Biophysical Observation

Effect of General Anesthetics on Sodium Transport in the Isolated Toad Bladder

*Nikaan B. Andersen, M.D.**

In the short-circuited toad bladder, cyclopropane and nitrous oxide gave rise to a dose dependent stimulation, and halothane to a dose dependent inhibition of active sodium transport. Ethyl ether produced a biphasic response: initial stimulation followed by inhibition of ion transport.

THE ANESTHETIC state has been correlated with a decreased passive sodium and potassium flux across the nerve cell membrane.[1, 2, 3, 4] The hypothesis that anesthetic agents in clinically used concentrations also inhibit the active transport of sodium and potassium across cellular membranes[5] was confirmed for local anesthetic agents in a study of active ion transport across red cell membranes.[6] This effect appeared to be similar to the effect of cardiac glycosides in the same model.[7, 8]

The present study was undertaken to test the effect of general anesthetic agents upon active ion transport. High concentrations of local anesthetics were found to damage the red cell membrane resulting in hemolysis and increased passive ion flux,[6] Since pilot studies indicated that this would be a greater problem with general anesthetic agents, a different model was used.

In 1951 it was shown that active sodium transport was solely responsible for the potential difference across frog skin.[9] However, while the original technique of the short-circuited frog skin,[9] with a few minor modifications, was used in this study, we chose to replace the frog skin with toad bladder. The toad bladder is a simple membrane consisting of a single layer of mucosal cells, supported by a minimum of connective tissue which is covered by a layer of serosal cells.[10]

* Assistant Professor of Anesthesiology, College of Medicine, University of Florida, Gainesville, Florida.

Accepted for publication December 7, 1965.

Methods

Toads (*Buffo marinus*) were pithed, and the bladder was removed and divided. Each bladder half was mounted between two symmetrical halves of a lucite chamber; one half bladder was treated, the other half served as the control. In each chamber half there were three inlets, two for electrodes and one for gas. Each chamber held 2 × 15 ml. solution. The diameter of the circular opening over which the bladder was mounted was 15 mm. The same bathing solution was used in both chamber halves, 115 m*M* NaCl, 3 m*M* KCl, 3 m*M* $NaHCO_3$, and 1 m*M* $CaCl_2$, adjusted to a *p*H of 8.0 with NaH_2PO_4. During several experiments *p*H was monitored at intervals, and a slow increase to *p*H 8.2–8.4 was noted in both the mucosal and serosal solutions.* Full activity of sodium transport in toad bladder has been shown at a *p*H from 7 to 9.[11] Total solutes in toad urine were found to range from 50 to 300 mosm./liter, and in toad serum from 200–300 mosm/liter.[12] Experiments were carried out at room temperature.

Despite the extreme thinness of the toad bladder, a potential of about 10–110 mv. can usually be measured across the wall (transbladder potential), the mucosal, or urinary side, being electronegative with respect to the serosal side. The electrical activity of

* In some of the later experiments, a different solution was used: 110 m*M* NaCl, 10 m*M* KCl, 1 m*M* $MgCl_2$, 0.25 m*M* $CaCl_2$ 0.9 m*M* NaH_2PO_4, 4.3 m*M* Na_2HPO_4, 5.3 m*M* THAM, 2.2 m*M* H_2SO_4, 6 g./l. glucose, 0.75 g./l. adenosin, adjusted to *p*H 8.0 with 0.3 molar THAM. This solution had no effect upon the response of the bladder to the anesthetic agents, but it prolonged the life of the bladders, seemed to account for a higher resting membrane potential (fewer bladders had to be discarded prior to the experiment), and maintained *p*H throughout an experiment without change.

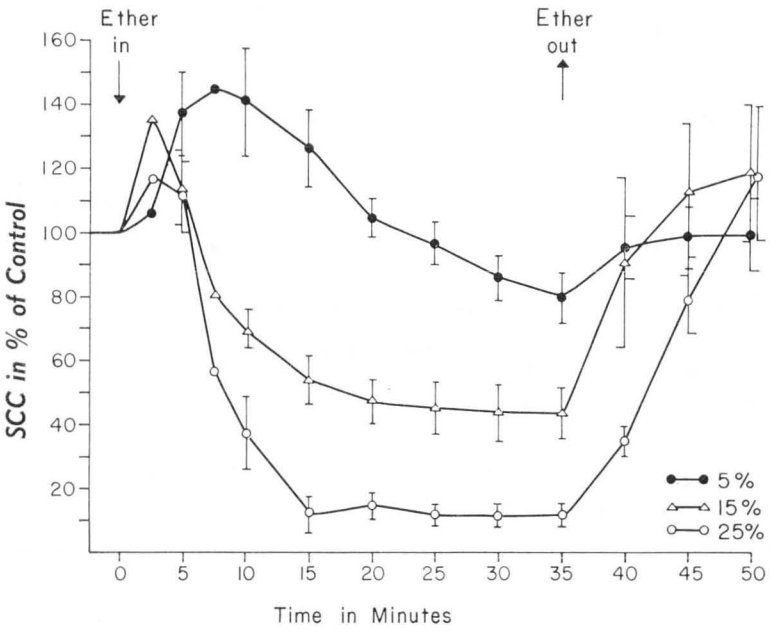

FIG. 1. Effect of ethyl ether upon short circuited current in toad bladder expressed in percentage of control.

be accounted for by active transport of sodium from the mucosal to serosal surface. When both surfaces of the membrane are bathed with the same Ringer's solution, and the transmembrane potential is nullified by an imposed external electromotive force, a current flows through the external circuit (short circuited current or SCC). The transbladder potential was measured with a Beckman expanded scale *p*H meter. The SCC was monitored with a d.c. microameter. The SCC was followed throughout an experiment and was accepted as a measurement of the active sodium transport according to Ussing and Zerahn [9] and Leaf *et al.*[12]

While the current stabilized, and during a control period of at least 15 minutes, oxygen 100 per cent or atmospheric air was bubbled through both chambers at a rate of 100–200 ml./min. At the start of an experiment, both halves of the test chamber were aerated with the gas under study, while the control was aerated as before. After 50 minutes, or when the SCC had stabilized at a new value, the test chamber was switched back to the previously given gas, *i.e.*, oxygen or air. The following sequences were tested:

Oxygen—Ethyl ether 5, 15 or 25 vol. per cent in oxygen—Oxygen
Oxygen—Halothane 2, 5 or 8 vol. per cent in oxygen—Oxygen
Oxygen—Cyclopropane 10, 30 or 60 vol. per cent in oxygen—Oxygen
Oxygen—Nitrous oxide 30 or 80 vol. per cent in oxygen—Oxygen
Oxygen—Air—Oxygen
Air—Oxygen—Air

The anesthetic gases and vapors were delivered from a Foregger anesthetic machine into a reservoir bag and from there circulated through the chamber by a perfusion pump (Sigma). The composition of the anesthetic mixture was measured at 5-minute intervals in an F and M gas chromatograph.

Results

During the control period SCC values ranged from 20 to 176 (mean 46.0) microamperes in the control bladders, and from 26 to 145 (mean 53.8) microamperes in the test bladders. The readings in the test bladders were expressed as percentage of the SCC in the control bladders and are shown as such in all figures.

Figures 1, 2, 3, and 4 show the effect of different concentrations of ethyl ether, halothane, cyclopropane, and nitrous oxide, respectively. Each curve represents the mean value

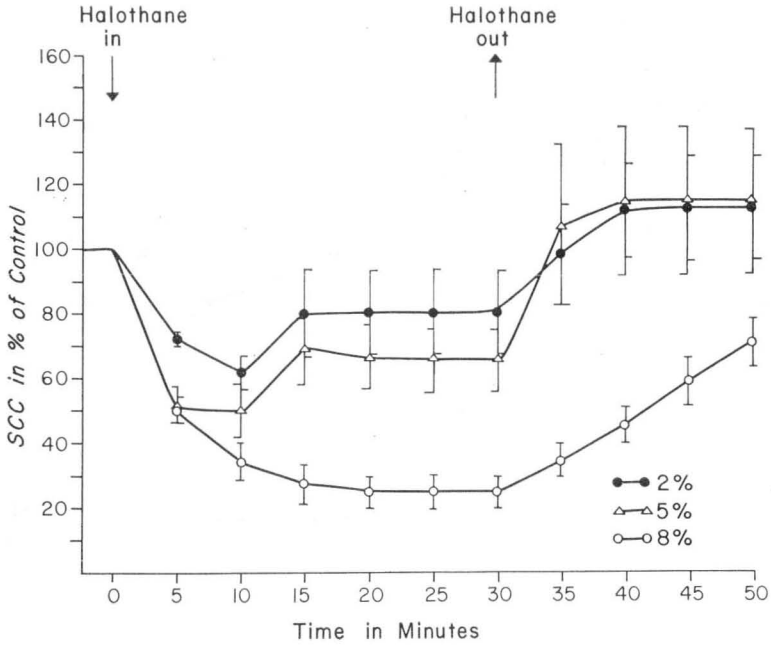

FIG. 2. Effect of halothane upon short circuited current in toad bladder expressed in percentage of control.

of five experiments, and the brackets indicate ± 1 standard error of the mean. Table 1 shows the significance of the deviations from the base line of the control as expressed by Student's paired *t*-test.

It is seen that 5 per cent ether stimulated the bladder, but that this stimulation wore off with time. After 35 minutes, significant depression was present. After initial slight stimulation 15 and 25 per cent ether depressed the

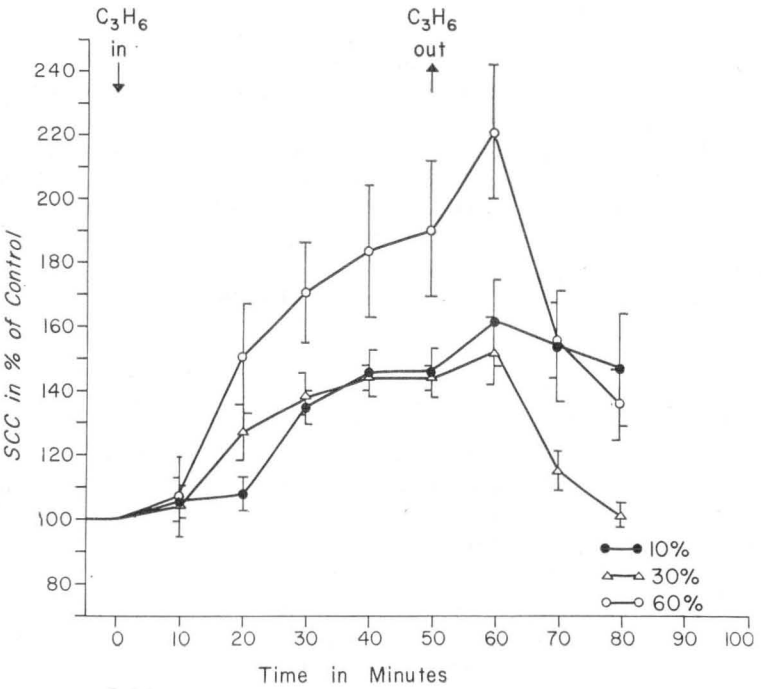

FIG. 3. Effect of cyclopropane upon the short circuited current in toad bladder expressed in percentage of control.

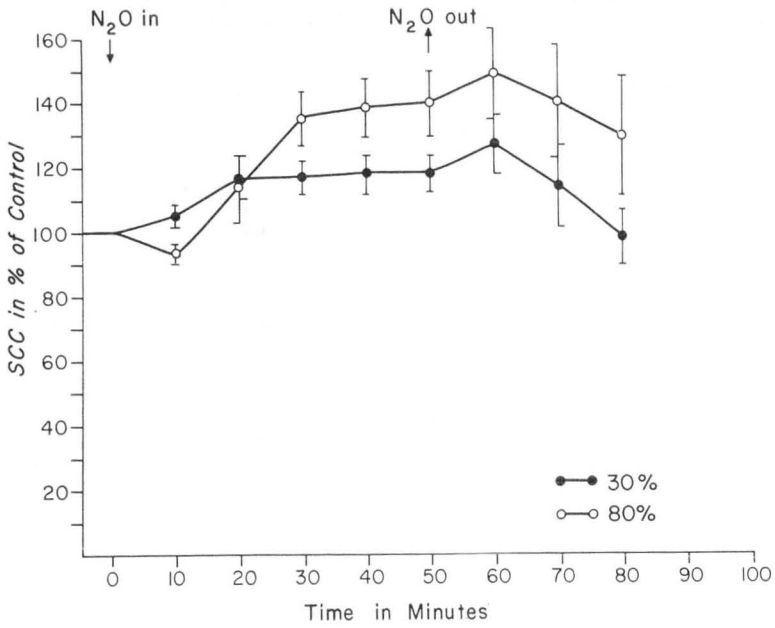

FIG. 4. Effect of nitrous oxide upon the short circuited current in toad bladder expressed in percentage of control.

bladder, but to different degrees. Halothane gave rise to a dose dependent depression at the concentrations tested. Cyclopropane had the opposite effect, *i.e.*, a dose dependent stimulation of the bladder, while nitrous oxide had an effect in all respects similar to that of cyclopropane.

In the bladders exposed to air after initial oxygen, the mean value of 5 experiments was 96.7 per cent ± 8.2 standard error of the mean, and in the bladders exposed to oxygen after initial stabilization with air the mean value of 5 experiments was 73.6 per cent ± 13.1 standard error of the mean.

The changes associated with anesthetic agents appeared to be fully reversible. Following discontinuation of the anesthetic, SCC values approached the base line, and changes became insignificant except in two instances (cyclopropane 60 per cent and halothane 8 per cent). With cyclopropane and nitrous oxide a short-lived increase in stimulation was noted immediately following discontinuation of the anesthetic. In the air/oxygen/air or oxygen/air/oxygen experiments, no change was noted when one replaced the other at the end of the experiment.

Discussion

On the basis of earlier studies it seems likely that the recorded SCC represented the active sodium transport of toad bladder. A direct relationship between SCC and active sodium transport in untreated tissue has been demonstrated by Ussing and Zerahn [9] for frog skin and by Leaf *et al.*[12] for toad bladder. Neither ouabain in the frog skin,[13] nor vasopressin [14] and aldosterone [15] in toad bladder changed this relation.

The initial stimulation in all the ether experiments (fig. 1) probably represents the effect of the initially small concentration of ether. As higher ether concentrations were attained, ether depressed sodium transport. Other explanations that assume the presence of different kinetic systems in the bladder cannot be ruled out.

The finding that halothane in the concentrations tested, and ether in concentrations above 4–5 per cent inhibited active sodium transport agree with our earlier report that local anesthetics inhibit active sodium and potassium transport in red blood cells [6] and with another study by Skou and Zerahn [16] in which undissociated local anesthetic base inhibited active transport of sodium in frog skin. This inhibi-

TABLE 1. Significance of Experimental Deviations from Base Line of Control in Figures 1, 2, 3, and 4 as Expressed by Students Paired *t*-Test

Agent	Conc. (per cent)	Time (minutes)	Mean Difference	Significance
Ether	5	2.5	+ 6.0	$0.4 > p > 0.3$
		7.5	+44.8	$0.05 > p > 0.025$
		20.0	+ 4.5	$0.5 > p > 0.4$
		35.0	−20.4	$0.05 > p > 0.025$
		50.0	− 0.6	$0.7 > p > 0.6$
	15	2.5	+35.0	$0.2 > p > 0.1$
		7.5	−19.8	$0.05 > p > 0.025$
		20.0	−52.8	$0.005 > p > 0.001$
		35.0	−56.4	$0.005 > p > 0.001$
		50.0	+18.6	$0.5 > p > 0.4$
	25	2.5	+17.2	$0.1 > p > 0.05$
		7.5	−44.2	$p = 0.05$
		20.0	−85.2	$0.001 > p$
		35.0	−88.4	$0.001 > p$
		50.0	+18.2	$0.5 > p > 0.4$
Halothane	2	10.0	−38.6	$0.005 > p > 0.001$
		30.0	−20.2	$0.3 > p > 0.2$
		45.0	+13.2	$0.5 > p > 0.4$
	5	10.0	−49.8	$0.005 > p > 0.001$
		30.0	−34.6	$0.025 > p > 0.02$
		45.0	+14.8	$0.6 > p > 0.5$
	8	10.0	−65.6	$0.001 > p$
		30.0	−75.2	$0.001 > p$
		45.0	−40.8	$0.01 > p > 0.005$
Cyclopropane	10	30.0	+34.4	$0.005 > p > 0.001$
		50.0	+45.6	$0.005 > p > 0.001$
		80.0	+46.0	$0.1 > p > 0.05$
	30	30.0	+37.8	$0.005 > p > 0.001$
		50.0	+44.8	$0.001 > p$
		80.0	+ 1.0	$0.8 > p > 0.7$
	60	30.0	+70.6	$0.02 > p > 0.01$
		50.0	+90.5	$0.02 > p > 0.01$
		80.0	+35.2	$0.05 > p > 0.025$
Nitrous Oxide	30	50.0	+18.2	$0.05 > p > 0.025$
		80.0	− 2.2	$0.9 > p > 0.8$
	80	50.0	+39.3	$0.02 > p > 0.01$
		80.0	+29.6	$0.2 > p > 0.1$

tion of ion transport explains the prolongation of the positive after-potential seen in guinea pig myocardial action potentials upon application of procaine amide.[17] As a result of decreased sodium and potassium transport in nerve tissue one would expect to see a slow, uniform depolarization, since the action potential represents "leak" of these ions along the concentration gradients. Such depolarization has been demonstrated with ether, barbiturates, chloral, and cocaine in the spinal cord of cats exposed to repeated asphyxiation. After about 10 minutes the asphyxial depolarization potential was further reduced.[18]

The mechanism responsible for this inhibition of ion transport is unknown. There may be enzymatic inhibition [19, 20] similar to the effect of cardiac glycosides; [21, 22] inhibition of aerobic metabolism, [23, 24] which is necessary for ion transport, [25] or it may be the result of membrane stabilization. [1, 2]

At present it is not easy to explain why general anesthetic agents, *e.g.*, cyclopropane, nitrous oxide, and ether below 5 per cent stimulate sodium transport. However, this finding agrees with an earlier report by Skou and Zerahn [16] in which the ionized local anesthetic amines increased active transport of sodium through frog skin, when added to the outer solution. These authors theorized that anesthetics increase the permeability to sodium of the outer membrane of the model, an effect similar to that of vasopressin. [10] It has been shown that ouabain has a biphasic effect upon toad bladder. [26] It is interesting that ether in this study also had a biphasic effect. The transient further stimulation following discontinuation of cyclopropane and nitrous oxide could mean that these compounds gave rise to simultaneous inhibition and stimulation, the stimulation prevailing, and the inhibition wearing off upon removing the gases. Perhaps two receptor sites are active, one mediating stimulation of sodium transport, more sensitive to some anesthetics and low ether concentrations, the other mediating inhibition, more sensitive to other anesthetics and higher ether concentrations. Further studies are required for the understanding of these relationships.

Dr. J. S. Gravenstein furnished help and advice in connection with this study. This study was supported in part by National Institutes of Health Grants GM-13029-01, TI GM 427-05 and HE 07467-02S1.

References

1. Shanes, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. Part I. The resting cell and its alteration by extrinsic factors, Pharmacol. Rev. **10**: 59, 1958.
2. Shanes, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. Part II. The action potential and excitation, Pharmacol. Rev. **10**: 165, 1958.
3. Skou, J. C.: The effect of drugs on cell membranes with special reference to local anesthetics, J. Pharm. Pharmacol. **13**: 204, 1961.
4. Pauling, L.: A molecular theory of general anesthesia, Science **134**: 15, 1961.
5. Andersen, N. B.: The effect of anesthetic agents on cellular membranes, Anesth. Analg. **43**: 49, 1964.
6. Andersen, N. B., and Gravenstein, J. S.: Effects of local anesthetics on sodium and potassium in human red cells, J. Pharmacol. Exp. Ther. **147**: 40, 1965.
7. Kahn, J. B., Jr., and Acheson, G. H.: Effects of cardiac glycosides and other lactones and of certain other compounds on cation transfer in human erythrocytes, J. Pharmacol. Exp. Ther. **115**: 305, 1955.
8. Glynn, I. M.: Sodium and potassium movements in human red cells, J. Physiol. **134**: 278, 1956.
9. Ussing, H. H., and Zerahn, K.: Active transport of sodium as the source of electric current in the short-circuited isolated frog skin, Acta Physiol. Scand. **23**: 110, 1951.
10. Leaf, A.: Some actions of neurohypophyseal hormones on a living membrane, J. Gen. Physiol. **43** (Suppl. 1): 175, 1960.
11. Frazier, H. S., and Leaf, A.: The electrical characteristics of active sodium transport in the toad bladder, J. Gen. Physiol. **46**: 491, 1963.
12. Leaf, A., Anderson, J., and Page, L. B.: Active sodium transport by the isolated toad bladder, J. Gen. Physiol. **41**: 657, 1958.
13. Koefoed-Johnsen, V.: The effects of g-strophanthidin (ouabain) on the active transport of sodium through the isolated frog skin, Acta Physiol. Scand. (Suppl.) **145**: 87, 1957.
14. Leaf, A., and Dempsey, E.: Some effects of mammalian neurohypophyseal hormones on metabolism and active transport of sodium by the isolated toad bladder, J. Biol. Chem. **235**: 2160, 1960.
15. Crabbé, J.: Stimulation of active sodium transport by the isolated toad bladder with aldosterone in vitro, J. Clin. Invest. **40**: 2103, 1961.
16. Skou, J. C., and Zerahn, K.: Investigations on the effect of some local anesthetics and other amines on the active transport of sodium through the isolated short-circuited frog skin, Biochim. Biophys. Acta **35**: 324, 1959.
17. Johnson, E. A.: The effects of quinidine, procaine amide, and pyrilamine on the membrane resting and action potential of guinea pig ventricular muscle fibers, J. Pharmacol. Exp. Ther. **117**: 237, 1956.
18. Van Harreveld, A., and Feigen, G. A.: Effect of some drugs on the polarization state of spinal cord elements, Amer. J. Physiol. **160**: 451, 1950.
19. Bonting, S. L., and Caravaggio, L. L.: Studies on sodium-potassium-activated adenosintriphosphatase. V. Correlation of enzyme activity with cation flux in six tissues, Arch. Biochem. **101**: 37, 1963.

20. Järnefelt, J.: Inhibition of the brain microsomal adenosinetriphosphatase by depolarizing agents, Biochim. Biophys. Acta **48**: 111, 1961.
21. Post, R. L., Merritt, C. R., and Kinsolving, C. R.: Membrane adenosin triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte, J. Biol. Chem. **235**: 1796, 1960.
22. Skou, J. C.: Further investigations on a Mg^{++} + Na^{+} activated adenosintriphosphatase, possibly related to the active linked transport of Na^{+} and K^{+} across the nerve membrane, Biochim. Biophys. Acta **42**: 6, 1960.
23. Quastel, J. H.: Effects of anesthetics, depressants, and tranquilizers on cerebral metabolism, *In:* Metabolic Inhibitors; a comprehensive treatise, Vol. 2, Ed. R. M. Hochster and J. H. Quastel. New York, Acad. Press, 1963, pp. 517–538.
24. Bunker, J. P., and Vandam, L. D.: Effects of anesthesia on metabolism and cellular functions. A workshop, Pharmacol. Rev. **17**: 183, 1965.
25. Houstmuller, A. J.: Uptake of potassium by erythrocytes in relation to their glucose consumption, Clin. Chim. Acta **4**: 606, 1959.
26. McClane, T. K.: A biphasic action of ouabain on sodium transport in the toad bladder, J. Pharmacol. Exp. Ther. **148**: 106, 1965.

MANNITOL The urinary response to intravenous administration of 100 ml. of 25 per cent mannitol is used to differentiate organic acute renal failure from oliguria of extrarenal causes. A positive response is noted as an increase in urine flow of over 0.20 ml./minute in a three-hour period following mannitol. The response was variable in patients with extrarenal oliguria while none of those with organic acute renal failure exhibited significant diuresis. There were no untoward cardiovascular complications following the mannitol in any of the patients. It is usually possible to sustain the mannitol diuresis, if it occurs, by continued administration of plasma expanding fluids. The mannitol diuresis is due to acute expansion of blood volume and a subsequent increase in glomerular filtration rate. (*Scheer, R. L.: Effect of Hypertonic Mannitol on Oliguric Patients, Amer. J. Med. Sci. 250: 483 (Nov.) 1965.*)

REGIONAL CHEMOTHERAPY A wide pneumatic thoracoabdominal tourniquet has been used for mid-torso occlusion during regional (pelvic) perfusion of chemotherapeutic drugs. By this means, the collateral circulation is easily and effectively occluded in most instances. A light plane of general endotracheal anesthesia was employed. Nonexplosive agents were required and on occasion a pump oxygenator was used. The agent chosen was less important than the concept of light anesthesia combined with muscle and chest-wall relaxation. Vigorous intermittent positive pressure respiration was imperative. For pelvic perfusions, a thermostatically controlled hypothermia mattress and blanket were used. (*Selvin, B. L.: Cancer Chemotherapy by Regional Perfusion: Anesthetic and Physiologic Problems, Surgery 58: 941 (Dec.) 1965.*)

FEMORAL NERVE INJURY Femoral nerve injury during pelvic surgery is a known but unpublicized entity. Three case reports and a review of the literature are presented. Injury is usually due to pressure exerted by retraction upon the nerve along its course through the false pelvis. Recovery of nerve function is delayed but commonly does occur. Common factors associated with such injuries are the use of self-retaining retractors, a transverse incision, and a thin or average habitus in the patient. Careful placement and padding of retractors and frequent release of retractor pressure during long procedures are advisable. (*Klinges, K. G., and others: Injury to the Femoral Nerve during Pelvic Operation, Obstet. Gynec. 25: 619 (May) 1965.*)