similar to that of rat liver homogenates(18).

Our inability to demonstrate the formation of *N*-hydroxy-AAF *in vivo* is consistent with the low carcinogenicity of AAF in the rainbow trout. However, in view of the low recoveries of the administered compounds as known metabolites in the urine and feces of the trout, these data do not preclude the excretion of *N*-hydroxylated metabolites in other forms or through another excretory path. Likewise, the inability of the trout liver preparations to *N*-hydroxylate AAF *in vitro* in these studies is difficult to interpret. Even in the rat and rabbit, species which excrete large amounts of *N*-hydroxy-AAF in the urine(16,17), this reaction proceeds poorly *in vitro* with liver preparations(20).

Summary. Administration of the carcinogen 2-acetylaminofluorene (AAF) orally or by injection into rainbow trout resulted in the excretion of 5- and 7-hydroxy-AAF in the urine and feces. These metabolites were also formed *in vitro* from AAF by trout liver homogenates or the microsomal fraction in the presence of TPNH or a TPNH-generating system. *N*-Hydroxylation of AAF was not demonstrated either *in vivo* or *in vitro*.

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Effect of Cocaine on Vagal Escape in the Open-Chest Dog.* (31691)

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It has been well established that cocaine can potentiate the cardiovascular responses to exogenously administered norepinephrine(1). Recent studies made in this laboratory have shown that cocaine can potentiate significantly the cardiac responses to endogenous norepinephrine released both by submaximal and supramaximal stimulation of the cardiac sympathetic nerves(2). The present investigation was conducted to explore further the

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relationship of cardiac catecholamines to cardiac rhythmicity(3-6) and to the phenomenon of vagal escape. If catecholamine release is involved in escape of the heart from vagus nerve inhibition, cocaine should promote or enhance vagal escape rhythms by potentiating the effects of endogenous catecholamines on pacemaker activity.

Methods. Dogs of either sex, weighing between 7 and 16 kg, were anesthetized with a combination of pentobarbital sodium (20 mg/kg) and barbital sodium (220 mg/kg) i.v. The animals were then placed on artificial respiration with room air. A left thoractomy was performed, the pericardium opened and a strain gauge arch sutured to the left ventricle for measurement of cardiac contractile force(7-8). Femoral arterial pressure was measured with a Statham P-23AA pressure transducer connected to an indwelling arterial catheter. Blood pressure, contractile force and standard lead II electrocardiograms were recorded simultaneously on a Grass polygraph. Heart rates were computed from the ECG tracings. Recto-sigmoid temperatures were maintained between 37 and 38°C by heating pads placed around the back and abdomen of the animals. Both vagi were isolated and cut. The crushed, distal end of the sectioned right vagus nerve was stimulated through bipolar platinum electrodes using a Grass SD-5 stimulator. Submaximal and supramaximal voltages were determined for each preparation at a frequency of 5 cycles per second (cps) by selecting the voltages required to produce minimal and maximal slowing of sinus rate. These same voltages (ranging from 1 to 20 volts) were employed throughout the remainder of the experiment. The right vagus nerve was stimulated for a period of 30 seconds with square wave pulses of 5 msec duration at frequencies of 5, 10, 20, 40 and 80 cps. These various frequencies were employed in a random order both before and after cocaine.

Cocaine hydrochloride, dissolved in Ringer's solution, was infused i.v. at a rate of 0.5 mg/kg/min for 10 minutes using a Harvard infusion pump. The dose of cocaine is expressed as the salt and the dose of l-norepinephrine bitartrate (Levophed) as the base. The results obtained in 12 experiments are expressed as mean values $\pm$ one standard error. Comparisons of results were made using the paired t test(9) and differences between values with $P<.05$ were considered as statistically significant.

Results. As shown in Table I both submaximal and supramaximal electrical stimulation of the sectioned, distal end of the right vagus nerve slowed progressively the rate of discharge from the sinoatrial (SA) node as the frequency of stimulation was increased from 5 to 20 cps for submaximal and from 5 to 10 cps for supramaximal voltages. With supramaximal stimulation, the slowing of SA rate was significantly greater at frequencies of 5 and 10 cps than with submaximal stimulation. When the right vagus was stimulated supramaximally at a frequency of 20 cps, sinus dominance was lost and a ventricular escape rhythm was established. In contrast, with submaximal stimulation, sinus dominance was maintained until the nerves were stimulated at a higher frequency, 40 cps. Despite the difference in the frequency of stimulation required for sufficient inhibition of the SA pacemaker to permit vagal escape, the magnitude of inhibition of sinus discharge required before an escape rhythm occurred was comparable for both modes of stimulation. With frequencies of 20 cps for supramaximal and 40 cps for submaximal stimulation, a regular escape rhythm was established after a variable period of cardiac asystole. The incidence and duration of asystole was greater with supramaximal stimulation than with submaximal stimulation. Furthermore, during vagal escape the rate of subsidiary pacemaker activity was significantly slower with supramaximal than with submaximal stimulation. Inspection of the ECG tracings indicated that the escape rhythm originated from the area of the A-V node.

The intravenous infusion of cocaine, 0.5 mg/kg/min for 10 minutes, decreased ventricular contractile force, blood pressure and heart rate (Table II). The cardiovascular responses to norepinephrine, 0.5 μg/kg i.v., were potentiated significantly by cocaine. The general pattern of the stimulation frequency-heart rate responses to submaximal and

TABLE I. Effect of Cocaine on Stimulus Frequency-Heart Rate Responses to Submaximal and Supramaximal Stimulation of the Right Vagus Nerves in Open-Chest Dogs (N = 12).

Frequency of vagal stimulation	Submaximal voltage					Supramaximal voltage				
	5 cps	10 cps	20 cps	40 cps	80 cps	5 cps	10 cps	20 cps	40 cps	80 cps
Before cocaine										
Sinus rate (beats/min ± S.E.)	110 ± 6	88 ± 10	56 ± 11	0	0	86 ± 5†	61 ± 6†	0†	0	0
Vagal escape rate (beats/min ± S.E.)	0	0	0	51 ± 9	74 ± 8	0	0	28 ± 6†	28 ± 8†	60 ± 10
Incidence of asystole‡	0	0	0	5/12	5/12	0	0	10/12	12/12	9/12
Duration of asystole (sec)	0	0	0	9	7	0	0	11	12	9
After infusion of cocaine, 5 mg/kg i.v.										
Sinus rate (beats/min ± S.E.)	112 ± 10	110 ± 12*	84 ± 12*	0	0	94 ± 6	78 ± 7*	0	0	0
Vagal escape rate (beats/min ± S.E.)	0	0	0	80 ± 12*	98 ± 12*	0	0	60 ± 10*	58 ± 12*	82 ± 10*
Incidence of asystole‡	0	0	0	3/12	3/12	0	0	4/12	6/12	3/12
Duration of asystole (sec)	0	0	0	5	4	0	0	5	6	5

* Significant difference from value obtained before cocaine; paired t, $P < .05$.
† Significant difference from value obtained with submaximal stimulation at the same frequency; paired t, $P < .05$.
‡ No. of animals showing asystole/No. of animals used.

TABLE II. Effect of Cocaine on Cardiovascular Responses to Norepinephrine, 0.5 μg/kg i.v., in Open-Chest Dogs (N = 12).*

	Heart rate (beats/min)	Blood pressure (mm Hg)	Contractile force (g)
Before cocaine			
Control	155 ± 5	125 ± 8	130 ± 7
Norepinephrine	176 ± 6	174 ± 9	183 ± 9
After infusion of cocaine, 5 mg/kg i.v.			
Control	141 ± 5†	112 ± 7†	115 ± 7†
Norepinephrine	205 ± 9†	215 ±13†	212 ±10†

* All values represent the mean ± S.E.
† Significant difference from values obtained before cocaine; paired t, $P < .05$.

supramaximal stimulation of the vagus was not altered by cocaine. However, after cocaine there was significantly less slowing of the sinus rate in response to submaximal and supramaximal stimulation at frequencies of 10 and 20 cps which elicited a fairly marked sinus bradycardia prior to cocaine. The maximal inhibition of sinus discharge after cocaine was comparable for both modes of stimulation, although as observed during control determinations, submaximal stimulation required a higher frequency (40 cps) to produce equivalent inhibition of sinus rate. The rates of subsidiary pacemaker discharge during vagal escape were significantly enhanced after cocaine, with the fastest rates of vagal escape occurring with submaximal stimulation. Cocaine reduced the incidence of asystole and shortened the period of asystole prior to establishment of an escape rhythm.

Discussion. It is well known that acetylcholine and catecholamines have opposing actions on the automaticity of cardiac fibers which are capable of acting as pacemakers (10). These opposing actions on pacemaker automaticity have been explained by observations that acetylcholine depresses the pacemaker potential, specifically the slow diastolic depolarization characteristic for pacemaker fibers, while catecholamines accelerate the rate of the slow diastolic depolarization(10,11). Stimulation of the vagus nerve supply to the heart can result either in sinus bradycardia or cardiac arrest by in-

hibiting SA pacemaker activity or by blocking conduction through the atrioventricular node. Acetylcholine is liberated in a quantal fashion from vagal nerve terminals by repetitive excitation. The amount of acetylcholine released and the magnitude of effect resulting is proportional to the frequency and/or intensity of nerve stimulation(12). Stimulation of the sympathetic nerve supply to the heart, on the other hand, may induce sinus tachycardia by enhancing SA pacemaker activity and transmission through the A-V node is also facilitated. Endogenous catecholamines are released from sympathetic nerve terminals and the amount of catecholamines released and the magnitude of effect resulting is dependent upon the frequency and intensity of nerve stimulation(13). The ability of the parasympathetic nervous system to influence the activity of the sympathetic nervous system and *vice versa* has been demonstrated (14,15). Furthermore, it has been reported that acetylcholine can bring about the release of catecholamines(16).

Escape from vagal inhibition can be explained simply from a functional standpoint. Inhibition of the SA pacemaker, that area of the heart which possesses the highest inherent automaticity, allows a normally suppressed "latent" or subsidiary pacemaker in the specialized conduction tissues of the heart to assume the role of pacemaker by generating impulses at its own rate of inherent automaticity. It has been found that procedures which deplete myocardial catecholamines or prevent their release also markedly retard escape(4-6). These findings may indicate that acetylcholine liberated during vagal stimulation actively stimulates subsidiary pacemaker activity *via* release of endogenous catecholamines.

The results of the present investigation support the hypothesis that adrenergic factors may play a prominent role in the phenomenon of vagal escape. This study has demonstrated that cocaine can markedly enhance the automaticity of subsidiary pacemakers and thus increase the rate of escape from vagal-induced asystole. Furthermore, there appeared to be good correlation between the frequency and intensity of vagal stimulation and both the magnitude of cocaine-induced enhancement of vagal escape rhythms and the cocaine-induced prevention of sinus slowing. Previous studies from this laboratory have demonstrated that cocaine potentiated the positive chronotropic, positive inotropic and pressor responses to endogenous norepinephrine released both by submaximal and supramaximal stimulation of the cardiac sympathetic nerves(2). Considerable evidence has accumulated in recent years to indicate that cocaine exerts its potentiating effect by blocking the uptake of norepinephrine by adrenergic nerve terminals(1), one of the physiologically important mechanisms by which the action of norepinephrine is terminated. Thus, interference with catecholamine uptake mechanisms by cocaine would make higher concentrations of catecholamines available to react with receptor sites in the heart.

The present results are consistent with the concept that acetylcholine released by vagus nerve stimulation actively stimulates pacemaker automaticity by releasing catecholamines in the myocardium. Cocaine prevents the rapid uptake of catecholamines and enhances the action of catecholamines on myocardial receptor sites. Thus, cocaine indirectly increases pacemaker automaticity and promotes and enhances escape from vagal inhibition.

Summary. Heart rate responses evoked by submaximal and supramaximal electrical stimulation of the right vagus nerves at stimulus frequencies ranging from 5 to 80 cps were determined before and after i.v., infusion of 5 mg/kg of cocaine in vagotomized, open-chest dogs. Comparison of stimulus frequency-heart rate response curves showed that cocaine enhanced the rate of ventricular escape from vagal inhibition at high frequencies of stimulation and opposed the slowing of sinus rate at lower frequencies of stimulation. Cocaine also decreased the incidence and duration of asystole induced by vagal nerve stimulation. The results of this study are consistent with the hypothesis that adrenergic factors may play a prominent role in the phenomenon of vagal escape.

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Rubella Virus Neutralization by Plaque Reduction.* (31692)

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Rubella virus assay in green monkey kidney cells utilizing the interference to enterovirus cytopathic effect (CPE)(1) has provided substantial information about the nature of this agent(2). The direct cytopathic effect of the virus on human amnion cells(3) and on cell lines derived from rabbit tissues has also been found useful in rubella virus assays (4,5,6). Interference with CPE of challenge enteroviruses(7,8,9) and Sindbis virus(10) by rubella virus has been used also to assay neutralizing antibodies to rubella virus. However, the test requires at least 7 days and the titers of antibodies obtained are dependent on a number of factors which include rubella virus dose, day of addition of challenge virus and presence of antibodies in the test sera not only to rubella virus but also to the challenge virus(7,10). Rubella virus antibody assay by the prevention of direct CPE also requires at least 7 days and requires the use of virus adapted to the cell line used(6,11,12).

The development of a hemadsorption-negative plaque assay(13) has provided a means of studying the neutralization of rubella virus more quantitatively. The present study was undertaken to develop a rapid method of detecting and quantitating rubella antibodies and to determine some of the parameters of neutralization.

Materials and methods. Tissue culture and media. African green monkey kidney (GMK) cells were cultivated by a technique previously described(14). BSC-1 cells(15) and BHK_{21} cells(16) were grown in 16-oz prescription bottles. Cells were grown in Eagle's basal medium supplemented with 10% fetal bovine serum, antibiotics (penicillin 100 units/ml and streptomycin 100 μg/ml) and 1.5 g bicarbonate/liter. Cells grown in plastic petri dishes were placed in the medium described above but containing 2% fetal bovine serum, 2.25 g/liter of bicarbonate and in addition, 50 units/ml of mycostatin. Serum and virus dilutions were made in tris buffer (pH 7.2) supplemented with 2% fetal bovine serum for virus stabilization(2).

Viruses. The strains of rubella virus used were isolated from thyroid (R-1) and lung (R-3) tissues of infants with congenital

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