the aggregates after TTX, 10^{-5} g/ml, was washed out.

We conclude that there is a dramatic increase in the TTX sensitivity of spontaneously beating whole hearts from chick embryos between days 4 and 7 (*12*). Similarly, the effectiveness of TTX in blocking electrically stimulated action potentials in chick heart ventricle has also been shown to be related to the age of the embryo (*13*). Tetrodotoxin is widely accepted as a specific inhibitor of the sodium channel in nerve and muscle (*6*, *14*), and yet is without effect on channels that can carry both sodium and calcium (*6*, *15*). The observed change with age in the sensitivity to TTX of hearts and aggregates is open to three interpretations: (i) The channels responsible for the early inward current of the action potential are not specific for sodium in young hearts, but the sodium specificity of these channels increases between 4 and 7 days of development. (ii) New sodium-specific channels appear between days 4 and 7. (iii) Sodium-specific channels are always present in hearts, but the access of TTX to these channels changes with development. It has been reported that cardiac tissue from 3- and 4-day embryos can be stimulated to generate action potentials in sodium-free medium (*13*), whereas cardiac tissue from later embryos (6-day and 19-day) are not excitable in 30 m*M* sodium (*4*). This evidence tends to favor an interpretation of the observations based on an increasing sodium specificity of the membrane channels rather than a change in accessibility to TTX.

An unexplained finding is the response of single cells as compared with aggregates. In most experiments single cells and aggregates were cultured from the same population of cells; hearts were trypsinized and dissociated into single cells, and then the cells were either plated or aggregated. The diminished sensitivity of isolated cells to TTX does not result merely from lack of cell-to-cell contact. Confluent monolayer sheets were also resistant to TTX, as has been noted (*16*). It may be that treatment with trypsin, or other culture procedures, alters the structure of the sodium channel at the membrane surface, and that this alteration results in the observed insensitivity to TTX. Monolayer cultured cells (singlets or confluent sheets) may be unable to restore the specificity of the sodium channels although reaggregated cells are capable of doing so. It is interesting that embryonic retinal cells in confluent monolayers lack the ability to respond to hydrocortisone induction of glutamine synthetase, whereas aggregates show an enzyme activity similar to that of intact tissue (*17*). Similarly, brain cell reaggregates have several enzymes with specific activities in the same range as in vivo embryonic brain, but much higher than in monolayers (*18*).

TERENCE F. MCDONALD
HOWARD G. SACHS
ROBERT L. DEHAAN
Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210

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11. Tetrodotoxin (Sankyo) was obtained from Calbiochem and from G. Camougis.
12. In an article which appeared after the completion of our work, K. Shigenobu and N. Sperelakis [*J. Mol. Cell. Cardiol.* **3**, 271 (1971)] reported that the spontaneous activity of embryonic chick hearts 5 days old and younger was insensitive to TTX at a dose of 2×10^{-5} g/ml, while hearts from embryos aged 8 days and older were sensitive to TTX at a dose of 1 to 4×10^{-6} g/ml.
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Possible Mechanism for the Antiarrhythmic Effect of Helium in Anesthetized Dogs

Abstract. *Breathing a mixture of 75 percent helium and 25 percent oxygen instead of 75 percent nitrogen and 25 percent oxygen reduced the occurrence of dangerous cardiac arrhythmias after ligation of the circumflex coronary artery in open-chest dogs anesthetized with pentobarbital. In dogs not subjected to circumflex ligation, the sensitivity of blood pressure, heart rate, and extrasystoles to epinephrine injected intravenously was not altered by the substitution of helium for nitrogen; however, helium did reduce the baseline heart rate and the concentration of endogenous plasma catecholamines. The antiarrhythmic effect of helium may thus be mediated by changes in sympathetic activity.*

It was recently reported that helium protects anesthetized dogs from ventricular fibrillation after ligation of coronary arteries (*1*). We repeated this work with particular attention to the control of body temperature and of other variables known to influence ventricular arrhythmias. Of 14 dogs anesthetized with pentobarbital, six control dogs breathed a mixture of 75 percent nitrogen and 25 percent oxygen (N_2-O_2), and eight dogs breathed 75 percent helium and 25 percent oxygen (He-O_2), beginning 10 minutes prior to ligation of the circumflex coronary artery. After ligation, all animals were observed for 2 hours [or until ventricular fibrillation (VF) occurred] for changes in the electrocardiogram and arterial blood pressure. During this time, arterial blood gases and *p*H were maintained by administration of bicarbonate or by changes in mechanical ventilation (*2*).

Dogs breathing He-O_2 had fewer premature ventricular contractions (Table 1) and a lower incidence of ventricular fibrillation than did the dogs breathing N_2-O_2, three of which died from this arrhythmia 15 minutes after ligation. At the time of ligation, the He_2-O_2 and N_2-O_2 groups were similar

in terms of key independent variables (shown in Table 2), and also with respect to hematocrit, serum calcium, glutamic-oxaloacetic transaminase (E.C. 2.6.1.1), and lactate dehydrogenase (not shown in Table 2). After ligation, there was no significant change in these variables except for a rise in serum potassium in the dogs breathing N_2-O_2; this was probably related to their high incidence of ventricular tachycardia. In each animal, the site of ligation was confirmed by postmortem examination. Injection of India ink or Schlesinger mass into the left main coronary artery showed that a major part of the left ventricular myocardium had been deprived of blood supply in both the control and experimental groups (*3*). Thus we confirmed the earlier finding that helium reduces postligation arrhythmias in anesthetized dogs and could not attribute this antiarrhythmic effect to experimental bias. We did not find evidence of increased collateral blood supply in the dogs breathing He-O_2 (*1*).

In order to explain the protective effect of helium, we first tested the hypothesis that breathing He-O_2 alters cardiovascular responsiveness to catecholamines. In four dogs anesthetized with 1.1 percent halothane in breathing mixtures and in which surgical procedures were limited to cannulation of the femoral artery and vein, we measured the changes in arterial blood pressure, heart rate, and premature ventricular contractions (PVC's) following successive intravenous doses of epinephrine (*4*). These responses were first measured during N_2-O_2 breathing, after which the dogs breathed a mixture of He-O_2 that yielded a similar arterial P_{O_2} (125 to 150 mm-Hg; arterial P_{CO_2} and *p*H were also stable). The cardiovascular responses to intravenous epinephrine were measured again during He-O_2 breathing. The animals then returned to breathing N_2-O_2, and the responses to intravenous epinephrine were measured a third time. The dose of epinephrine which produced two or more PVC's was similar during breathing of N_2-O_2 and of He-O_2 (1.6 ± 1.0 μg/kg, 2.0 ± 0.8 μg/kg, respectively, $P > .10$), as was the effect of a standard epinephrine dose (1 μg/kg) on mean blood pressure, pulse pressure, and heart rate. Thus, it does not appear likely that helium alters cardiovascular sensitivity to catecholamines, as judged from the responses to exogenous epinephrine.

Could helium reduce arrhythmias by altering sympathetic stimulation to the heart directly, or by decreasing circulating catecholamine levels? To test the latter possibility, we measured the concentration of epinephrine and norepinephrine in arterial plasma (*5*) in three of the dogs anesthetized with halothane, after they had breathed mixtures of N_2-O_2 or of He-O_2 for at least 30 minutes. Blood was drawn 30 minutes or more after the intravenous injections of epinephrine. Arterial concentrations of catecholamines were measured during N_2-O_2 breathing in three other dogs anesthetized with 1.1 percent halothane but not given exogenous epinephrine. In both groups, the body temperature and arterial blood gases were kept the same with N_2-O_2 and He-O_2 breathing. While the dogs were breathing He-O_2 (Fig. 1), the concentration of epinephrine and norepinephrine in their plasma was 58 and 64 percent of the corresponding values during the preceding period of N_2-O_2 breathing ($P < .025$). In some animals, catecholamine concentrations returned to their initial values when the breathing mixture was switched back to N_2-O_2, although in other instances (Fig. 1) the concentrations stayed the same or dropped further. The heart rate was slower during He-O_2 breathing than during N_2-O_2 breathing (112 and 119 beats per minute, respectively, $P < .05$), and increased to 115 beats per minute when N_2-O_2 breathing was resumed.

Table 1. Occurrence of VF and PVC's in open-chest dogs anesthetized with pentobarbital and mechanically ventilated with either 75 percent N_2 and 25 percent O_2 (N_2-O_2), or 75 percent He and 25 percent O_2 (He-O_2). Results are expressed as means ± S.D. The number of PVC's was measured 0 to 15 minutes after ligation of the circumflex coronary artery.

Arrhythmia	N_2-O_2	He-O_2
Incidence of VF	3/6	0/8*
PVC's/min	82 ± 124	4 ± 10†

* $P < .05$ (*11*). † $P < .01$.

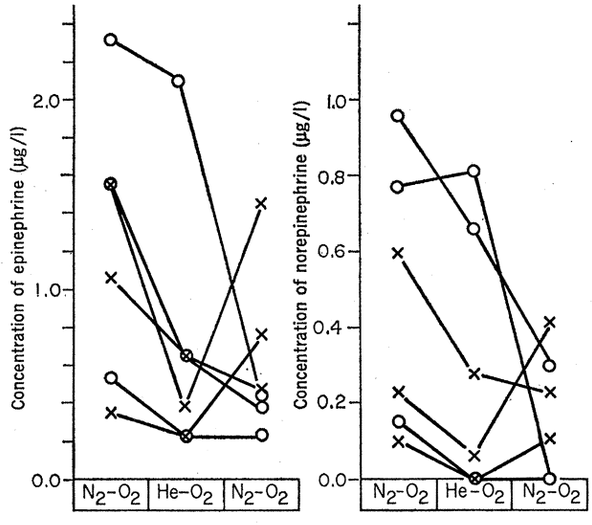

Fig. 1. Concentration of catecholamines in arterial plasma during N_2-O_2 and He-O_2 breathing in dogs anesthetized with 1.1 percent halothane in the breathing mixture. Lower concentrations of epinephrine and norepinephrine were found during He-O_2 breathing both in animals previously injected with epinephrine (×—×), and in animals that received no epinephrine (○—○).

Table 2. Similarity of independent variables known to influence ventricular arrhythmias, during and after ligation of the circumflex coronary artery in open-chest dogs that were anesthetized with pentobarbital and mechanically ventilated with either 75 percent N_2 and 25 percent O_2 (N_2-O_2), or 75 percent He and 25 percent O_2 (He-O_2). Results are expressed as means ± standard deviation, and do not differ significantly from controls except where indicated.

Indeependent variable	N_2-O_2		He-O_2	
	Initial	Ligation + 15 minutes	Initial	Ligation + 15 minutes
Body weight, kg	15.6 ± 2.8		15.1 ± 2.2	
Temperature, °C (esophageal)	38.3 ± 0.5	38.4 ± 0.5	38.2 ± 0.5	38.2 ± 0.5
Arterial blood				
Mean pressure, mm-Hg	97 ± 20	86 ± 20	108 ± 27	94 ± 39
P_{o_2}, mm-Hg	124 ± 19	124 ± 15	115 ± 17	120 ± 17
P_{co_2}, mm-Hg	38 ± 3	38 ± 4	39 ± 5	37 ± 6
*p*H	7.39 ± .04	7.38 ± .04	7.36 ± 0.04	7.37 ± 0.05
Potassium, meq/liter	3.9 ± 0.3	4.4 ± 0.3*	3.6 ± 0.3	3.8 ± 0.3

* $P < .025$.

Arterial blood pressure was unchanged by He-O_2 breathing.

The relationship between adrenergic activity and cardiac arrhythmias in experimental myocardial infarction is well known. Increases in circulating catecholamine concentrations, and an increase in sensitivity to their arrhythmic action, have both been demonstrated (*7*). The increased secretion of catecholamines after experimentally induced infarction is abolished by adrenalectomy, which is also followed by a return of normal cardiac rhythm. Cardiac sympathectomy protects against ventricular fibrillation after coronary artery ligation in anesthetized dogs (*7*). Thus, an agent which reduces sympathetic activity or circulating catecholamines might also reduce the likelihood of ventricular arrhythmias. We have some evidence of both effects and we suggest the possibility of a causal relationship.

These effects of helium are puzzling (*8*). In the traditional view, helium has no physiological effects except those attributable to its physical properties, such as its density, its thermal conductivity, or its acoustic velocity (*9*). Such properties are not likely to account for helium's antiarrhythmic action for several reasons: (i) any benefit in reduced airway resistance due to its low density would be partly offset by its higher viscosity (*10*); (ii) the effect of any change in the hydrodynamic properties of the breathing mixture was minimized, both in earlier work (*1*) and in ours, by the use of mechanical ventilation; (iii) the effect of thermal conductivity was minimized in our study by maintaining constant body temperature; (iv) maximal protection against ventricular fibrillation occurs with only 20 percent helium in the breathing mixture (*1*). This last finding also provides evidence that the putative helium effect is not merely due to the absence of nitrogen. It seems likely therefore that the antiarrhythmic property of helium in the anesthetized dog represents a pharmacologic action whose mechanism may involve altered adrenergic activity.

LAWRENCE RAYMOND
RICHARD B. WEISKOPF
MICHAEL J. HALSEY, ALAN GOLDFIEN
EDMOND I. EGER III
JOHN W. SEVERINGHAUS
Cardiovascular Research Institute and *Departments of Medicine, Anesthesia* and *Obstetrics and Gynecology, University of California, San Francisco 94122*

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Specific Triiodothyronine Binding Sites in the Anterior Pituitary of the Rat

Abstract. *Studies with* L-[^{125}I]*triiodothyronine and* L-[^{125}I]*thyroxine, and with equilibrium dialysis of plasma proteins indicate that rat pituitary binds* L-*triiodothyronine 9.8 times as strongly as it does* L-*thyroxine. Injection of even small doses of nonradioactive* L-*triiodothyronine reduces the pituitary/plasma ratio of radioactive* L-*triiodothyronine, an indication of the existence of pituitary binding sites with a limited capacity for* L-*triiodothyronine. Limited capacity binding sites for* L-*thyroxine could not be demonstrated.*

The principal site of the hormonal feedback regulating secretion of the thyroid gland appears to be situated within the cells of the adenohypophysis, although ancillary sites within the hypothalamus have not been excluded (*1*). Selective localization of L-triiodothyronine (T_3) and L-thyroxine (T_4) in the adenohypophysis of the rat has previously been noted (*2*). In order to analyze the mechanism by which thyroid hormones interact within the pituitary to modulate the secretion of thyroid-stimulating hormone (TSH) we performed experiments to define and quantitate the kinetics of interchange of T_4 and T_3 between the plasma and the adenohypophysis of the rat. The results of these studies reveal the existence of a set of pituitary binding sites, apparently specific for T_3, which have a high affinity and a low capacity for this iodothyronine.

The kinetics of interchange of thyroid hormones between tissues and plasma were analyzed according to techniques previously described (*3*). Male Sprague-Dawley rats (150 to 250 g), on a diet of Wayne laboratory chow, were injected intravenously with a combined dose of either [^{125}I]T_3 (60 to 80 μc/μg) and [^{131}I]albumin (0.5 to 1 μc/mg), or [^{125}I]T_4 (50 to 70 μc/μg) and [^{131}I]albumin (0.5 to 1