak 3 yielded an unchanged pattern, compared with the control chromatogram (fig. 5, middle trace). Peak 4, however, disappeared from the trace and was replaced by a new peak, 4a, with $R_f = 0.31$ (fig. 5, lower trace). This indicated the possibility of enzymatic attack. In separate experiments, an average of 58.6 per cent of the original radioactivity associated with peak 4 could be recovered in the form of peak 4a, with the remainder representing volatile materials.

In two control experiments peak 4 was similarly treated without the presence of β -glucuronidase. Under these circumstances, the original starting material was obtained, plus small amounts of a third polar product. No peak 4a was observed. Since in both instances, *i.e.*, with and without treatment by β -glucuronidase, new products are formed, it is possible that the original enzymatic conversion of peak 4 to 4a by β -glucuronidase may be followed by a secondary oxidation reaction. This is reasonable in light of the radiochromatographic evidence of the increased polarity of peak 4a.

An additional aliquot of peak 3 was subjected to alkaline hydrolysis with 1N NaOH in a sealed glass vial at 60 C for 30 minutes. The radioactivity was re-extracted into ether and subjected to thin-layer radiochromatography. Only the original starting material was obtained.

Aliquots (5,000 d.p.m.*) of the various peaks were then taken to dryness, dissolved in 10 μl dimethyl sulfoxide, and injected into a gas chromatograph equipped with flame ion-

* Disintegrations/min.

TABLE 3. Concentration of Radioactivity (Nonvolatile Metabolites) in Liver and Intestine Following the Intravenous Injection of 25 μc of ^{14}C -Diethyl Ether*

Tissue	Organ Weight (mg)	Radioactivity (count/min/mg)	Total Organ Radioactivity (count/min)	Percentage Injected Radioactivity†
Liver	1,478 \pm 112	399 \pm 47	589,720	2.15
Intestine (plus contents)	2,546 \pm 569	157 \pm 31	399,719	1.46
				3.61

* Mice sacrificed at 120 minutes. Data represent duplicate determinations in each of three mice (\pm SE).

† Correction for machine efficiency (41.2 per cent).

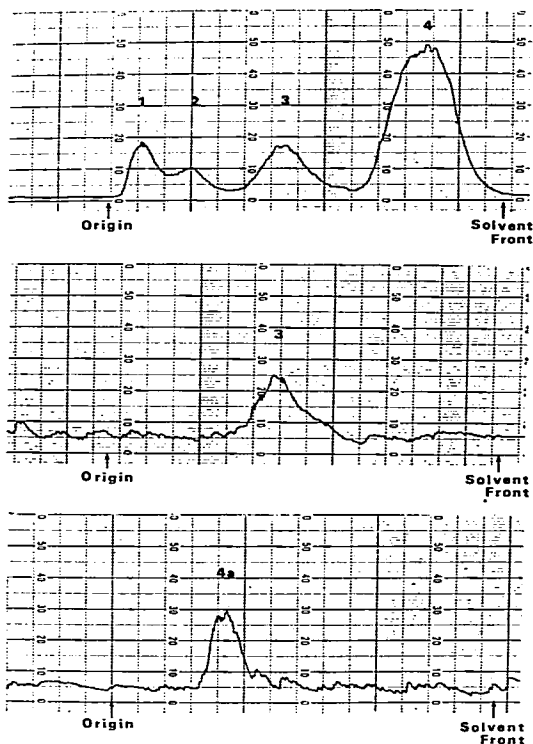


Fig. 5. Upper trace: Thin-layer radiochromatogram from liver extract of mouse sacrificed 120 minutes following ten-minute exposure to ^{14}C -diethyl ether. Solvent system: petroleum ether, 90; diethyl ether, 10; acetic acid, 1. R_f peak 1 = 0.08; R_f peak 2 = 0.21; R_f peak 3 = 0.43; R_f peak 4 = 0.80. Middle trace: Thin-layer radiochromatogram following preliminary treatment of peak 3 with 8,000 units β -glucuronidase for 24 hours at 37 C. No change compared with control trace. Lower trace: Thin-layer radiochromatogram following preliminary treatment of peak 4 with 8,000 units β -glucuronidase for 24 hours at 37 C. Peak 4 has been replaced by peak 4a (R_f = 0.31).

ization and radioactivity detectors. A 1 per cent S.E. 30-column was utilized and temperature programmed in ranges up to 240 C. Although a number of solvent-extracted hydrocarbons could be detected with the flame ionization detector, no evidence of radioactivity was found in association with these peaks. Methylated and esterified derivatives were then prepared in an attempt to render these compounds susceptible to direct chromatographic analysis. With these techniques a number of radioactive peaks were isolated and subsequently identified by mass spectrometric methods. These are to be reported elsewhere.†

† In preparation.

Discussion

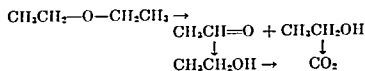
During the first ten minutes of anesthesia, the uptake of diethyl ether in the mouse appears to follow closely the pattern of circulation. At the end of this time most blood/tissue partition coefficients approach a value of unity, although the relative concentration of radioactivity in general body fat lags considerably due to the slow circulation to this depot. By 15 minutes, the blood/tissue partition coefficient for general body fat has approached unity, but by this time the partition coefficient for brown fat has reached 3.21. This high concentration reflects a rapid circulation to brown fat as well as a favorable partition co-

efficient, which combine to produce continued transfer of anesthetic to the brown fat depot.† The tissue/blood radioactivity ratios for both liver and kidney continued to increase throughout the experiment, reflecting their metabolic and excretory functions.

The rapidly increasing ratio of liver/blood radioactivity represents an accumulation of nonvolatile metabolites within this organ. These nonvolatile metabolites increase in amount from 10.8 per cent immediately after anesthesia to 49.2 per cent at 15 minutes. By 120 minutes all liver radioactivity is nonvolatile. Although the two-hour liver/blood radioactivity ratio for diethyl ether is higher than that observed for chloroform, the absolute amount of nonvolatile metabolite present in the liver (and intestine) is greater following chloroform anesthesia.³ Furthermore, Van Dyke *et al.*² have shown that the production of volatile metabolite (¹⁴CO₂) decreases rapidly after the first hour in the case of diethyl ether, but maintains a steadier production (for at least 12 hours) with chloroform. Therefore, one would anticipate, with a longer observation period following the administration of anesthesia (*i.e.*, more than two hours), a greater percentage of chloroform would be converted to nonvolatile metabolites than would occur with diethyl ether. These variations in rates of metabolism of the two anesthetics may represent specific biochemical factors or reflect differences in physical-chemical properties. It has been emphasized that the high lipid solubility of chloroform affords an extensive storehouse for this drug, retaining it within the body for prolonged periods of availability to biochemical attack.³

The precise mechanism for the biologic degradation of diethyl ether has not been established, although Van Dyke *et al.*² have demonstrated its *in-vivo* metabolism to CO₂ and to undefined nonvolatile urinary products. *In-vitro* studies by Bray,⁴ Axelrod,⁵ and Van Dyke⁶ indicate that certain of the ethers are cleaved under the catalysis of liver microsomal enzymes. In a recent review of the metabo-

lism of anesthetic drugs Greene⁷ points out that diethyl ether may be cleaved through the addition of a hydroxyl group to form ethanol and acetaldehyde. The suggested mechanism includes:



Although this scheme serves to provide a mechanism for the metabolism of diethyl ether to carbon dioxide, additional pathways are needed to explain the formation of the nonvolatile liver metabolites demonstrated in the present study. The likelihood is that these products represent conjugated materials. In the present study we suggest the presence of a glucuronide formed from diethyl ether to account for the major nonvolatile metabolite extracted from the liver. Recently, evidence has been obtained for the formation of urinary glucuronides for two other ethers, *i.e.*, methoxyflurane⁸ and fluorene.⁹ Although the data presented in the present study indicate that the liver is importantly involved in the metabolism of diethyl ether to nonvolatile metabolites, further investigations are needed to establish the identification of the remaining nonvolatile ether metabolites. In addition, much work will be necessary before it will be possible to translate the above results of animal studies to the clinical situation.

The authors' appreciation is expressed to the Royal Veterinary College for use of the facilities of the Department of Pharmacology and Chemistry, and to Med. Kand. Kristler Green and Dr. Bengt Samuelsson for helpful advice. Certain aspects of metabolite identification were carried out at the Pharmacology Institute, University of Zurich.

References

1. Goodman, L. S., and Gilman, A.: *The Pharmacological Basis of Therapeutics*. Ed. 3. New York, Macmillan, 1965, p. 63.
2. Van Dyke, R. A., Chenoweth, M. B., and Van Poznak, A.: Metabolism of volatile anesthetics—I, *Biochem. Pharm.* 13: 1239, 1964.
3. Cohen, E. N., and Hood, N.: Application of low-temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. I. Chloroform, *ANESTHESIOLOGY* 30: 306, 1969.

† With chloroform anesthesia, transfer of the anesthetic to both general body fat and brown fat is more rapid, reflecting the much higher fat/blood partition coefficient for chloroform (20.0) compared with that for diethyl ether (5.0).

4. Bray, H. G., Craddock, V. M., and Thorpe, W. V.: Metabolism of ethers in the rabbit, *Biochem. J.* 60: 225, 1955.
5. Axelrod, J.: The enzymatic cleavage of aromatic ethers, *Biochem. J.* 63: 634, 1956.
6. Van Dyke, R. A.: Metabolism of volatile anesthetics. III. Induction of microsomal dechlorinating and ether-cleaving enzymes, *J. Pharmacol. Exp. Ther.* 154: 346, 1966.
7. Greene, N. M.: The metabolism of drugs employed in anesthesia. Part II, *ANESTHESIOLOGY* 29: 327, 1968.
8. Van Dyke, R. A.: Metabolic pathways and anesthetic detoxification. In: *Proceedings of the Symposium on Toxic Effects of Anesthetics*, Baltimore, Williams and Wilkins Co., 1968, p. 63.
9. Blake, D. A., Rozman, R. S., Cascorbi, H. H., and Krantz, J. C., Jr.: Anesthesia LXXI: Biotransformation of Fluoroxene. I. Metabolism in mice and dogs *in vivo*, *Biochem. Pharmacol.* 16: 1237, 1967.

Nervous System

IRREVERSIBLE COMA Our primary purpose is to define irreversible coma as a new criterion for death. There are two reasons why there is need for a definition: (1) Improvements in resuscitative and supportive measures have led to increased efforts to save those who are critically injured. Sometimes these efforts have only partial success, so that the result is an individual whose heart continues to beat but whose brain is irreversibly damaged. The burden is great on patients who suffer permanent loss of intellect, on their families, on the hospitals, and on those in need of hospital beds already occupied by these comatose patients. (2) Obsolete criteria for the definition of death can lead to controversy in obtaining organs for transplantations. (*Ad Hoc Committee of the Harvard Medical School to Examine the Definition of Brain Death: A Definition of Irreversible Coma, J.A.M.A.* 205: 337 (Aug.) 1968.)

ELECTRICAL ANESTHESIA The parallel disappearance of responsiveness and of cortical-evoked responses suggests that electroanesthesia is largely a cortical phenomenon. Since pulses of relatively high frequency are used, it appears likely that synaptic mechanisms may be affected. Spreading depression does not appear to be a likely explanation, since the recovery time for evoked potentials is short and the impedance values do not change significantly. The disappearance of cortical recruiting responses and of somato-sensory, visual, and auditory potentials suggests that cortical processing of afferent impulses is markedly affected. (*Larson, S. J., and Sances, A.: Physiologic Effects of Electroanesthesia, Surgery* 64: 281 (July) 1968.)

TETANUS Alimentation during tetanus is extremely difficult, since the caloric requirement is 7,000 to 8,000 calories per day for an adult. The old belief that the high caloric requirement is due to muscle spasm is no longer tenable, since complete curarization does not change this requirement and does not give any improvement in nitrogen balance. During intensive therapy for tetanus, the authors use a mixture of synthetic amino acids combined with carbohydrates and ethyl alcohol administered through a catheter inserted via the jugular vein into the vena cava. Emulsions of fat have been unsatisfactory. As soon as possible, feedings through a nasogastric tube are added. (*Schultis, K., and L'Allemand, H.: The Nutrition of Tetanus Patients, Der Anaesthetist* 17: 196 (June) 1968.)