Anesthesiology
V 39, No 1, July 1973

Sympathetic Excitation during Nitrous Oxide–Halothane Anesthesia in the Cat

Atsuo F. Fukunaga, M.D., and Robert M. Epstein, M.D.†*

The effect of 70 per cent nitrous oxide on preganglionic sympathetic activity was studied by recording the compound action potential from the splanchnic nerve in the cat. Substitution of nitrous oxide for an equal concentration of nitrogen in the inspired mixture during steady-state halothane–nitrogen–oxygen anesthesia caused significant increases in splanchnic-nerve discharge and mean arterial pressure and a moderate increase in heart rate. When the carotid sinus and aortic baroreceptor nerves were sectioned, these responses were further accentuated and were accompanied by a 71 per cent incidence of cardiac arrhythmias. With continued halothane anesthesia, decerebration by transection of the brainstem at the midcollicular level completely suppressed the responses to nitrous oxide. Following the removal of halothane in decerebrate preparations, nitrous oxide acted as a depressant, producing marked decreases in heart rate in animals with intact baroreceptors and significant decreases in splanchnic-nerve activity in debuffered animals. These results indicate that the increase in sympathetic activity produced by nitrous oxide is neurally mediated and dependent on actions at suprapontine levels, the loci of which are undetermined. The study further suggests that the direct effect of nitrous oxide is depressant to vasomotor neurons at the brainstem or spinal level. (Key words: Nitrous oxide; Halothane; Sympathetic-nerve activity; Preganglionic splanchnic nerve; Central nervous system actions of anesthetics.)

EXPERIMENTAL STUDIES of the effects of nitrous oxide have been few. It has been believed that nitrous oxide exerts minimal effects on cardiovascular activity. This opinion, however, is largely based on traditional clinical experience. The low potency of the drug itself, and the technical difficulties of a suitable experimental preparation, have impeded extensive studies of the effects of nitrous oxide upon the autonomic nervous system and the cardiovascular system.

The advantage of combining nitrous oxide with other anesthetic agents has been supported by studies in man, and its clinical value is well established.[1] Recently, several groups of investigators have reported that the addition of nitrous oxide to halothane–oxygen anesthesia in man produces vasoconstriction and other signs of sympathetic activation.[2–4] Similar effects were also observed when nitrous oxide was combined with diethyl ether and fluroxene.[5, 6] The nature of this sympathetic activation is not known. It may be a direct action of nitrous oxide itself on blood-vessel tone, be neurally mediated, or be a result of interaction with the primary agent.

The present study was undertaken to investigate further the effects of nitrous oxide on the sympathetic nervous system by recording the compound nerve action potentials of the preganglionic splanchnic nerves in cats.

Methods

Nineteen adult cats, weighing 2.5–4.9 kg, were studied. Each cat was initially anesthetized with halothane in oxygen, which was delivered into a small-animal box. When the cat was suitably anesthetized, a tracheotomy was performed. Both femoral arteries (one for continuous monitoring of arterial pressure, the other for the arterial blood sampling) and one vein were cannulated. Drugs were injected and fluids replaced through the femoral-vein catheter.

The animal's head was placed in a stereotaxic head-holder and kept in its normal posi-

* Research trainee, supported by USPHS Research Training Grant GM 00056-13.

† Professor of Anesthesiology. Present address: Professor and Chairman, Department of Anesthesiology, University of Virginia, Charlottesville, Virginia.

Received from the Department of Anesthesiology, Columbia University College of Physicians and Surgeons, New York, New York. Accepted for publication January 17, 1973. Supported in part by USPHS Grant GM 09069-10, National Institute of General Medical Sciences, NIH. Awarded first prize in the Residents' Research Essay Contest of the American Society of Anesthesiologists for 1972; presented at the 1972 meeting.

tion throughout the experiment, except when the surgical preparation was performed. Intermittent positive-pressure respiration was instituted by means of a Frumin nonrebreathing valve and a modified Frumin-Lee respirator with pediatric bellows which allowed constant-pressure ventilation. In order to maintain stable alveolar ventilation, end-tidal gas, 1 ml of each breath, was sampled continuously from the trachea by a time-phased sampling pump actuated by the respirator. The sample was passed through a Beckman LB-1 infrared CO_2 analyzer and the percentage of CO_2 was displayed on a polygraph (Offner Dynograph System—Invengineering). The rate and tidal volume were further adjusted to maintain Pa_{CO_2} at 30–35 torr as measured with a CO_2 electrode. The output of the end-tidal gas sample was collected in a 10-ml glass syringe and then analyzed by a Mayo Vapor Analyzer (Ohio Medical Products) for end-expired halothane concentration. Arterial blood pressure was measured with a Statham strain-gauge transducer, P23De. Mean arterial blood pressure was obtained by electrical damping. Heart rate was recorded using a cardiotachometer coupler triggered by the arterial pulse. The EKG was recorded from the forelimbs of the cat (lead 1). These recordings were all displayed on the polygraph. Halothane was vaporized with oxygen flowing through a Foregger Copper Kettle. Following the initial surgical procedure, a mixture of 30 per cent oxygen and 70 per cent nitrogen or 30 per cent oxygen and 70 per cent nitrous oxide was used as a diluent gas. The inspiratory oxygen concentration of the mixture was monitored with a Beckman Pauling oxygen analyzer, Model D.

Room temperature was kept at about 27 C, and esophageal temperature was maintained at 36–38 C with the aid of an electric heating pad placed under the animal's body. A small dose of pancuronium bromide (Pavulon, Organon, Inc.), 0.1–0.2 mg, was given intravenously as needed to prevent movement during recording of neural activity.

Splanchnic-nerve Dissection

The left greater splanchnic nerve was located in the left vertebrocostal triangle by blunt dissection and retraction. The nerve was freely exposed without interference by the surrounding tissues. A small part of the diaphragmatic muscle was removed at the left vertebrocostal corner and the left chest was opened to allow adequate space for the placement of the electrode, minimizing respiratory movement of the chest. The celiac ganglion was located in the fat retroperitoneally above the kidney. The nerve was cut at its entrance to the celiac ganglion, desheathed, immersed in a pool of mineral oil, and covered with cotton.

Decerebration

The animal was decerebrated under halothane–oxygen anesthesia. Through a left parietal craniotomy midcollicular transection was performed with a thin metal spatula. The forebrain was removed by suction. The basilar artery and other bleeders were secured with Codman Vesclips and oxidized cellulose (Oxycel, Parke-Davis). The surgical decerebration procedure was completed in less than 10 minutes with minimal blood loss (total suction volumes, including brain tissue, averaged 10–15 ml). The animals recovered quickly in spite of this major intervention, the blood pressure stabilizing near its predecerebration level within 30 minutes following a transient fall at the time of transection. The carotid arteries were neither tied nor clamped.

Baroreceptor Denervation

Through a midline ventral approach, the carotid sinus nerves and the aortic baroreceptor nerves in conjunction with the vagi were located at a high level in the neck and severed. After surgical denervation was completed, the animal was tested with carotid occlusion, or with a small iv dose of epinephrine or histamine, to assure that the preparation had been released from the buffer mechanisms.

Nerve Recording

Nerve action potentials were recorded from a multifiber strand of the left greater splanchnic nerve, which was placed on a bipolar platinum wire electrode (diameter 22 gauge, interelectrode distance 2 mm) connected to a Grass preamplifier (Model P 15) set to a bandwidth of 100–3,000 Hz. The output signals were led to an oscilloscope and an ultraviolet-writing oscillograph (Model 1706-Honeywell) and were stored on magnetic tape along with

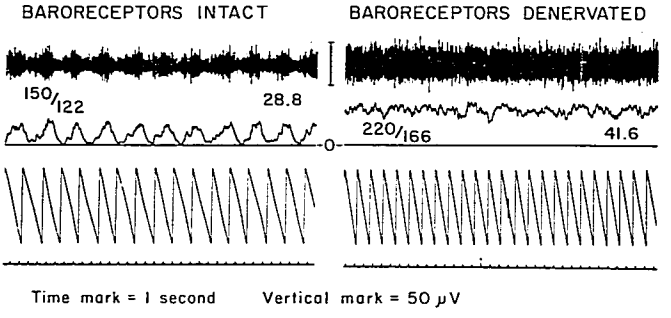

FIG. 1. Preganglionic splanchnic nerve discharge before and after baroreceptor denervation in the same cat. *Upper trace:* compound action potential. *Middle trace:* smoothed splanchnic discharge. Zero line: background after procaine. The lower panel displays the output of the self-resetting integrator used to quantitate the smoothed action potential. Numbers on each panel give prevailing arterial blood pressure (*left*) and integrated value in arbitrary units (constant for each study) for splanchnic nerve activity over one minute's recording (*right*) Tracing for demonstration of method; cat under chloralose anesthesia.

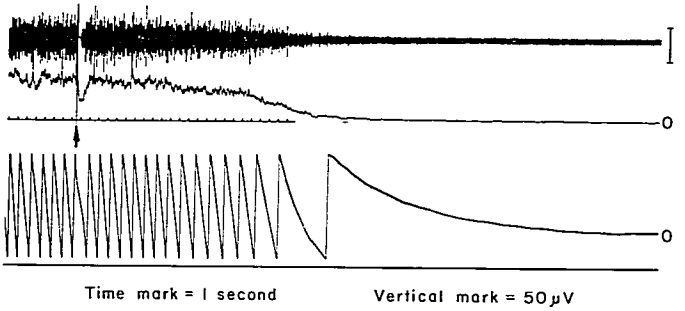

FIG. 2. Application of procaine (*arrow*), showing signal-to-noise ratio after anesthesia of the splanchnic nerve. Traces as in figure 1. Note that the integrator counts are zero when the procaine effect is complete.

blood pressure and EKG data. The action potentials were also rectified and averaged with an electromyograph-integrating circuit (fig. 1, "smoothed splanchnic nerve").

In order to obtain a quantitative index of the magnitude of neural activity, the smoothed neural signal was integrated (fig. 1) using a self-resetting integrator designed by A. S. J. Lee. In order to determine the noise level, two or three drops of 1 per cent procaine were placed on the nerve central to the electrodes at the end of the experiment (fig. 2). The noise level after addition of procaine was chosen as zero neural activity, and the integrator baseline was set to this level. Neural activity was recorded from a multifiber strand so that all recorded activities above baseline were included. Audio monitoring of the nerve discharge was used throughout the experiment.

All measurements were taken from original recordings or (resetting integrator) from data on magnetic tape played at the original speed.

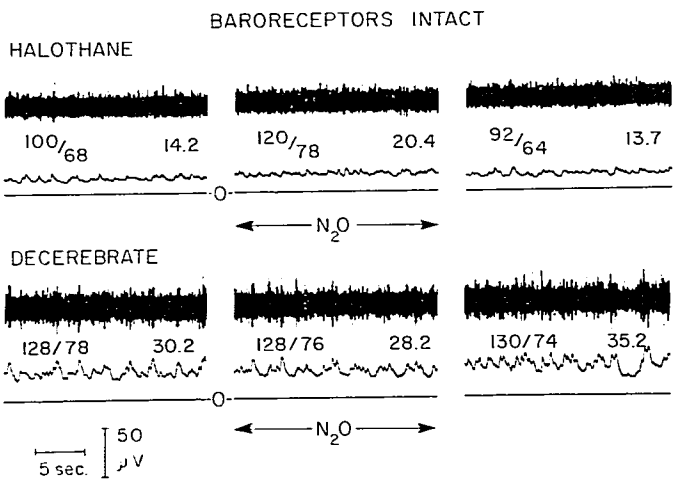

FIG. 3. Effects of nitrous oxide on splanchnic nerve discharge in a single cat. Baroreceptors intact. Upper trace: compound action potential. Lower trace: smoothed splanchnic discharge. Zero line: background after procaine. Figures give prevailing arterial blood pressure (*left*) and integrated value in arbitrary units for splanchnic nerve activity over one minute's recording (*right*). Left panels, control on 70 per cent nitrogen; center panels, after 70 per cent N_2O for 10 minutes; right panels, after 20 minutes' recovery. Vertical scale refers to compound action potential; smoothed value is in arbitrary units.

For display only, the figures here published are taken from taped data replayed at ⅛ or 1/16 the original speed and recorded on a Brush 220 ink recorder. This speed change permits reproduction of the original high-frequency signals on the ink recorder.

EXPERIMENTAL PROTOCOL

The animals were divided into two groups, those with intact baroreceptors (12 cats) and those with denervated baroreceptors (seven cats). Each group was then observed in three stages: intact brain during halothane anesthesia, decerebrate with halothane background, and decerebrate with no background anesthetic.

All the animals were ventilated with the gas mixture of oxygen (30 per cent)–nitrogen (70 per cent) throughout the experiment, except during those times when nitrogen was replaced by nitrous oxide (70 per cent) with the same concentration of oxygen (30 per cent). At each stage, after control values had been obtained, the animals were immediately exposed to nitrous oxide for 20 minutes, and the data were recorded after 5, 10, 15 and 20 minutes of exposure to nitrous oxide. The measurements of recovery values were made after nitrous oxide had been discontinued for about 15–30 minutes.

Measurements were started after the animals had been exposed to halothane for at least two hours. When nitrous oxide was added to the stable halothane anesthesia the inspired halothane concentration was reduced in order to maintain a constant alveolar concentration of halothane.[7, 8] Thus, the same alveolar concentration of halothane was maintained before, during, and after the addition of nitrous oxide. In the final stage, halothane was discontinued for at least 90 minutes. The study proceeded only when the end-tidal halothane concentration approached zero (<0.05 per cent) and

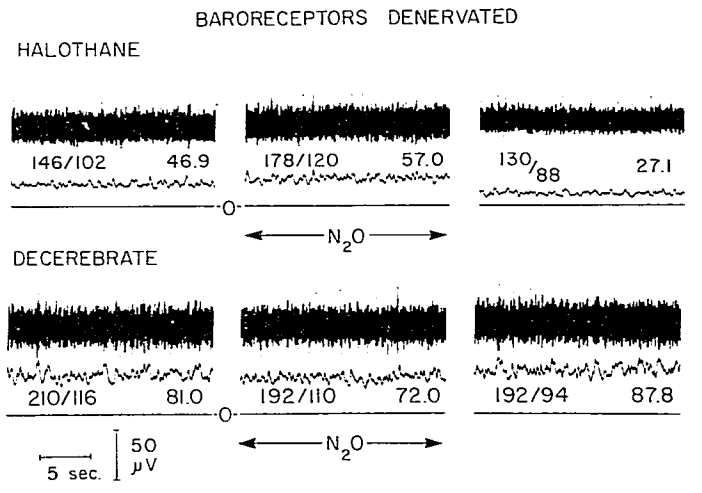

FIG. 4. As in figure 3, baroreceptors denervated.

arterial blood pressure and neural activity were stable. Nitrous oxide was then administered by simply substituting it for nitrogen.

All data were tested with Student's t test for paired data, with $P < 0.05$ taken to be significant. The data are expressed as mean ± SE.

Results

Sympathetic nerve action potentials recorded from preganglionic splanchnic nerves showed continuous spontaneous discharges combined with respiratory and cardiac rhythms. After denervation of the carotid and aortic baroreceptor nerves, the discharge pattern was modified; the pattern manifested a more continuous discharge, and usually the discharge rates were higher than before denervation (fig. 1). Figures 3 and 4 demonstrate the effect of nitrous oxide on splanchnic-nerve discharge. Figure 3 shows the effects in a single animal with intact baroreceptor nerves, before and after decerebration. In figure 4, the same procedures following baroreceptor denervation are shown. As can be observed in figures 5 and 6, changes in splanchnic-nerve activity showed a good positive correlation with changes in arterial blood pressure. Tables 1 and 2 show the control values and the results after administration of nitrous oxide in baroreceptor-intact and debuffered cats, respectively.

The end-tidal CO_2 concentration was maintained in the range of 4.0–5.0 per cent. All data reported were obtained at Pa_{CO_2}'s below 44 torr and at Pa_{O_2}'s above 100 torr. Pa_{CO_2}'s in all cats during control periods before the administration of nitrous oxide were 29.4 ± 1.1 torr, while the values of the arterial blood gases during administration of nitrous oxide were Pa_{CO_2} 32.7 ± 0.9 torr and Pa_{O_2} 120.4 ± 9.2 torr, with *p*H 7.36 ± 0.03. This reflects an appreciable increase in Pa_{O_2} with the uptake of nitrous oxide, a change observed to be equal in all experimental groups (table 3). The esophageal temperatures during measurements were 36.9 ± 0.14 C.

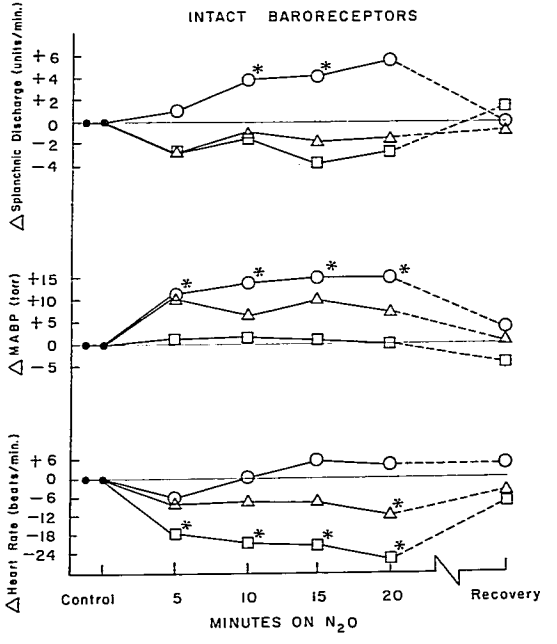

FIG. 5. Changes of nerve activity, mean arterial blood pressure, and heart rate following administration of nitrous oxide to cats with intact baroreceptors. ○—○, intact brain, halothane anesthesia; △—△, decerebrate, halothane anesthesia; □—□, decerebrate, no anesthetic. * = Significantly different from control.

ANIMALS WITH INTACT BRAINS

The addition of nitrous oxide to steady-state halothane anesthesia (0.9 per cent) caused a significant increase in sympathetic activity (+13 per cent, $P < 0.025$). This change was accompanied by elevated mean arterial blood pressure (+15 per cent, $P < 0.001$) and a moderate increase in heart rate (table 1). The changes were further magnified when the baroreceptors were denervated (table 2). Despite the already-elevated control values (resting level) the absolute changes in neural discharge and mean arterial pressure were even more pronounced when nitrous oxide was added (figs. 5 and 6). Increases in neural activity and mean arterial pressure 5, 10, 15 and 20 minutes after exposure to nitrous oxide were all significant statistically.

DECEREBRATE ANIMALS

Decerebration with steady-state halothane anesthesia caused marked decreases in the resting level of the action potentials (almost 50 per cent) in both intact and debuffered preparations (tables 1 and 2). The administration of nitrous oxide produced no significant change in neural activity or blood pressure in either intact or debuffered animals, but it caused significant declines in the heart rates of those with intact baroreceptors (table 1).

When halothane was eliminated from the decerebrate preparation, the resting level of neural activity increased markedly from 17.3 to 40.4 units/min (133 per cent) in animals with intact baroreceptors. In the animals which were debuffered and decerebrated, arterial pressures began to rise sharply 15–20

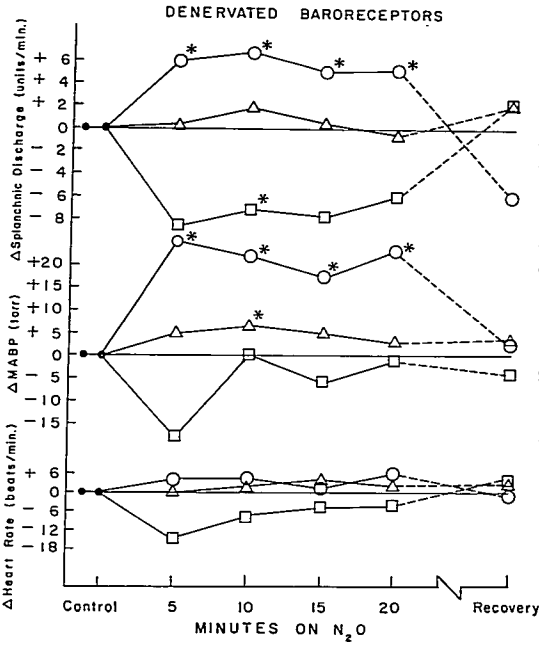

FIG. 6. As in figure 5, cats with denervated baroreceptors.

minutes after halothane was discontinued, and often overshot 200 torr. Because of the impending danger of high pressure in the brainstem and rupture of blood vessels leading to massive hemorrhage, the blood pressure was controlled by withdrawing blood through the femoral-artery catheter and later reinfusing it through the vein as needed to maintain systolic pressure below 170 torr prior to administration of nitrous oxide. The amounts of blood withdrawn ranged from 12 to 67 ml, averaging 24.3 ± 8.2 ml. Under these conditions, splanchnic activity increased from 22.9 to 64.7 units/min (182 per cent) following elimination of halothane in debuffered decerebrate animals.

Inhalation of nitrous oxide in these preparations depressed all neural activity. Heart rates of animals with intact baroreceptors were markedly depressed (−12 per cent, $P < 0.01$), and there was a significant decrease in splanchnic-nerve discharge in the debuffered animals (−9.3 per cent, $P < 0.05$). Arterial pressures showed no significant alteration (tables 1 and 2).

CHANGES DURING RECOVERY

Recovery values were measured after administration of nitrous oxide had been discontinued for 18.0 ± 0.6 minutes. In animals with intact baroreceptors, neural activity, blood pressure, and heart rate recovered almost to control levels (fig. 5). In those with denervated baroreceptors the pattern differed. Although blood pressure and heart rate recovered to near control levels, neural activity did not. When, before decerebration, nitrous oxide increased sympathetic activity in the debuffered animals, the recovery values were below control. On the other hand, after decerebration nitrous

TABLE 1. Effects of 70 Per Cent Nitrous Oxide, Baroreceptors Intact

	Time (min)	Number of Cats	Mean Arterial Blood Pressure (torr) (Mean ± SE)	Heart Rate (min^{-1}) (Mean ± SE)	Integrated Splanchnic Discharge* (Per Cent Δ) (Mean ± SE)	Halothane End-expired Concentration (Per Cent) (Mean)
Intact brain, halothane anesthesia	Control	10	103.8 ± 7.9	183.0 ± 9.4	(34.8 ± 6.1)	0.89
	5	9	112.2 ± 8.3†	181.3 ± 8.5	6.0 ± 5.3	0.94
	10	10	117.8 ± 7.9‡	183.3 ± 7.9	10.2 ± 3.6†	0.91
	15	10	119.0 ± 8.0§	188.4 ± 7.9	13.4 ± 5.2†	0.89
	20	8	117.5 ± 9.9‡	192.0 ± 10.2	17.0 ± 8.0	0.89
	Recovery	10	107.4 ± 6.3	186.3 ± 8.1	0.3 ± 7.3	0.86
Decerebrated, halothane anesthesia	Control	5	83.2 ± 8.4	159.6 ± 8.4	(17.3 ± 4.9)	0.90
	5	4	91.0 ± 13.4	154.5 ± 10.5	−16.0 ± 3.2	1.01
	10	5	89.2 ± 13.0	152.4 ± 9.9	−2.8 ± 6.2	0.97
	15	4	90.5 ± 16.0	154.5 ± 12.5	−2.8 ± 9.4	0.91
	20	5	90.0 ± 12.3	147.6 ± 9.9†	−3.1 ± 7.3	0.91
	Recovery	5	83.6 ± 10.2	154.8 ± 6.9	7.2 ± 13.4	0.91
Decerebrated, no background anesthetic	Control	6	126.0 ± 9.9	189.0 ± 5.5	(40.4 ± 10.4)	0
	5	5	126.0 ± 12.8	168.0 ± 10.2†	−7.1 ± 3.2	0
	10	6	128.0 ± 10.1	168.0 ± 8.2‡	−4.6 ± 2.0	0
	15	6	127.3 ± 10.5	167.0 ± 9.6‡	−7.3 ± 5.1	0
	20	4	131.5 ± 14.3	156.0 ± 10.9†	−5.0 ± 3.4	0
	Recovery	6	121.6 ± 7.9	181.0 ± 10.2	2.5 ± 4.9	0

* Control values of the integrated splanchnic discharge, expressed as units/min, in parentheses.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

oxide depressed neural activity in the same animals, but during recovery it increased to above control (fig. 6). No attempt was made, therefore, to "correct" the observed values for a supposed drift of the baseline values.[9] Figure 7 illustrates typical changes in the responses to nitrous oxide at different stages of the experimental preparations in a single animal.

CARDIAC EFFECTS

The addition of nitrous oxide to steady-state halothane anesthesia generated a very high incidence of cardiac arrhythmias when the brain was intact. Principally ventricular premature beats, they occurred in five of seven debuffered animals (71 per cent) and in two of ten of those with intact baroreceptors (20 per cent). Decerebration abolished all the cardiac irregularities in both groups.

Discussion

In the present study, efferent discharge of the preganglionic splanchnic nerve was used as an indicator of the outflow from the central sympathetic nervous system. The importance of the splanchnic nerve and the splanchnic region in blood pressure regulation is widely known, and there is much evidence validating the use of this nerve for studying the effects of circulatory changes.[10-15] The records of the compound nerve action potentials from the splanchnic nerve showed good correlation with changes in arterial pressure and heart rate (figs. 5 and 6).

The effects of nitrous oxide were examined by measuring the nerve action potentials in several experimental preparations. Halothane was chosen for a background anesthetic, and effects before and after decerebration were compared. Nitrous oxide alone, with no anesthetic, was then tested in decerebrate preparations. Our results confirmed that the addition of nitrous oxide to steady-state halothane anesthesia causes a significant increase of splanchnic-nerve activity.

There is a possibility, however remote, that the changes observed resulted from a nonspecific increment in the depth of anesthesia

TABLE 2. Effects of 70 Per Cent Nitrous Oxide, Baroreceptors Denervated

	Time (min)	Number of Cats	Mean Arterial Blood Pressure (torr) (Mean ± SE)	Heart Rate (min^{-1}) (Mean ± SE)	Integrated Splanchnic Discharge* (Per Cent Δ) (Mean ± SE)	Halothane End-expired Concentration (Per Cent) (Mean)
Intact brain, halothane anesthesia	Control	7	114.8 ± 7.4	190.2 ± 8.0	(45.0 ± 3.0)	0.87
	5	7	139.7 ± 10.1‡	194.2 ± 5.7	15.2 ± 6.0†	0.87
	10	7	136.8 ± 10.4‡	194.5 ± 5.3	15.4 ± 5.7†	0.87
	15	7	132.0 ± 11.3†	191.7 ± 6.5	11.5 ± 4.9†	0.85
	20	7	138.2 ± 11.3‡	196.2 ± 7.6	12.8 ± 5.8†	0.86
	Recovery	7	116.8 ± 8.9	189.1 ± 8.9	−11.2 ± 8.9	0.80
Decerebrated, halothane anesthesia	Control	7	77.1 ± 5.8	144.0 ± 11.7	(22.9 ± 3.3)	0.80
	5	7	82.2 ± 7.5	144.0 ± 11.7	5.3 ± 5.7	0.81
	10	7	84.2 ± 6.9†	145.7 ± 11.3	12.2 ± 8.2	0.78
	15	7	82.2 ± 6.7	147.0 ± 12.2	10.4 ± 10.5	0.80
	20	7	80.0 ± 5.1	145.7 ± 10.7	1.4 ± 10.4	0.77
	Recovery	7	79.7 ± 6.5	145.7 ± 11.7	9.6 ± 10.1	0.77
Decerebrated, no background anesthetic	Control	7	132.0 ± 7.8	234.8 ± 9.6	(64.7 ± 8.1)	0
	5	7	114.2 ± 11.9	220.8 ± 17.2	−13.2 ± 6.9	0
	10	7	132.0 ± 8.3	226.7 ± 11.7	−9.3 ± 4.4†	0
	15	7	126.5 ± 7.6	228.0 ± 10.9	−10.3 ± 5.9	0
	10	7	131.1 ± 10.0	228.8 ± 10.8	−6.5 ± 5.9	0
	Recovery	7	127.7 ± 6.1	238.1 ± 5.8	5.0 ± 8.8	0

* Control values of the integrated splanchnic discharge, expressed as units/min, in parentheses.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

secondary to the addition of nitrous oxide, rather than from a specific action of this drug. The difference may be philosophical, but one can argue first that its known properties suggest that simple deepening of anesthesia with, say, halothane, would depress, not augment, sympathetic outflow. Conversely, lightening of halothane anesthesia while adding nitrous oxide to produce a constant anesthetic potency of the mixture would constitute the withdrawal of a sympathetic depressant and vitiate the meaning of the observed stimulation. For these reasons we are forced to conclude that the effects observed represent a qualitative rather than simply a quantitative action of nitrous oxide.

This neurally mediated response can account for the sympathetic activation pattern found

TABLE 3. Changes in $Paco_2$ with Induction of Nitrous Oxide (mean ± SE)

	Intact Baroreceptors		Baroreceptors Denervated	
	Control $F_{IN_2} = 0.7$	During N_2O $F_{IN_2O} = 0.7$	Control $F_{IN_2} = 0.7$	During N_2O $F_{IN_2O} = 0.7$
Intact brain, halothane anesthesia	27.9 ± 2.3 (8)	31.2 ± 2.5 (7)	29.7 ± 2.6 (7)	33.5 ± 2.1 (7)
Decerebrated, halothane anesthesia	31.7 ± 0.2 (2)	34.0 ± 1.0 (4)	29.3 ± 3.6 (7)	32.9 ± 3.3 (7)
Decerebrated, no background anesthesia	28.3 ± 2.4 (4)	30.6 ± 0.9 (5)	31.7 ± 3.1 (5)	33.7 ± 1.9 (7)

* Measurements were made immediately before nitrous oxide was begun and 10–15 minutes after induction. Recovery values in most cases were monitored by end-tidal gas analysis only.
† Numbers in parentheses are numbers of cats.

previously in man [2-6] and in the dog.[16] These reports showed that the addition of nitrous oxide causes increased mean arterial and right atrial pressures and systemic vascular resistance, dilated pupils, increased esophageal temperature, and elevated serum norepinephrine levels.

Increased sympathetic activity induced by nitrous oxide, however, occurred only in the animals with intact brains. It disappeared after transection of the brainstem at the midcollicular level in both intact and debuffered preparations. In the decerebrate animal, nitrous oxide, on the contrary, depressed the splanchnic-nerve discharge. It is inferred through this observation that the direct effect of nitrous oxide is depressant to the vasomotor neurons at the brainstem or spinal level. Assuming nitrous oxide exerts a dual action on the central sympathetic nervous system, the predominant effect on total outflow observed in the periphery seems to depend mainly on suprapontine structures.

Traditionally, it has been believed that the major integrative mechanisms for central cardiovascular regulation reside in the lower brainstem (medullary vasomotor center), while higher neural structures, such as the hypothalamus, serve in modulating these bulbar mechanisms.[17] The activity of medullary vasomotor neurons (pressor and depressor) is also modified by impulses from the peripheral baroreceptors.[15] The vasomotor center, in turn, sends impulses to the vasomotor neurons at the spinal level, regulating the total outflow to the periphery.

Other investigators, using various techniques to record nervous activity, have found that some anesthetics affect the medullary vasomotor neurons to cause an increase in sympathetic outflow,[9, 19] while others depress them.[20, 21] The interpretation of these findings has been based on analysis of possible effects limited to the lower level of the brainstem in conjunction with baroreceptor reflex mechanisms. No quantitative comparison of anesthetic effects on components of neural activity between the suprapontine and the lower brainstem has been reported. In the present study we observed a consistent decrease in the resting level of splanchnic-nerve activity with transection at the midcollicular level. The changes showed decreases of as much as 50 per cent in both intact and debuffered animals (tables 1 and 2). This seems to demonstrate that higher neural structures participate in determining the total splanchnic-nerve outflow under the conditions of these experiments.

Studies indicate that electrical stimulation of localized areas of the hypothalamus can alter peripheral sympathetic outflow and cardiovascular activity.[22-25] These induced hypothalamic activities are also modulated by the impulses from the baroreceptor reflex system.[26, 27] There are numerous possible sites rostral to the level of decerebration that can participate in the excitation of the sympathetic autonomic system. These include areas of the neocortex, limbic system, diencephalon, and mesencephalon.[28] Thus, it seems reasonable to believe that the whole neural structure from the cortex through the hypothalamus and the midbrain to the medulla constitutes a functional unit, and the whole structure participates, to some extent, in the integrative mechanism of the lower brainstem.

The hypothalamic region is more likely to be concerned with cardiovascular control than any of the other higher structures. Ranson and Magoun [22] reported that increments in arterial pressure and other signs of sympathetic activity were observed with stimulation of the hypothalamus in the cat. Bronk *et al.*[23] also demonstrated effects of hypothalamic stimulation on sympathetic discharge. They found that as increasing intensities of stimulation, arterial blood pressure and sympathetic discharge to the heart were similarly increased. By stimulating different parts of the hypothalamus, Folkow *et al.*[29] identified regions which could preferentially increase either the epinephrine or the norepinephrine content of the adrenal medullary secretion. Hess *et al.*[30] found regular increases in blood pressure with site-specific stimulation of the hypothalamus posterior and caudodorsal to the entrance of the aqueduct of Sylvius and the field of the nucleus of the posterior commissure. Furthermore, Pitts *et al.*, by measuring single-unit discharges in the inferior cardiac nerve, clearly demonstrated interacting effects between hypothalamic stimulation and the baroreceptor reflexes. Baroreceptor stimulation depressed the sympathetic discharges evoked by hypothalamic stimulation.

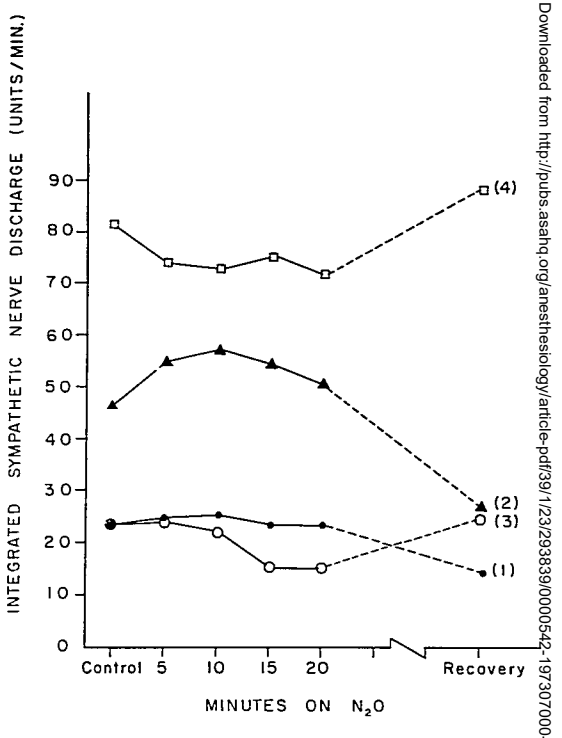

FIG. 7. Sympathetic responses to nitrous oxide at different stages of experimental preparation in a single cat. ●—●, intact brain–intact baroreceptors with halothane (0.9 per cent) anesthesia; ▲—▲, intact brain–denervated baroreceptors with halothane (0.9 per cent) anesthesia; ○—○, decerebrate–denervated baroreceptors with halothane (0.9 per cent) background; □—□, decerebrate–denervated baroreceptors with no anesthetic. Figures indicate the order in which steps are performed. Note that nitrous oxide produced sympathetic stimulation when the brain was intact, whereas its effect was depressant following decerebration.

This change is compatible with the differences between our intact and debuffered animals. Although it is speculative, nitrous oxide may stimulate the posterior hypothalamus directly. It is impossible, however, to exclude the alternative possibility that nitrous oxide may release the posterior hypothalamus from inhibition arising elsewhere.

Millar *et al.*[31] have investigated the effects of nitrous oxide on central sympathetic discharge and arterial blood pressure during halothane anesthesia by recording the nerve action potential from preganglionic cervical sympathetic nerves. They observed increased cervical sympathetic discharges in three of four animals. Their data, however, are not significant statistically. They reported a decrease in arterial blood pressure when nitrous oxide was added to halothane anesthesia, and infused norepinephrine to oppose the reduction in blood pressure in some animals, but it is unclear how markedly the arterial pressure was affected and how long it was depressed. It should be noted that in normal animals a reduction in blood pressure alone causes a significant increase in sympathetic outflow arising from the barostatic reflex system. Fortunately, this blood pressure change did not appear in our studies.

In contrast to the above data, we observed consistent rises of arterial blood pressure dur-

ing inhalation of nitrous oxide with halothane in both normal and debuffered preparations with intact brains. These rises were always accompanied by concomitant increases in sympathetic outflow. In preliminary studies using a constant inspiratory halothane concentration, transient depression of arterial blood pressure was noticed when halothane uptake was enhanced by the addition of nitrous oxide, the so-called "second-gas effect."[7, 8] Inspiratory halothane was adjusted, therefore, to maintain constant end-tidal halothane concentration. The fact that the inspiratory halothane concentration was not corrected in Millar's experiment also might have contributed to the hypotension reported. In addition, his data were collected shortly after addition of nitrous oxide (average 5.7 minutes). Any changes occurring during this period of rapid uptake are very difficult to interpret. In our experience, stability has not been obtained so soon. Millar gave 2 per cent halothane for 16 to 20 minutes before addition of nitrous oxide, while our studies were performed using light halothane anesthesia (0.8–1.0 per cent), which was continued until cardiovascular adaptation to halothane was established. We chose a light level of halothane anesthesia for the following reasons: 1) Although Smith *et al.*[2] had reported that the addition of nitrous oxide stimulates cardiovascular activity more during deep than during light halothane anesthesia, in our studies in cats we observed that deep halothane anesthesia depressed splanchnic-nerve activity substantially. When end-tidal halothane exceeded 1.6 per cent, neural activity often could not be differentiated from the noise level. 2) Decerebration itself caused a further reduction in splanchnic-nerve outflow. The technique used in the present experiment allowed us to record action potentials from the same nerve for many hours without interruption, and in most cases we were able to record from the same nerve strand before and after decerebration without moving the electrode. This would not have been possible if the effects of deep anesthesia had been superimposed on those of decerebration.

After the withdrawal of nitrous oxide, splanchnic-nerve activity returned to values not statistically different from the control for each stage. The largest individual changes were reductions seen in the debuffered preparation during halothane anesthesia (*cf.* fig. 4). It is likely that this was the result of the ablation of peripheral reflex stimulation of sympathetic discharge together with the withdrawal of the central sympathetic stimulation associated with nitrous oxide. This combination of events occurred only in this stage, of the six studied. In any event, it is clear that the reduction was not due to nerve damage, since the response always increased markedly after the subsequent decerebration (fig. 4).

Neural components which influence cardiac arrhythmias during the inhalation of some anesthetics have been previously reported. Beattie *et al.*,[32] by transecting the brainstem at various levels, concluded that the hypothalamus is important in the genesis of cardiac arrhythmias during chloroform anesthesia. Allen *et al.*[33] reported the abolition by surgical decerebration of cardiac arrhythmias caused by cyclopropane–epinephrine, as did Katz[34] for the halopropane arrhythmias. In the present study a high incidence of cardiac arrhythmias was manifested during the administration of nitrous oxide. This was particularly so in the baroreceptor-denervated animals, with an incidence of 71 per cent, but after decerebration no cardiac irregularity was evidenced in any experimental group. Presumably it is the increased catecholamine release stimulated by nitrous oxide in the brain-intact animals which triggers arrhythmias in the presence of the halogenated anesthetic. (It was, of course, impossible in these studies to test the effect of nitrous oxide in the absence of halothane and with an intact brain.)

The addition of nitrous oxide to halothane increases Pa_{CO_2} with either controlled or spontaneous ventilation.[7, 8] Kitahata has shown that the increase is real and can be attributed to a second-gas effect on CO_2.[35] In the present study it was statistically significant, but the average increase of 3–4 torr seems too small to be the cause of the substantial changes in sympathetic outflow. Furthermore, since the increase of Pa_{CO_2} was observed in every phase of the experiment (table 3), the increased sympathetic activity seen only in brain-intact animals probably should not be attributed to it. Unfortunately, altering ventilation so as to prevent the small increase in Pa_{CO_2} would intro-

duce a different but equally confounding change in baseline conditions. Addition of small amounts of CO_2 to the inspired gas during control states, its removal during the early phases of nitrous oxide uptake, and the use of constant ventilation represent a theoretical solution to the problem, but would require a technical *tour de force* that seems impractical. Further studies of the effects of the addition of small amounts of CO_2 without nitrous oxide to cats with intact brains during halothane anesthesia would help to resolve the question.

Among the various muscle relaxants, gallamine has been widely used in neuropharmacologic studies. In the present study its use was undesirable, since gallamine has been shown to exert vagal and sympathetic actions.[36, 37] Recently, in a comparative study, it was reported that pancuronium has minimal effects on the cardiovascular system and autonomic nervous system.[38] In the present experiment no change in nerve activity, blood pressure, or heart rate was observed with the use of small doses of pancuronium. This seems to support the report of Buckett *et al.*[38] and lends confidence to the use of pancuronium in studies intended to elucidate the autonomic actions of anesthetics.

In summary, the present work confirms a sympathetic stimulating action of nitrous oxide in the cat which is compatible with related observations in man, ascribes the effect to its action on the central nervous system, and demonstrates the dependence of this action on the integrity of forebrain structures. The nature of this action, be it excitatory or an inhibitory release, is as yet unknown, as is its locus.

The authors acknowledge with thanks the diligent and intelligent assistance in the performance of these studies afforded by Messrs. Mott T. Greene and Steven R. Cross.

References

1. Saidman LJ, Eger EI II: Effect of nitrous oxide and of narcotic premedication on the alveolar concentration of halothane required for anesthesia. ANESTHESIOLOGY 25:302–306, 1964
2. Smith NT, Eger EI II, Stoelting RK, et al: The cardiovascular and sympathomimetic responses to the addition of nitrous oxide to halothane in man. ANESTHESIOLOGY 32:410–421, 1970
3. Hornbein TF, Martin WE, Bonica JJ, et al: Nitrous oxide effects on the circulatory and ventilatory responses to halothane. ANESTHESIOLOGY 31:250–260, 1969
4. Bahlman SH, Eger EI II: Smith NT, et al: The cardiovascular effects of nitrous oxide in halothane anesthesia in man. ANESTHESIOLOGY 35:274–285, 1971
5. Smith NT, Eger EI II, Gregory CA, et al: The cardiovascular responses to the addition of nitrous oxide to diethyl ether in man. Can Anaesth Soc J 19:42–48, 1972
6. Smith NT, Eger EI II: Cullen BF, et al: The cardiovascular responses to the addition of nitrous oxide to fluroxene in man. Br Anaesth 44:142–149, 1972
7. Epstein RM, Rackow H, Salanitre E, et al: Influence of the concentration effect on the uptake of anesthetic mixtures: Second gas effect. ANESTHESIOLOGY 25:364–371, 1964
8. Stoelting RK, Eger EI II: An additional explanation for the second gas effect: A concentrating effect. ANESTHESIOLOGY 30:273–277, 1969
9. Price HL, Warden JC, Cooperman LH, et al: Central sympathetic excitation caused by cyclopropane. ANESTHESIOLOGY 30:426–438, 1969
10. Bradley SE: The hepatic circulation, Handbook of Physiology, Section 2, Circulation, volume 2. Edited by J Field. Washington, D. C., American Physiological Society, 1963, pp 1387–1438
11. Kremer M, Wright S: The effects on blood pressure of section of the splanchnic nerves. Q J Exp Physiol 21:319–335, 1932
12. Kahn N, Mills E: Centrally evoked sympathetic discharge: A functional study of medullary vasomotor areas. J Physiol (Lond) 191:339–352, 1967
13. Tang PC, Maire FW, Amassian VE: Respiratory influence on the vasomotor center. Am J Physiol 191:218–224, 1957
14. Dontas AS: Effects of protoveratrine, serotonin and ATP on afferent and splanchnic nerve activity. Circ Res 3:363–373, 1955
15. Gernandt B, Liljestrand G, Zotterman Y: Efferent impulses in the splanchnic nerve. Acta Physiol Scand 11:230–247, 1946
16. Smith NT, Corbascio AN: The cardiovascular effects of nitrous oxide during halothane anesthesia in the dog. ANESTHESIOLOGY 27:560–566, 1966
17. Wang SC: Bulbar regulatory mechanisms, Effects of Anesthetics on the Circulation. Edited by HL Price, PJ Cohen. Springfield, Charles C Thomas, 1964, pp 16–31
18. Alexander RS: Tonic and reflex functions of medullary sympathetic cardiovascular centers. J Neurophysiol 9:205–217, 1946
19. Skovsted P, Price HL: Central sympathetic excitation caused by fluroxene. ANESTHESIOLOGY 32:210–217, 1970

20. Skovsted P, Price ML, Price HL: The effects of halothane on arterial pressure, preganglionic sympathetic activity and barostatic reflexes. ANESTHESIOLOGY 31:507–514, 1969
21. Skovsted P, Price HL: The effects of methoxyflurane on arterial pressure, preganglionic sympathetic activity and barostatic reflexes. ANESTHESIOLOGY 31:515–521, 1969
22. Ranson SW, Magoun HW: The hypothalamus. Ergeb Physiol 41:56–163, 1939
23. Bronk DW, Pitts RF, Larrabee MG: Role of the hypothalamus in cardiovascular regulation. Res Publ Assoc Res Nerv Ment Dis 20:323–341, 1940
24. Manning JW, Peiss CN: Cardiovascular responses to electrical stimulation in the diencephalon. Am J Physiol 198:366–370, 1960
25. Folkow B, Johansson B, Öberg B: A hypothalamic structure with a marked inhitory effect on tonic sympathetic activity. Acta Physiol Scand 47:262–270, 1959
26. Pitts RF, Larrabee MG, Bronk DW: An analysis of hypothalamic cardiovascular control. Am J Physiol 134:359–383, 1941
27. Hilton SM, Spyer KM: Participation of the anterior hypothalamus in the baroreceptor reflex. J Physiol (Lond) 218:271–293, 1971
28. Smith OA Jr: Cardiovascular integration by the central nervous system, Physiology and Biophysics. Nineteenth edition. Edited by TC Ruch, HD Patton. Philadelphia, W. B. Saunders, 1966, pp 684–689
29. Folkow B, von Euler US: Selective activation of noradrenaline and adrenaline producing cells in the cat's adrenal gland by hypothalamic stimulation. Circ Res 2:191–195, 1954
30. Hess WR: Diencephalon, Autonomic and Extrapyramidal Function. New York, Grune and Stratton, 1954, pp 12–24
31. Millar RA, Warden JC, Cooperman LH, et al: Central sympathetic discharge and mean arterial pressure during halothane anesthesia. Br J Anaesth 41:918–927, 1969
32. Beattie J, Brow GR, Long CNH: The hypothalamus and the sympathetic nervous system. Proc Assoc Res Nerv Ment Dis 9:249–316, 1928
33. Allen CR, Stutzman JW, Meek WJ: The production of ventricular tachycardia by adrenaline in cyclopropane anesthesia. ANESTHESIOLOGY 1:158–166, 1940
34. Katz RL: Neural factors affecting cardiac arrhythmias induced by halopropane. J Pharmacol Exp Ther 152:88–94, 1966
35. Kitahata LM, Taub A, Conte AJ: The effects of nitrous oxide on alveolar carbon dioxide tension: A second-gas effect. ANESTHESIOLOGY 35:607–611, 1971
36. Riker WF Jr, Wescoe WC: The pharmacology of flaxedil, with observations on certain analogs. Ann NY Acad Sci 54:373–394, 1951
37. Brown BR Jr, Crout JR: The sympathomimetic effect of gallamine on the heart. J Pharmacol Exp Ther 172:266–273, 1970
38. Buckett WR, Marjoribanks CFB, Marwick FA, et al: The pharmacology of pancuronium bromide (ORG.NA 97), a new potent steroidal neuromuscular blocking agent. Br J Pharmacol Chemother 32:671–682, 1968

Renal Function

TRANSPLANTATION AND RENAL BLOOD FLOW Intrarenal distribution of blood flow was determined serially with radioxenon after renal transplantation in 20 patients whose renal arteries had been catheterized at the time of operation. Prompt diuresis was associated with greater-than-normal renal blood flow. The presence of oliguria was indicative of an absent rapid- or cortical-flow component for a variable interval before diuresis finally appeared. Acute rejection episodes were associated with significant reduction in renal cortical perfusion, one of the earliest manifestations of rejection. Some patients had intermittent reductions in blood flow, which were interpreted as representing subthreshold rejection episodes. In several patients it was impossible to remove the renal-artery catheter easily at the end of the study; this problem was treated by applying gentle traction to the catheter, cutting it at the skin level and allowing it to retract into the retroperitoneal space. Complications such as sepsis or thrombosis may or may not have been aggravated by the presence of the catheter. In no case did the use of the intra-arterial catheter or its removal result in loss of kidney function or a life-threatening episode. However, a much higher therapeutic yield is necessary before this form of monitoring can be recommended for routine use. *(Hollenberg, N. K., and others: Relationships between Intrarenal Perfusion and Function: Serial Hemodynamic Studies in the Transplanted Human Kidney, Medicine 51: 95, 1972.)*