RELATIONSHIP BETWEEN SYMPATHOMIMETIC AMINES AND METHYLXANTHINES INDUCING CARDIAC ARRHYTHMIAS

I. UEDA, M.D., PH.D., R. W. LOEHNING, M.D., PH.D., H. UEYAMA, M.D., PH.D.

RECENT reports have shown that catechol amines which stimulate phosphorylase activity of cardiac tissue also increase myocardial contractile force [1-6] and that sympathomimetic amines which have no effect on enzyme activity do not affect contractile force.[3, 5] A correlation apparently exists in that only those catechol amines which stimulate contractile force will initiate ventricular irregularities with certain hydrocarbon anesthetic agents. It has also been shown that the phosphorylase and contractile force stimulation by catechol amines can further be enhanced by methylxanthines [1, 7, 8] and that caffeine sodium benzoate sensitizes the myocardium to epinephrine resulting in ventricular irregularites.[9]

The above findings suggest that the glycogenolytic enzyme system may in some manner be related to catechol amine induced cardiac irregularities.

This study was undertaken to (1) clarify the relationship between methylxanthines and sympathomimetic amines in their capacity to induce cardiac irregularities and (2) to initiate an investigation of the administration of other chemical agents known to act on the glycogenolytic enzyme system, and ascertain their influence on induced cardiac irregularities.

METHOD

Mongrel dogs were anesthetized with pentobarbital 20-35 mg./kg. and given gallamine triethiodide for relaxation. The animals were respired with a constant volume ventilator (Palmer Ideal Pump) with sufficient volumes of room air to maintain end-tidal P_{CO_2} levels of approximately 35 mm. of mercury. Carbon dioxide tensions were determined with a Beckman LB1 analyzer. To eliminate the central effects of methylxanthines, this study included animals in which the spinal cord had been severed at the foramen magnum after bilateral carotid artery ligation and vagotomy. One milliliter of 1 per cent lidocaine hydrochloride was applied at the site of section of spinal cord and vagi to eliminate cardiac irregularities which occur at the time of section. Additional pentobarbital was not given after neural sections and a period of 3–4 hours was allowed for stabilization. Arterial pressure was determined from a catheter in the femoral artery and recorded simultaneously with the ECG on a Visicorder and a multichannel recording system (ENSCO).

All drugs were given in single doses through an indwelling catheter in the distal inferior vena cava. Commercially available epinephrine hydrochloride (adrenaline) was used in all experiments. Caffeine and theophylline in their base form are relatively insoluble in water at room temperature but dissolve readily when heated in a water bath and remain in solution when cooled to body temperature. Theophylline hydrochloride and caffeine sodium benzoate were used in several animals without apparent variations from their base form. Dosages of xanthines are expressed in their base form. Sodium fluoride was diluted from a stock solution. For depletion of cardiac catechol amines, reserpine (Serpasil) in doses of 0.1 mg./kg. was given intraperitoneally 24 and 48 hours before the experiments. A single dose of 0.1 mg./kg. has been reported to deplete cardiac catechol amines.[10]

RESULTS

Arrhythmias Following Epinephrine in "Spinal" and "Intact" Animals. Epinephrine 5 μg./kg. was given to 14 animals within 30 minutes after induction of anesthesia with 25–35 mg./kg. of pentobarbital. A similar dose of epinephrine was given to 9 dogs 3–4

Accepted for publication July 5, 1961. Dr. Ueda is Research Associate, Dr. Loehning is Assistant Professor, and Dr. Ueyama is Research Assistant, Division of Anesthesiology, University of Utah College of Medicine, Salt Lake City, Utah.

TABLE 1

INCIDENCE OF ARRHYTHMIA AFTER 5 μG./KG. OF EPINEPHRINE IN "INTACT" AND "SPINAL" DOGS

	Total Number of Dogs	Sinus Tachycardia	Bigeminy	A-V Block
"Intact" Animals	14	9	4	1
"Spinal" Animals	9	6	3	0

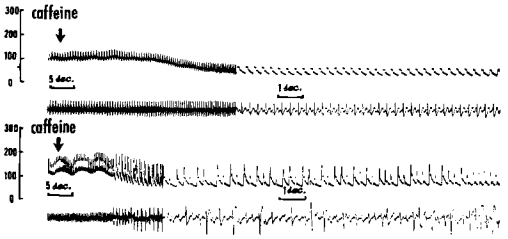

FIG. 1. Effect of caffeine 50 mg./kg. on arterial pressure and ECG (Dogs no. 8 above and 2 below).

hours after section of spinal cord at the foramen magnum following bilateral carotid artery ligation and vagotomy. Ventricular arrhythmias other than bigeminy and A-V block in one animal were not observed in either group (table 1). The arterial pressure rose from an average of 120/71 to 275/140 mm. of mercury in the "intact" group and from an average of 112/68 mm. of mercury to 260/193 in the "spinal" series.

Effect of Caffeine on ECG in "Spinal" and "Intact" Animals. Caffeine in doses of 25–100 mg./kg. was given to a series of dogs anesthetized with pentobarbital. A direct relationship was found between blood pressure, time since last adminstration of barbiturate, and severity of ventricular irregularities. Severe arrhythmias occurred in animals with blood pressures over 150/100 mm. of mercury and in animals which had not received barbiturates within 30 minutes before methylxanthine injection (fig. 1, table 2). Two animals, given 20 mg./kg. of pentobarbital followed by caffeine after 45 minutes, developed ventricular fi-

TABLE 2

IRREGULARITIES OCCURRING AFTER METHYLXANTHINE AND FOLLOWED WITHIN 15 MINUTES BY EPINEPHRINE 5 μG./KG.

Dog	Weight (kg.)	Elapsed Time (minutes) after Barbiturate	Blood Pressure before Methylxanthines	Methylxanthines (mg./kg.)	Irregularities with Methylxanthines	Irregularities with Epinephrine
			Caffeine	Caffeine		
P-1	15.0	45	220/170	35	V.F.	—
P-2	12.0	45	190/160	40	V.F.	—
2	25.0	6 hours	180/140	35	M.V.R.	V.T.
3	12.5	6 hours	130/80	35	M.V.R.	M.V.R.
4	11.5	7 hours	230/125	100	M.V.R.	M.V.R.
5	12.0	10	140/70	100	V.E.S.	V.F.
6	13.5	30	200/120	100	M.V.R.	M.V.R.
7	13.0	10	120/80	100	V.E.S.	V.F.
8	12.0	10	130/80	100	S.T.	Bigeminy
9	11.0	10	100/50	100	N.S.R.	V.F.
10	11.5	40	150/100	100	Bigeminy	V.F.
11	10.0	15	140/80	100	S.T.	M.V.R.
13	10.0	10	100/60	100	N.S.R.	V.F.
14	15.5	10	100/60	100	S.T.	V.F.
			Theophylline	Theophylline		
20	8.0	15	120/70	50	S.T.	V.F.
23	10.0	15	110/70	50	S.T.	M.V.R.
24	12.0	30	160/100	50	V.E.S.	V.F.
26	8.5	15	150/90	50	V.E.S.	V.F.
38	12.0	15	150/80	25	S.T.	V.F.

S.T., sinus tachycardia; V.E.S.; occasional ventricular extrasystoles; M.V.R., multifocal ventricular rhythm; V.T., ventricular tachycardia; V.F., ventricular fibrillation; N.S.R., normal sinus rhythm.

TABLE 3

IRREGULARITIES IN "SPINAL" ANIMALS AFTER ADMINISTRATION OF CAFFEINE OR THEOPHYLLINE 50 MG./KG. AND FOLLOWED BY EPINEPHRINE 5 μG./KG.

Dog	Weight (kg.)	Blood Pressure	Irreg. with Caffeine	Irreg. with Epinephrine	Dog	Weight (kg.)	Blood Pressure	Irreg. with Theophylline	Irreg. with Epinephrine
16	25.0	100/70	S.T.	V.F.	21	8.5	120/70	S.T.	V.T.
17	15.5	120/70	S.T.	V.F.	22	22.0	110/70	S.T.	V.T.
18	14.5	120/70	S.T.	V.T.	25	11.0	110/70	S.T.	V.F.
29	12.5	130/80	S.T.	V.F.	27	10.5	140/80	N.S.R.	V.F.
30	14.0	140/80	N.S.R.	V.F.	37	11.5	140/80	S.T.	V.F.

S.T., sinus tachycardia; V.F., ventricular fibrillation; V.T., ventricular tachycardia; N.S.R., normal sinus rhythm.

brillation. The animals in the "spinal" group did not develop irregularities following methylxanthine adminstration (table 3). Animals in the "spinal" series received only an induction dose of barbiturate and were allowed to stabilize 3–4 hours after spinal cord sections and before menthylxanthine was given.

Irregularities Following Epinephrine in Animals Pretreated with Methylxanthines. Two to fifteen minutes following administration of either caffeine or theophylline, epinephrine, 5 μg./kg. given intravenously, initiated severe ventricular irregularities including a high incidence of ventricular fibrillation (fig. 2, table 2). Animals in the theophylline series were given barbiturate 15 minutes before theophylline to prevent irregularities which may occur following administration of the methylxanthine. A high incidence of ventricular fibrillation was also observed in the "spinal" series pretreated with caffeine or theophylline and followed by epinephrine (table 3).

Effect of Caffeine and Caffeine-Epinephrine in Animals Pretreated with Reserpine. Six dogs were given reserpine 100 μg./kg. intraperitoneally 48 and 24 hours prior to injection of caffeine. These animals were sedated from reserpine and required only 10–20 mg./kg. of pentobarbital to induce anesthesia. The anesthetized animals were found to have a bradycardia and hypotension. Following the administration of gallamine 50 mg. in two animals and atropine 0.1 mg./kg. in the remaining animals, systolic pressures rose to 160–190 mm. of mercury and heart rates increased to 150–180 per minute. Ventricular irregularities did not follow administration of caffeine 50–200 mg./kg.; however, ventricular fibrillation was produced in all 5 animals when epinephrine 5 μg./kg. was adminstered after caffeine (table 4).

TABLE 4

EFFECT OF ATROPINE OR GALLAMINE FOLLOWED BY CAFFEINE AND EPINEPHRINE ON DOGS PRETREATED WITH RESERPINE

Dog	Weight (kg.)	Pento-barbital (mg./kg.)	Blood Pressure			Caffeine (mg./kg.)	Irregularities with Caffeine	Irregularities with Epinephrine (5 μg./kg.)
			After Barbiturate	After Gallamine	After Atropine			
31	10	15	110/60	190/120	—	100	S.T.	—
				180/120	—	200	S.T.	V.F.
32	11	15	100/60	170/130	—	200	N.S.R.	V.F.
47	17	20	100/65	—	180/130	50	S.T.	V.F.
48	21	10	100/70	—	160/120	50	S.T.	V.F.
49	18	15	110/80	—	180/130	50	N.S.R.	V.F.

S.T., sinus tachycardia; V.F., ventricular fibrillation; N.S.R., normal sinus rhythm.

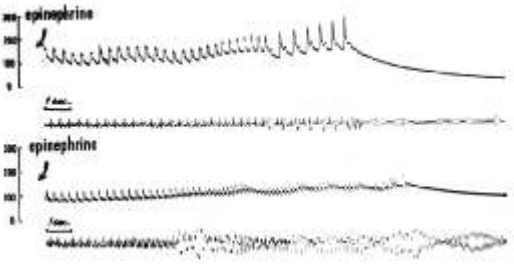

FIG. 2. Ventricular fibrillation following epinephrine 5μg./kg. in animals pretreated with caffeine (upper) and theophylline (lower). Arterial pressure in mm. Hg is also presented.

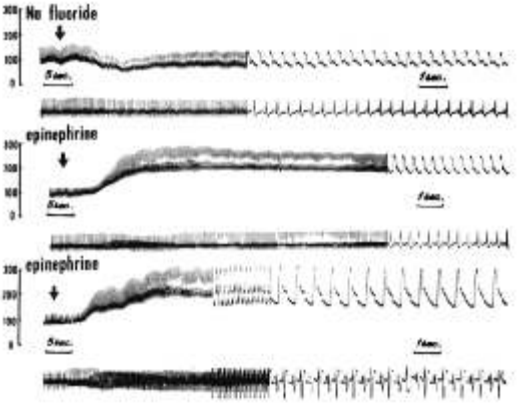

FIG. 3. Effect of epinephrine 10 μg./kg. before (lower tracing) and after (middle) sodium fluoride. A transient hypotension following sodium fluoride is shown in the upper tracing.

Methoxamine in Animals Pretreated with Caffeine. Methoxamine, a sympathomimetic amine which does not influence cardiac contractility or myocardial phosphorylase activity[3, 5] was given in doses of 0.02–0.2 mg./kg. to 5 dogs which had received caffeine. Cardiac irregularities did not occur with doses of methoxamine of 0.1 mg./kg., however, a short period of bigeminal rhythm was found in both animals receiving 0.2 mg./kg. (table 5).

Protective Effect of Sodium Fluoride on Epinephrine Induced Irregularities. Sodium fluoride, an agent shown to block enzyme activity in the glycogenolytic and glycolytic enzyme systems,[7] was given to 5 animals in doses of 50 mg./kg. A slight hypotension followed the administration of sodium fluoride but without disturbance of normal cardiac rhythm. A subsequent injection of 10 μg./kg. of epinephrine did not result in cardiac arrhythmias (fig. 3). Control animals receiving epinephrine 10 mg./kg. without sodium fluoride developed ventricular irregularities (table 6).

DISCUSSION

Dikshit[11] working with dogs reported that caffeine applied at the diencephalic site caused ventricular arrhythmias and concluded that the irregularities were caused by action on hypothalamic centers. Absence of ventricular irregularities in our "spinal" animals given methylxanthines might be attributed to (1) blockade of autonomic impulses from higher centers, or (2) the relative hypotension present after spinal cord section. Dikshit also demonstrated that barbital given intracerebrally protected the dog from irregularities produced by caffeine applied at the dience-

TABLE 5

ADMINISTRATION OF CAFFEINE FOLLOWED BY METHOXAMINE TO DOG ANESTHETIZED WITH PENTOBARBITAL AND GALLAMINE

Dog	Weight (kg.)	Blood Pressure	Caffeine (mg./kg.)	Irregularities with Caffeine	Methoxamine (μg./kg.)	Blood Pressure after Methoxamine	Irregularities
40	17	100/50	50	N.S.R.	20	150/70	S.T.
41	18	120/70	50	N.S.R.	100	260/140	S.T.
42	18	160/110	50	M.V.R.	200	250/120	Bigeminy
43	16	110/70	50	N.S.R.	200	310/150	Bigeminy
45	19.5	120/70	50	N.S.R.	100	270/140	S.T.
46	15.5	130/70	50	S.T.	100	260/130	S.T.

N.S.R., normal sinus rhythm; S.T., sinus tachycardia; M.V.R., multifocal ventricular rhythm.

TABLE 6

ABSENCE OF VENTRICULAR IRREGULARITIES FOLLOWING SODIUM FLOURIDE

Dog	Weight (kg.)	Epinephrine (μg./kg.)	Irregularities with Epinephrine	Sodium Fluoride (mg./kg.)	Irregularities with Sodium Fluoride	Epinephrine (μg./kg.)	Irregularities with Epinephrine
28	10.5	5	Bigeminy	50	N.S.R.	5	N.S.R.
33	13.0	10	Bigeminy	50	N.S.R.	10	N.S.R.
34	7.0	10	M.V.R.	50	N.S.R.	10	N.S.R.
35	8.0	10	M.V.R.	50	N.S.R.	10	N.S.R.
36	10.0	10	Bigeminy	50	N.S.R.	10	N.S.R.

N.S.R., normal sinus rhythm; M.V.R., multifocal ventricular rhythm.

phalic site. In our "intact" series barbiturate given intravenously within 30 minutes before caffeine protected the animals from irregularities. The mechanism by which barbiturate protected the animals from irregularities was not determined, whether it be through central or direct myocardial activity or from the associated hypotension. Several authors [11–13] have shown that irregularities induced by epinephrine in animals anesthetized with cyclopropane are related to the degree of hypertension. Levy [14] however, concluded from his experiments with chloroform and epinephrine that the production of arrhythmias was not related to hypertension. Cummings and Hays [15] reported that isoproterenol produces a fall in blood pressure and cardiac irregularities with cyclopropane anesthesia in dogs and concluded that hypertension *per se* was not the cause of ventricular irregularities. All of the dogs in our studies receiving reserpine developed a rise in heart rate and blood pressure following gallamine or atropine. Although the above animals were hypertensive, ventricular irregularities were not observed. Animals with the same degree of hypertension without reserpine pretreatment developed ventricular arrhythmias following caffeine (table 2). Our evidence suggests that enhanced myocardial or circulating catechol amines are indispensible in the production of cardiac irregularities with caffeine. Section of the cord with, presumably, resulting low autonomic discharge, or reserpine pretreatment to deplete body catechol amines, protected against ventricular irregularities. When epinephrine was given following methylxanthines in the animals treated in the manner described immediately above, a high incidence of ventricular fibrillation resulted.

Many authors have found that catechol amines which augment cardiac contractile force also increase glycogen phosphorylase activity.[1–6, 16] Mayer and Moran [5] have shown that epinephrine, norepinephrine, isoproterenol, and cardiac sympathetic stimulation increase myocardial contractility and enzyme activity and that methoxamine has no such effect. Kukovets and co-workers [3] found that methoxamine, metanephrine, and phenylephrine (except in large doses) did not stimulate the enzyme system or cardiac contractile force. The present study demonstrates that methoxamine in doses of 0.1 mg./kg. do not initiate cardiac irregularities with methylxanthines (table 5).

Sutherland and Cori [17] have shown that epinephrine does not stimulate phosphorylase activity by a direct action but rather through accumulation of cyclic mononucleotide, adenosine 3′5′ phosphoric acid (3′5′ AMP) (fig. 4). This accumulation is caused by accelerating the conversion of adenosine triphosphate (ATP) to 3′5′ AMP. An excess of 3′5′ AMP stimulates the conversion of active phosphorylase from the inactive form.[7, 8, 16] Phosphorylase activity may also be increased by methylxanthines as has been shown in liver and heart homogenates,[7, 8] and Hess and Haugard [1] in isolated perfused rat heart. Methylxanthines do not act by accelerating ATP to 3′5′ AMP but by inhibiting diesterase which hydrolyzes 3′5′ AMP to adenosine 5′ monophosphate (5′ AMP) (fig. 4). Mayer and Moran,[5] however, using dogs with open chests and *in situ* hearts did not observe a sig-

nificant stimulation in phosphorylase activity after theophylline hydrochloride, although they did note the heart rate was increased. These authors assumed that this variance from the work of others resulted from differences in freezing techniques, species, or drugs. We wish to propose another explanation for the failure of Mayer and Moran to show a stimulation of phosphorylase activity, namely, that these authors used superficial right ventricle strips while others used homogenates from total hearts. Samples of superficial right ventricle would contain relatively little conductive tissue. Conductive tissue has been shown to contain a high content of glycogen and the remainder of the heart relatively little glycogen.[18] Therefore, it may be expected that the conductive system would be more susceptible than the remainder of the heart to agents which influence activity of the glycogenolytic enzyme system. We suggest that it may be in the conductive system that cardiac irregularities are initiated through stimulation of glycogenolysis. When catechol amines stimulate production of 3′5′ AMP and methylxanthines block degradation to 5′ AMP, phosphorylase activity is increased leading to a rapid breakdown of glycogen (fig. 4).

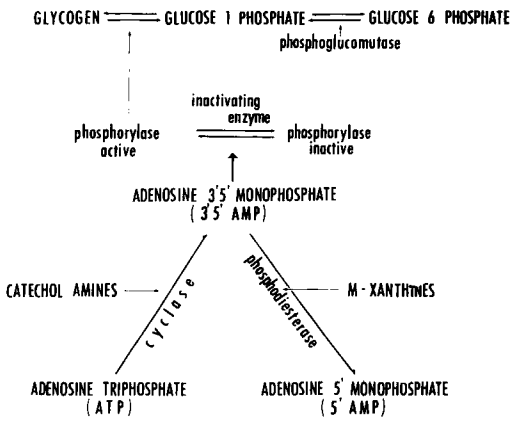

FIG. 4. Glycogenolytic enzyme system—site of action of catechol amines and methylxanthines is shown.

Administration of sodium fluoride was followed by a decreased capacity of epinephrine to induce irregularities. It has been shown that sodium fluoride increases active phosphorylase by blocking the inactivating enzyme, and that sodium fluoride inhibits glycolysis by blocking phosphoglucomutase (fig. 4).[7] The protection to ventricular irregularities demonstrated with sodium fluoride suggests that an intact glycolytic system is important to initiate arrhythmias.

Although much remains to be clarified, one might speculate on the existence of a similar mechanism of action for both hydrocarbon anesthetics and methylxanthines in their capacity to sensitize the heart to catechol amines. The hypothesis suggested above is at the present time under investigation in our laboratory.

SUMMARY

Caffeine 25–100 mg./kg., given to dogs anesthetized with pentobarbital and gallamine, initiated ventricular irregularities in animals when arterial pressures were above 150/100 mm. of mercury and the animals had not been given barbiturates within 30 minutes prior to injection of methylxanthines. Dogs were protected from ventricular arrhythmias induced by methylxanthines by: (1) barbiturate given within 30 minutes prior to the injection of methylxanthines (2) pretreatment with reserpine (3) spinal cord section at the foramen magnum and bilateral vagotomy. Intravenous epinephrine 5 μg./kg. given to the animals described above resulted in a high incidence of ventricular fibrillation. Increasing circulating or tissue catechol amine levels are thought to be necessary for the production of cardiac irregularities after intravenous methylxanthines. Methoxamine 0.1 mg./kg. did not initiate arrhythmias after caffeine. Pretreatment with sodium fluoride 50 mg./kg. protected dogs from ventricular irregularities induced by epinephrine 10 μg./kg. A possible mechanism by which methylxanthines and epinephrine produce ventricular arrhythmias has been discussed.

Supported by Grants H–5678 and RG–6677 (C1) from the National Institutes of Health, U. S. Public Health Service.

REFERENCES

1. Hess, M. E., and Haugaard, N.: Effect of epinephrine and aminophylline on phosphorylase activity of perfused contracting heart muscle, J. Pharmacol. Exp. Ther. 122: 169, 1958.

2. Mayer, S. E., and Moran, N. C.: Relationship between myocardial phophorylase and contractile force, Fed. Proc. **18**: 419, 1959.
3. Kukovets, W. R., Hess, M. E., Shanfeld, J., and Haugaard, N.: Action of sympathomimetic amines on isometric contraction and phosphorylase activity of isolated rat heart, J. Pharmacol. Exp. Ther. **127**: 122, 1959.
4. Belford, J., and Feinleib, M. R.: Phosphorylase activity of heart muscle under various conditions affecting force of contraction, J. Pharmacol. Exp. Ther. **127**: 257, 1959.
5. Mayer, S. E., and Moran, N. C.: Relation between pharmacologic augmentation of cardiac contractile force and activation of myocardial glycogen phosphorylase, J. Pharmacol. Exp. Ther. **129**: 271, 1960.
6. Lacroix, E., and Leusen, I.: Influence of sympathomimetic amines on phosphorylase activity of isolated perfused rat hearts, Arch. Int. Pharmacodyn. **76**: 482, 1960.
7. Berthet, J., Sutherland, E. W., and Rall, T. W.: Assay of glucagon and epinephrine with use of liver homogenates, J. Biol. Chem. **229**: 351, 1957.
8. Butcher, R. W., Jr., and Sutherland, E. W.: Enzymatic inactivation of adenosine 3′5′ phosphate by preparations from heart, Pharmacologist **1**: 63, 1959.
9. Mezzasalma, G., Spunti patogenetic sulle aritmie sperimentali da caffeina-adrenalina, Boll. Soc. Ital. Biol. Sper. **32**: 1637, 1956.
10. Waud, D. R., Kottegoda, S. R., and Krayer, O.: Threshold dose and time course of norepinephrine depletion of mammalian heart by reserpine, J. Pharmacol. Exp. Ther. **124**: 340, 1960.
11. Dikshit, B. D.: Production of cardiac irregularities by excitation of hypothalamic centres, J. Physiol. **81**: 382, 1934.
12. Moe, G. K., Malton, S. D., Rennick, B. R., and Freyburger, W. A.: Role of arterial pressure in induction of idioventricular rhythms under cyclopropane anesthesia, J. Pharm. Exp. Ther. **93**: 319, 1948.
13. Dresel, P. E., MacCannell, K. L., and Nickerson, M.: Cardiac arrhythmias induced by minimal doses of epinephrine in cyclopropane-anesthetized dogs, Circulat. Res. **8**: 948, 1960.
14. Levy, A. G.: Genesis of ventricular extrasystoles under chloroform with special reference to consecutive ventricular fibrillation, Heart **5**: 299, 1914.
15. Cummings, J. R., and Hays, W.: Cardiovascular studies of adrenergic and ganglionic stimulating drugs administered during cyclopropane, ANESTHESIOLOGY **17**: 314, 1956.
16. Rall, T. W., and Sutherland, E. W.: Formation of cyclic adenine ribonucleotide by tissue particles, J. Biol. Chem. **232**: 1065, 1958.
17. Sutherland, E. W., and Cori, C. F.: Effect of hyperglycemic glycogenolytic factor and epinephrine on liver phosphorylase, J. Biol. Chem. **188**: 531, 1951.
18. Uhley, H. N., Reich, S. S., and Rivkin, J.: Radioautography of conduction system of dog's heart with I^{131}, Amer. J. Physiol. **198**: 859, 1960.

CARCINOID SYNDROME In patients suspect of a serotonin releasing carcinoid, a typical effect may be provoked by the intravenous injection of epinephrine or norepinephrine. In four patients, the intravenous injection of 2 μg. of epinephrine or 10 μg of norepinephrine produced a typical facial flush and resulted in a marked rise in the free plasma serotonin levels. (*Peart, W. S., Andrews, T. M., and Robertson, J. I. S.: Carcinoid Syndrome, Lancet 1: 577 (Mar. 18) 1961.*)

VITAMIN B-15 Pangamic acid (vitamin B-15) can produce a neuromuscular blocking action in the rabbit and chicken and hypotension in the anesthetized dog. Neostigmine methylsulphate is an effective antagonist to the neuromuscular blockade produced in the rabbit. These effects appear to be qualitatively similar to those reported for thiamine hydrochloride. (*Marshall, F. N., Adamson, R. H., and Long, J. P.: Some Pharmacologic Properties of Pangamic Acid (Vitamin B-15), Proc. Soc. Exp. Biol. Med. 107: 420 (June) 1961.*)