of acetate, log y = 6.57433 – 0.002123 x; (c) for total acetate concentration per ml of rumen liquor, y = 0.08152 – 0.00006292 x; where x is time in minutes following administration of radioacetate.

The rate of change of specific activity of acetate, that is, rate of dilution, was used to compute the rate of acetic acid production. The decrease in specific activity of the radioacetic acid, due to dilution by acetic acid synthesized calculated over the straight line part of the curve (33 minutes to 455 minutes), was found to be at the rate of 50% per 130 minutes. On the basis of the total acetic acid present per ml of rumen liquor at the start of this period (33 minutes after radioacetic acid administration) and this rate of dilution over the 7-hour straight line period of isotope dilution, it was calculated that 0.126 millimole of acetic acid were synthesized per ml of rumen liquor per 7 hours. This amounts to 0.00756 g or 0.0264 Kcals of acetic acid. If one assumes a rumen content of 5 liters, this then would amount to 132 Kcals, which is approximately 30% of a 140 lb. sheep's 7-hour maintenance requirement.

Summary. It has been found by the technic of isotope dilution using acetate-1-C^{14} that the normal sheep on an all-hay ration produced acetic acid in its rumen by bacterial synthesis at the rate of 0.126 millimole per ml of rumen liquor for the 7-hour period following feeding. Radioactivity was found in the other rumen volatile fatty acid (propionic, butyric and higher acids) demonstrating their formation from acetic acid in the rumen.

1. Phillipson, A. T., *J. Exp. Biol.,* 1942, v19, 186.
2. Munch-Petersen, E., *Austr. Vet. J.,* 1957, v33, 292.
3. Danielli, J. F., Hitchcock, M. W. S., Marshall, R. A., Phillipson, A. T., *J. Exp. Biol.,* 1945, v22, 75.
4. McCarthy, R. D., Holter, J. B., Shaw, J. C., Heuter, F. G., McCarthy, Jeanne, *Maryland Agr. Exp. Sta. Misc. Pub.,* 1957, v291, 33.
5. Barcroft, J., McAnally, R. A., Phillipson, A. T., *J. Exp. Biol.,* 1944, v20, 120.
6. Oppermann, R. A., Nelson, W. O., Brown, R. E., *J. Dairy Sci.,* 1957, v40, 779.

Received April 10, 1959. P.S.E.B.M., 1959, v101.

Effect of Methylphenidate on Cardiovascular Actions of Pressor Amines. (25072)

HENRY J. POVALSKI* AND E. D. GOLDSMITH
Dept. of Biology, Graduate School of Arts and Science, and College of Dentistry, N. Y. University and Research Department, CIBA Pharmaceutical Products, Inc., Summit, New Jersey

Fröhlich and Loewi showed that cocaine potentiated pressor responses to epinephrine (1). Later, Tainter and Chang found cocaine blocked tyramine pressor responses(2). More recently, Fleckenstein and Bass(3) and Fleckenstein and Stöckle(4) demonstrated that cocaine altered responses of cat nictitating membrane to a series of sympathomimetic amines of the phenylalkylamine type, in such a way that contractions produced by one group of amines were augmented, contractions elicited by a second group were not affected and contractions produced by a third group were decreased. Recently Maxwell, *et al.*(5) demonstrated that the central nervous system stimulant, methylphenidate, potentiates pressor actions induced by epinephrine and norepinephrine while blocking and antagonizing pressor responses to amphetamine and ephedrine, respectively. In consideration of this, and the fact that the structures and primary pharmacological characteristics of cocaine and methylphenidate are markedly different, an attempt has been made to ascertain whether the effects of methylphenidate on cardiovascular actions of various phenylalkylamines would closely parallel those of cocaine.

Materials and methods. Mongrel dogs of both sexes weighing 7 to 15 kg were used. The anesthesia was Na pentobarbital, an initial dose of 30 mg/kg intraperitoneally fol-

* Taken from dissertation submitted in partial fulfillment of requirements for Degree of Master of Science.

TABLE I. Effect of 15 mg/kg Methylphenidate I.V. on Mean Blood Pressure of the Dog.*

Control B.P. (mm Hg)	B. P. after methylphenidate (mm Hg)
120	115
130	120
100	100
120	100
110	100
90	100
105	105
100	100
120	120
115	115
111 ± 3.86†	108 ± 2.81†

* The difference between avg values was significant only at the 43% level by "t" test. The authors consider a level of significance of 5% or better to be required.
† Avg ± S.E.

lowed by 0.1 mg/kg/min infusion throughout the experiment. Blood pressures were determined by an Anderson glass-capsule manometer(6). Injections were administered intravenously. All drugs were in aqueous solution. Experiments were conducted in two ways: 1) An initial low dose of each pressor amine selected to produce a blood pressure elevation of at least 50 mm Hg was given. Fifteen to 30 minutes later 15 mg/kg of methylphenidate HCl was administered. Seven to 10 minutes elapsed before repeating the initial doses. In cases where pressure responses to repeated control doses of amines were blocked, dosage was increased 2 to 40 times to induce the return of amine responses to control levels. When repeated control responses were potentiated or were not altered by methylphenidate, no multiple doses were given. 2) To rule out the possibility of tachyphylaxis in studies of amines other than catecholamines, experiments were run in which the effects of initial doses and doses of amines given only after methylphenidate administration, were obtained in separate animals.

Results. Effect of methylphenidate on blood pressure. Administration of methylphenidate caused no significant change in mean blood pressure. Table I is a comparison of mean blood pressure levels before and after 15 mg/kg methylphenidate I.V. in 10 anesthetized dogs.

Pressor amine responses potentiated or not affected by methylphenidate. Pressor response to d - 1 norepinephrine HCl 4γ/kg, 1-epinephrine bitartrate 4γ/kg, epinine HCl 25γ/kg, cobefrine HCl 4γ/kg, synephrine tartrate 0.5 mg/kg and neosynephrine HCl 25γ/kg were obtained in 3 to 4 animals for each amine. All responses were at least 50 mm Hg. After administration of 15 mg/kg of methylphenidate, the above doses of amines were repeated with results that pressor responses to norepinephrine (Fig. 1A), epinine and cobefrine were clearly potentiated, while pressor actions of epinephrine, synephrine and neosynephrine were not significantly affected (Fig. 1A and Table II). These 6 amines will be referred to as Group I.

Pressor amine responses reversibly blocked by methylphenidate. Control pressor responses to ephedrine HCl 0.6 mg/kg, propadrine HCl 0.5 mg/kg and paredrine HBr 0.25 mg/kg were obtained in 4 to 9 animals for each amine (Table II). The doses were repeated in 2 to 7 of these animals after injection of 15 mg/kg methylphenidate and their effects were markedly antagonized. This blockade, however, could be overcome by increasing the doses of amines from 2 to 20 times (Fig. 1B). It is interesting that the blockade of ephedrine by methylphenidate could be overcome by increasing the dose level, despite the fact that animals are well known to exhibit tachyphylaxis to ephedrine.

In the second type of experiment 2 to 3 dogs were given above doses of ephedrine, propadrine and paredrine, for the first time, only after administration of methylphenidate. As in above experiments the pressor responses were markedly diminished, and this antagonism could be overcome by increasing the doses of amines from 2 to 20 times. Since the results of both experiments were similar the total number of animals following pretreatment with methylphenidate were grouped together and analyzed in Table II.

These 3 amines will be referred to as Group II.

Pressor amine responses irreversibly blocked by methylphenidate. Control responses to amphetamine sulfate 0.3 mg/kg, methamphetamine HCl 0.5 mg/kg, vonedrine HCl 0.5 mg/kg, tyramine HCl 0.25 mg/kg

TABLE II. Effect of Methylphenidate on Pressor Amine Responses.

Amine	Dose/kg (γ)	Controls: No. of animals	Controls: Mean pressor response ± S.E. (mm Hg)	Methylphenidate pretreated, 15 mg/kg I.V.: No. of animals	Methylphenidate pretreated, 15 mg/kg I.V.: Mean pressor response ± S.E. (mm Hg)	Level of signif. of differences in pressor responses before and after methylphenidate*† (%)
Group 1			x_1		x_2	
Norepinephrine	4	5	85 ± 12.04	5	135 ± 13.04	2
Epinephrine	4	7	80 ± 11.95	7	106 ± 9.91	13
Epinine	25	7	73 ± 8.79	7	109 ± 10.49	2
Cobefrine	4	5	75 ± 9.75	5	113 ± 7.00	1.4
Synephrine	500	4	86 ± 21.64	4	79 ± 20.65	80
Neosynephrine	25	3	67 ± 12.02	3	73 ± 4.42	60
Group 2						
Ephedrine	600	6	85 ± 10.88	4	11 ± 3.75	<.1
Propadrine	500	7	93 ± 5.86	5	20 ± 2.42	"
Paredrine	250	9	85 ± 8.29	9	2 ± 1.21	"
Group 3						
Amphetamine	300	4	91 ± 13.90	4	1 ± 1.25	<.1
Methamphetamine	500	5	73 ± 2.00	4	1 ± 1.25	"
Vonedrine	500	6	77 ± 13.27	4	1 ± .72	.7
Tyramine	250	6	94 ± 13.74	4	4 ± 2.40	<.1
Phenylethylamine	500	4	108 ± 12.50	5	10 ± 1.58	"
Paredrinol	250	6	98 ± 15.79	4	1 ± 1.25	.1

* Changes considered significant when level of significance is equal to or better than 5%.

† Significance of results determined by "t" test of Snedecor(9) $t = \frac{x_1 - x_2}{S_{x_1 - x_2}}$.

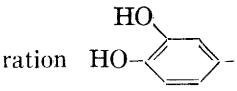
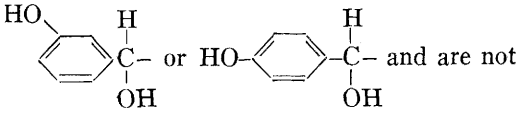
x = mean.

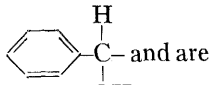
(Fig. 1C), phenylethylamine 0.5 mg/kg and paredrinol 0.25 mg/kg were obtained in 4 to 7 animals for each amine. In 2 to 3 of these animals, for each amine doses were repeated after injection of 15 mg/kg of methylphenidate and the responses were severely inhibited. However in contrast to amines in Group II this blockade could not be overcome by increasing the dose levels from 2 to 40 times (Fig. 1C).

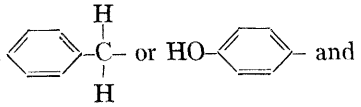
In a second set of experiments, comprising 2 animals for each amine the above doses were given for the first time only after methylphenidate administration. The results were similar to those where the amines elicited markedly reduced pressor responses which could not be surmounted by increasing the dose levels. Since the results of both experiments were similar, all animals following pretreatment with methylphenidate were grouped together and analyzed as a unit (Table II).

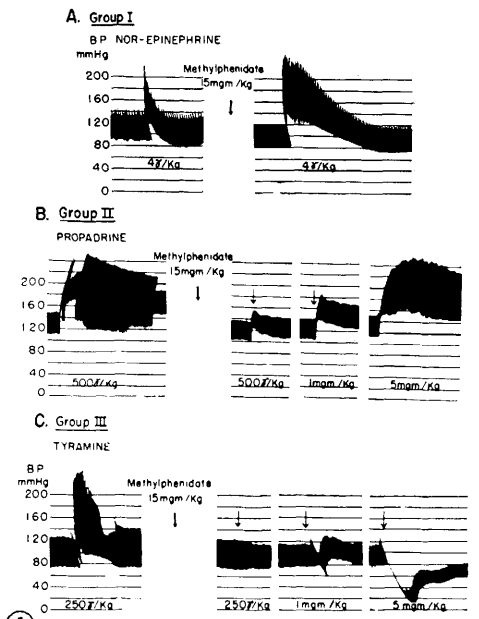
These amines will be referred to as Group III.

Discussion. Our experiments show that methylphenidate alters cardiovascular responses to a series of pressor phenylalkylamines in 3 ways: 1) by potentiating or not affecting amine pressor responses; 2) by reversibly blocking amine pressor responses; 3) by irreversibly blocking amine pressor responses.

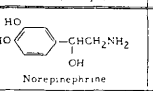
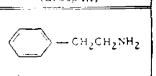
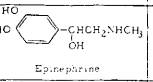
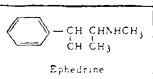
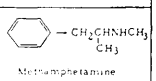
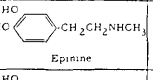
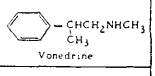
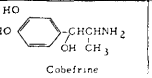
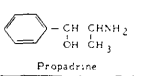
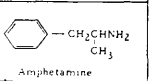
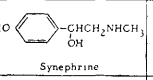
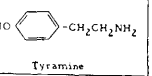
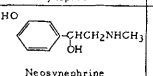
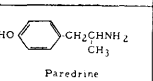
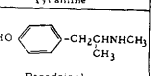
The amines in group I possess the configuration  and are augmented, or  and are not significantly affected (Fig. 2).

Group II amines possess  and are blocked but this antagonism can be overcome by giving higher doses (Fig. 2).

Group III is composed of amines with the configurations  and



①

Potentiated or Not Affected (Group I)	Reversibly Blocked (Group II)	Irreversibly Blocked (Group III)
 Norepinephrine		 Phenylethylamine
 Epinephrine	 Ephedrine	 Methamphetamine
 Epinine		 Vonedrine
 Cobefrine	 Propadrine	 Amphetamine
 Synephrine		 Tyramine
 Neosynephrine	 Paredrine	 Paredrinol

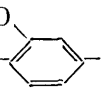
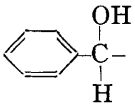
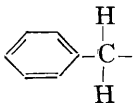
②

FIG. 1. Effect of intrav. methylphenidate on pressor responses to phenylalkylamines in mongrel dogs, anesthetized with pentobarbital.

FIG. 2. Effect of methylphenidate on pressor responses to sympathomimetic amines.

are blocked to an extent which cannot be overcome by administration of higher doses (Fig. 2). Paredrine with its configuration containing HO–  is the only sympathomimetic amine which has a structure belonging in group III but is affected by methylphenidate as are Group II amines (Fig. 2). No obvious explanation can be given for this seemingly anomalous occurrence.

This differential effect of methylphenidate, a central nervous stimulant, on pressor amine responses assumes more generalized importance with the knowledge that 3 other compounds of different primary pharmacological action and chemical configuration, also influence the activity of pressor amines similarly:

1) Fleckenstein and Bass(3) and Fleckenstein and Stöckle(4) have shown that intravenous injections of cocaine, a local anesthetic, affects responses of cat nictitating membrane to amines in such a way as to create 3 classifications. They are: 1) pyrocatechol derivatives possessing  which are enhanced, 2) intermediate substances with  which are not essentially affected and 3) neurosympathicomimetics containing  which are greatly antagonized.

2) Recently 2 compounds have been shown to affect pressor amine responses in almost identical manner as methylphenidate. One is the anti-hypertensive agent hexahydro-1-azepinepropionamidoxime (Su-4029) demonstrated by Maxwell *et al.*(7). The other reported by same authors(8) is the tranquilizing agent, reserpine.

The degree of similarity in influence of these 4 drugs on pressor amine activity is very notable. The structures of these compounds and their prominent pharmacological characteristics, as yet, offer no clue as to this parallelism.

Summary. Methylphenidate alters the pressor responses in the dog to a series of phenylalkylamines. The amines affected by this central nervous system stimulant are influenced in such a way as to fall into 3 distinct categories, *i.e.*, 1) potentiated or not al-

tered, 2) reversibly blocked and 3) irreversibly blocked. The differences in structure of pressor phenylalkylamines comprising the 3 groups are reviewed along with reference to other compounds which affect pressor action of these amines in a similar manner.

Grateful acknowledgement is expressed to Dr. Robert A. Maxwell for his valuable advice.

1. Fröhlich, A., Loewi, O., *Arch. Exp. Path. u. Pharmakol.,* 1910, v62, 158.

2. Tainter, M. L., Chang, D. K., *J. Pharm. and Exp. Therap.,* 1927, v30, 193.

3. Fleckenstein, A., Bass, H., *Arch. Exp. Path. u. Pharmakol.,* 1953, v220, 143.

4. Fleckenstein, A., Stöckle, D., *ibid.,* 1955, v224, 401.

5. Maxwell, R. A., Plummer, A. J., Ross, S. D., Paytas, J. J., Dennis, A. D., *Arch. Int. Pharmacodyn.,* 1957, v112, 26.

6. Anderson, F. F., *J. Lab. and Clin. Med.,* 1941, v26, 1520.

7. Maxwell, R. A., Plummer, A. J., Daniel, A. I., Schneider, F., Povalski, H., *J. Pharm. and Exp. Therap.,* 1958, v124, 127.

8. Maxwell, R. A., Povalski, H., Plummer, A. J., *ibid.,* 1958, v125, 178.

9. Snedecor, G. W., *Statistical Methods,* 5th Edi., 1956, Iowa State College Press, Ames, Iowa, p90.

Received April 13, 1959. P.S.E.B.M., 1959, v101.

Effect of Oxygen Tension on Sodium Transport Across Isolated Frog Skin. (25073)

HERMAN FALSETTI (Introduced by Wallace O. Fenn)

Dept. of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The transport of the sodium ion across the frog skin is an example of active transport (Huf, Wills and Cooley(1); Ussing and Zerahn(2)) and requires an adequate supply of oxygen. Production of the necessary energy depends on the integrity of oxidative enzymes in the skin. Cass(3) has shown that high oxygen tensions (8-35 atmos.) inhibit carbon dioxide production by frog muscle, and Gerschman *et al.*(4) have postulated that high oxygen tensions, like ionizing radiation, damage enzyme systems, possibly by increasing the concentration of free radicles in the tissue. The present study was undertaken to see whether a high pressure of oxygen inhibits sodium transport in the frog skin as it does other vital processes.

Method. The preparation used was essentially that described by Huf *et al.*(1). The skin was stripped from the hind legs of a frog and tied off at the distal ends to make 2 bags. Each bag was suspended "inside out" from the end of a piece of glass tubing inserted in the neck of the bag. Well-aerated Ringer's solution was introduced into the skin bag and the bag was completely immersed in similar Ringer's solution. The levels of the 2 solutions were the same inside and out to avoid hydrostatic effects. The preparation from one leg of each frog was placed in a simulated bomb kept at room pressure and temperature ($19 \pm 1°C$) for 24 hours as a control and the skin bag from the other leg was placed for an equal period in the same room in a bomb where the pressure was elevated to 8-12 atmospheres using either 100% oxygen or 2.5% oxygen in nitrogen. Gas was introduced slowly over a 20 minute period to minimize rise in temperature due to compression. All gas that entered the bomb was passed through a carbon dioxide absorber to avoid depression of sodium transport by high carbon dioxide tension(2). The volume and sodium content of the Ringer's solution in the bag was measured at beginning and end of experiment. The area of the skin was measured by placing it on squared paper and counting the squares. Sodium analysis was carried out by flame photometry. The transport of sodium out of the bag was calculated as microequivalents per square centimeter per hour. (μ eq/cm^2/hr.).

Results. The transport in each experimental preparation was calculated as a per-