## 8412

# 8412<br>AUGMENTATION OF PHARMACOLOGICAL PROPERTIES OF<br>TECHOLAMINES BY O-METHYL TRANSFERASE INHIBITORS 8412<br>AUGMENTATION OF PHARMACOLOGICAL PROPERTIES OF<br>CATECHOLAMINES BY O-METHYL TRANSFERASE INHIBITORS AUGMENTATION OF PHARMACOLOGICAL PROPERTIES OF<br>TECHOLAMINES BY O-METHYL TRANSFERASE INHIBITOI<br>D. W. WYLIE, S. ARCHER AND A. ARNOLD

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New Yo<br>Received for publication<br>Maclagan and Wilkinson (1954) first showed<br>at phenolic hydroxyl groups could be methyl-Received for pul<br>Maclagan and Wilkinson (1954) first show<br>that phenolic hydroxyl groups could be meth<br>ated in man. Subsequently, Axelrod (19 Maclagan and Wilkinson (1954) first showed<br>that phenolic hydroxyl groups could be methyl-<br>ated in man. Subsequently, Axelrod (1957)<br>demonstrated methylation at the 3-hydroxy Maclagan and Wilkinson (1954) first showed<br>that phenolic hydroxyl groups could be methyl-<br>ated in man. Subsequently, Axelrod (1957)<br>demonstrated methylation at the 3-hydroxy<br>position of epinephrine and norepinephrine *in* position of epinephrine and norepinephrine *in*<br>
with a behavior of epinephrine and norepinephrine *in*<br>
witro and in vivo, and later (1958) that the amines *eritrophenome in and subsequently, Axelrod (1957)*<br>demonstrated methylation at the 3-hydroxy<br>position of epinephrine and norepinephrine *in*<br>*vitro* and *in vivo*, and later (1958) that the amines<br>undergo oxidative deamin demonstrated methylation at the 3-hydroxy<br>position of epinephrine and norepinephrine in<br>witro and in vivo, and later (1958) that the amines<br>undergo oxidative deamination only after they<br>have been methylated. The m-O-methy position of epinephrine and norepinephrine<br>position of epinephrine and norepinephrine<br>witro and in vivo, and later (1958) that the<br>undergo oxidative deamination only aft<br>have been methylated. The m-O-methy<br>nephrine (norme position of ephrephrine and notephrephrine<br>vitro and in vivo, and later (1958) that the ami<br>undergo oxidative deamination only after t<br>have been methylated. The m-O-methylnor<br>nephrine (normetanephrine) formed by meth;<br>tio betto and the two, and later (1500) that the animes<br>undergo oxidative deamination only after they<br>have been methylated. The m-O-methylnorepi-<br>nephrine (normetanephrine) formed by methyla-<br>tion possesses only  $\frac{1}{2600}$  have been methylated. The *m*-O-methylnorepinephrine (normetanephrine) formed by methylation possesses only  $\frac{1}{2600}$  of the activity of nor-<br>epinephrine, suggesting that this metabolic process is the means of inactiva mephrine (normetanephrine) formed by methyla-<br>tion possesses only  $\frac{1}{2600}$  of the activity of nor-<br>epinephrine, suggesting that this metabolic oprocess is the means of inactivation of these  $\frac{4}{3}$ <br>amines (Evarts *e* tion possesses only  $\frac{1}{2600}$  of the activity of nor-<br>epinephrine, suggesting that this metabolic<br>process is the means of inactivation of these<br>amines (Evarts *et al.*, 1958).<br>An O-methyl transferase (OMT) inhibitor<br>wo in possesses only  $>500$  of the activity of hori-<br>inephrine, suggesting that this metabolic<br>ocess is the means of inactivation of these<br>innes (Evarts *et al.*, 1958).<br>An O-methyl transferase (OMT) inhibitor<br>ould prolong t

process is the means of inactivation of these<br>amines (Evarts *et al.*, 1958).<br>An O-methyl transferase (OMT) inhibitor<br>would prolong the activity of epinephrine and<br>many substances have been reported to do this;<br>Bacq, for An O-methyl transferase (OMT) inhibitional product and product and product and product and product and product and the same substance (1936a, b), described augmentation of epinephrine effects by polyphenols. All O-methyr transictast (ONT) immediately<br>would prolong the activity of epinephrine and<br>many substances have been reported to do this;<br>Bacq, for instance (1936a, b), described augmen-<br>tation of epinephrine effects by pol many substances have been reported to do the category of the category of the category of the category and the category and the category polyphenols. The category are also methyled at the 3-hydroxy position (Booth  $et$ many substances have been reported to do this,<br>Bacq, for instance (1936a, b), described augmen-<br>tation of epinephrine effects by polyphenols. As<br>polyphenols of the catechol type are also methyl-<br>ated at the 3-hydroxy posit Bacq, for instance (1966a, 9), described algebration of epinephrine effects by polyphenols. As<br>polyphenols of the catechol type are also methylated at the 3-hydroxy position (Booth *et al.*, 1955; Archer *et al.*, 1960) it polyphenols of the catechol type are also methylated at the 3-hydroxy position (Booth *et al.*, 1955; Archer *et al.*, 1960) it occurred to us that the augmentation observed by Bacq could result from competitive affinity ated at the 3-hydroxy position (Booth *et al.*, 1955; Archer *et al.*, 1960) it occurred to us that the augmentation observed by Bacq could result from competitive affinity for the same en-<br>zyme system (Wylie *et al.*, 19 ated at the 0-hydroxy position (B)<br>1955; Archer *et al.*, 1960) it occurred<br>the augmentation observed by Bac<br>sult from competitive affinity for the<br>zyme system (Wylie *et al.*, 1959).<sup>1</sup>

Include the Higher of Solid Multipleta of Solid Multipleta and Arnold, A.:<br>
10.5 ml of borate buffer (pH 10.0). The formed<br>
<u>metanephrine</u> was extracted into 20 volumes of<br>
14 Wylie, D. W., Archer, S. and Arnold, A.:<br>
Phar Sult from competitive affinity for the same en-<br>
zyme system (Wylie *et al.*, 1959).<sup>1</sup><br>
METHODS. In vitro. The O-methylation of procedure of<br>
epinephrine was estimated by the procedure of<br>
Axelrod and Tomchick (1958). Th zyme system (Wylie *et al.*, 1959).<sup>1</sup><br>METHODS. *In vitro*. The O-methylation of<br>epinephrine was estimated by the procedure of<br>Axelrod and Tomchick (1958). The procedure<br>depends on the incubation of 1.5  $\mu$ mol of *l*-epi METHODS. In vitro. The O-methylation of epinephrine was estimated by the procedure of Axelrod and Tomchick (1958). The procedure depends on the incubation of 1.5  $\mu$ mol of l-epinephrine-D-bitartrate, 50  $\mu$ mol magnesium METHODS. In vitro. The O-methylation of<br>epinephrine was estimated by the procedure of<br>Axelrod and Tomchick (1958). The procedure Tai<br>depends on the incubation of 1.5  $\mu$ mol of *l*-epi-<br>nephrine-D-bitartrate, 50  $\mu$ mol m epinephrine was estimated by the procedure of Axelrod and Tomchick (1958). The procedure depends on the incubation of 1.5  $\mu$ mol of *l*-epinephrine-D-bitartrate, 50  $\mu$ mol magnesium chloride, 1.5  $\mu$ mol of S-adenosyl-m Axelrod and Tomchick (1958). The procedure<br>depends on the incubation of 1.5  $\mu$ mol of *l*-epi-<br>nephrine-D-bitartrate, 50  $\mu$ mol magnesium<br>chloride, 1.5  $\mu$ mol of S-adenosyl-methionine (Cal.<br>Found. for Biochem. Res.), 5 depends on the incubation of 1.5  $\mu$ mol of *l*-epi-<br>nephrine-D-bitartrate, 50  $\mu$ mol magnesium<br>chloride, 1.5  $\mu$ mol of S-adenosyl-methionine (Cal.<br>Found. for Biochem. Res.), 50  $\mu$ mol phosphate<br>buffer (pH 7.8), enzyme nephrine-D-bitartrate, 50  $\mu$ mol magnesium<br>chloride, 1.5  $\mu$ mol of S-adenosyl-methionine (Cal. ment<br>Found. for Biochem. Res.), 50  $\mu$ mol phosphate<br>buffer (pH 7.8), enzyme and water to 5.0 ml at<br>staps were preincubated chloride, 1.5  $\mu$ mol of S-adenosyl-methionine ((Found. for Biochem. Res.), 50  $\mu$ mol phosph<br>buffer (pH 7.8), enzyme and water to 5.0 ml<br>37°C. Inhibitors were preincubated in the syst<br>prior to the addition of epinephrine syl-methodic incomes and water to 5.0 ml at<br>sylect cold. Fresh solutions were made every 4<br>sy<sup>o</sup>C. Inhibitors were preincubated in the system<br>prior to the addition of epinephrine and S-adeno-<br>syl-methionine for 10 minutes buffer (pH 7.8), enzyme and water to 5.0 ml at  $37^{\circ}$ C. Inhibitors were preincubated in the system<br>prior to the addition of epinephrine and S-adeno-<br>syl-methionine for 10 minutes. At the end of the<br>incubation period of 37°C. Inhibitors were preincubated in the system<br>prior to the addition of epinephrine and S-adeno-<br>syl-methionine for 10 minutes. At the end of the<br>incubation period of 30 or 60 minutes, the reaction<br>in a 1.0-ml aliquot w prior to the addition of epinephrine and S-adeno-<br>syl-methionine for 10 minutes. At the end of the<br>incubation period of 30 or 60 minutes, the reaction<br>in a 1.0-ml aliquot was stopped by the addition of<br>0.5 ml of borate bu syl-methionine for 10 minutes. At the end of the<br>incubation period of 30 or 60 minutes, the reaction<br>in a 1.0-ml aliquot was stopped by the addition of<br>0.5 ml of borate buffer (pH 10.0). The formed<br>metanephrine was extrac

fork<br>on February 27, 1960<br>ethylene dichloride-isoamyl alcohol  $(2\%)$  and<br>extracted, in turn, into 2.0 ml HCl  $(0.1 \text{ N})$ . The on February 27, 1960<br>ethylene dichloride-isoamyl alcohol  $(2\%)$  and<br>extracted, in turn, into 2.0 ml HCl  $(0.1 \text{ N})$ . The<br>metanephrine was estimated fluorimetrically<sup>2</sup> at on February 27, 1960<br>ethylene dichloride-isoamyl alcohol (2%) and<br>extracted, in turn, into 2.0 ml HCl (0.1 N). The<br>metanephrine was estimated fluorimetrically<sup>2</sup> at<br>335 m<sub>H</sub> after activation at 285 m<sub>H</sub>. ethylene dichloride-isoamyl alco<br>extracted, in turn, into 2.0 ml HC<br>metanephrine was estimated fluor<br>335 m $\mu$  after activation at 285 m $\mu$ .<br>In vivo. Spinal cats were prepare hylene dichloride-isoamyl alcohol  $(2\%)$  and<br>tracted, in turn, into 2.0 ml HCl  $(0.1 \text{ N})$ . The<br>etanephrine was estimated fluorimetrically<sup>2</sup> at<br>5 m<sub> $\mu$ </sub> after activation at 285 m $\mu$ .<br>*In vivo*. Spinal cats were prepare

epinephrine, suggesting that this metabolic of the brain, graded submaximal doses  $(0.5 \text{ to } 4.0 \mu\text{g})$  of epinephrine and norepinephrine were<br>amines (Evarts *et al.*, 1958).<br>An O-methyl transferase  $(OMT)$  inhibitor subm extracted, in turn, into 2.0 ml HCl (0.1 N). The<br>metanephrine was estimated fluorimetrically<sup>2</sup> at<br>335 m $\mu$  after activation at 285 m $\mu$ .<br>*In vivo*. Spinal cats were prepared by sectioning<br>the cord at C2 and destroying metanephrine was estimated fluorimetrically<sup>2</sup> at 335 m $\mu$  after activation at 285 m $\mu$ .<br>
In vivo. Spinal cats were prepared by sectioning<br>
the cord at C2 and destroying the brain by means<br>
of a rod introduced through t 335 m $\mu$  after activation at 285 m $\mu$ .<br>
In vivo. Spinal cats were prepared by sectioning<br>
the cord at C2 and destroying the brain by means<br>
of a rod introduced through the foramen magnum.<br>
Following destruction of the b the cord at C2 and destroying the brain by means<br>of a rod introduced through the foramen magnum.<br>Following destruction of the brain the animals<br>were allowed to recover from the anesthesia. The<br>blood pressure was recorded b the cord at C2 and destroying the brain by means<br>of a rod introduced through the foramen magnum.<br>Following destruction of the brain the animals<br>were allowed to recover from the anesthesia. The<br>blood pressure was recorded b of a rod introduced through the foramen magnum.<br>Following destruction of the brain the animals<br>were allowed to recover from the anesthesia. The<br>blood pressure was recorded by catheterization of<br>the carotid artery. One hour Following destruction of the brain the animals<br>were allowed to recover from the anesthesia. The<br>blood pressure was recorded by catheterization of<br>the carotid artery. One hour following destruction<br>of the brain, graded sub were allowed to recover from the anesthesia. The<br>blood pressure was recorded by catheterization of<br>the carotid artery. One hour following destruction<br>of the brain, graded submaximal doses  $(0.5 \text{ to}$ <br> $4.0 \mu$ g) of epinephr blood pressure was recorded by catheterization of<br>the carotid artery. One hour following destruction<br>of the brain, graded submaximal doses  $(0.5 \text{ to}$ <br>4.0  $\mu$ g) of epinephrine and norepinephrine were<br>administered intrave the carotid artery. One hour following destruction<br>of the brain, graded submaximal doses  $(0.5 \text{ to}$ <br> $4.0 \mu g)$  of epinephrine and norepinephrine were<br>administered intravenously and an appropriate<br>submaximal dose was repea of the brain, graded submaximal doses  $(0.5 \text{ to } 4.0 \mu g)$  of epinephrine and norepinephrine were administered intravenously and an appropriate submaximal dose was repeated until it produced equal pressor responses consecu 4.0  $\mu$ g) of epinephrine and norepinephrine were administered intravenously and an appropriate submaximal dose was repeated until it produced equal pressor responses consecutively. The OMT inhibitors were given either be administered intravenously and an appear and the submaximal dose was repeated until it jequal pressor responses consecutively. Thin initiative were given either before or amines and the pressor effects compared Supramaxima bmaximal dose was repeated until it produced<br>ual pressor responses consecutively. The OMT<br>hibitors were given either before or with the<br>ines and the pressor effects compared.<br>Supramaximal stimuli were applied to the pre-<br>n equal pressor responses consecutively. The OMT<br>inhibitors were given either before or with the<br>amines and the pressor effects compared.<br>Supramaximal stimuli were applied to the pre-<br>ganglionic sympathetic nerve trunk so th

inhibitors were given either before or with the<br>amines and the pressor effects compared.<br>Supramaximal stimuli were applied to the pre-<br>ganglionic sympathetic nerve trunk so that equal<br>contractions of the nictitating membra amines and the pressor effects compared.<br>Supramaximal stimuli were applied to the pre-<br>ganglionic sympathetic nerve trunk so that equal<br>contractions of the nictitating membrane were<br>obtained. Following intravenous administ Supramaximal stimuli were applied to the pre-<br>ganglionic sympathetic nerve trunk so that equal<br>contractions of the nictitating membrane were<br>obtained. Following intravenous administration<br>of the inhibitor, the same stimulu ganglionic sympathetic nerve trunk so that equal<br>contractions of the nictitating membrane were<br>obtained. Following intravenous administration<br>of the inhibitor, the same stimulus was applied<br>and the height and duration of t obtained. Following intravenous administration<br>of the inhibitor, the same stimulus was applied<br>and the height and duration of the contractions<br>compared with the controls.<br>White male rats of 80 to 100  $g$  were used for tained. Following intravenous administration<br>the inhibitor, the same stimulus was applied<br>d the height and duration of the contractions<br>mpared with the controls.<br>White male rats of 80 to 100 g were used for<br>travenous toxi

the augmentation observed by Bacq could re-<br>sult from competitive affinity for the same en-<br>zyme system (Wylie *et al.*, 1959).<sup>1</sup> White male rats of 80 to 100 g were used for<br>intravenous toxicity determinations. The anim of the inhibitor, the same stimulus was applied<br>and the height and duration of the contractions<br>compared with the controls.<br>White male rats of 80 to 100 g were used for<br>intravenous toxicity determinations. The animals<br>were and the height and duration of the contractio<br>compared with the controls.<br>White male rats of 80 to 100 g were used f<br>intravenous toxicity determinations. The anima<br>were observed for 24 hours for death. The calc<br>lations of compared with the controls.<br>White male rats of 80 to 100 g were used for<br>intravenous toxicity determinations. The animals<br>were observed for 24 hours for death. The calcu-<br>lations of the LD50 were made by the Miller and<br>Tai White male rats of 80<br>intravenous toxicity detainer observed for 24 hours<br>alations of the LD50 were<br>Tainter method (1944).<br>L-Epinephrine bitartra travenous toxicity determinations. The animals<br>re observed for 24 hours for death. The calcu-<br>tions of the LD50 were made by the Miller and<br>uinter method (1944).<br>*l*-Epinephrine bitartrate and *l*-norepinephrine<br>tartrate m

were observed for 24 hours for death. The calculations of the LD50 were made by the Miller a<br>Tainter method (1944).<br> *l*-Epinephrine bitartrate and *l*-norepinephri<br>
bitartrate monohydrate were used in all experients and t lations of the LD50 were made by the Miller and<br>Tainter method (1944).<br> *l*-Epinephrine bitartrate and *l*-norepinephrine<br>
bitartrate monohydrate were used in all experi-<br>
ments and the doses reported are of the base; the Tainter method (1944).<br> *l*-Epinephrine bitartrate and *l*-norepinephrine<br>
bitartrate monohydrate were used in all experi-<br>
ments and the doses reported are of the base; the<br>
solutions contained  $0.01\%$  ascorbic acid and *l*-Epinephrine bitartrate and *l*-norepinephrine<br>bitartrate monohydrate were used in all experi-<br>ments and the doses reported are of the base; the<br>solutions contained  $0.01\%$  ascorbic acid and were<br>kept cold. Fresh solu hours. lutions contained 0.01% ascorbic acid and were<br>pt cold. Fresh solutions were made every 4<br>urs.<br>RESULTS. Under *in vitro* conditions we found<br>at a series of polyphenols markedly inhibited

kept cold. Fresh solutions were made every 4<br>hours.<br>RESULTS. Under *in vitro* conditions we found<br>that a series of polyphenols markedly inhibited<br>the rate of methylation of epinephrine (table 1), hours.<br>RESULTS. Under *in vitro* conditions we found<br>that a series of polyphenols markedly inhibited<br>the rate of methylation of epinephrine (table 1),<br>and there was approximately a 16-fold difference RESULTS. Under *in vitro* conditions we found<br>that a series of polyphenols markedly inhibited<br>the rate of methylation of epinephrine (table 1),<br>and there was approximately a 16-fold difference<br>in their activities. Cocaine, RESULTS. Under *in viero* conductions we round<br>that a series of polyphenols markedly inhibited<br>the rate of methylation of epinephrine (table 1),<br>and there was approximately a 16-fold difference<br>in their activities. Cocaine augments of polyphenois markedly inhibited<br>the rate of methylation of epinephrine (table 1),<br>and there was approximately a 16-fold difference<br>in their activities. Cocaine, which powerfully<br>augments epinephrine effects on s and there was approximately a 16-fold difference<br>in their activities. Cocaine, which powerfully<br>augments epinephrine effects on smooth muscle<br><sup>2</sup> Aminco-Bowman spectrophotofluorometer.

preparations, did not, however, affect methylation.

Adrenalone  $(\omega$ - [N-methylamino]-3, 4 dihypreparations, did not, however, affect methy<br>tion.<br>Adrenalone ( $\omega$ -[N-methylamino]-3,4 di<br>droxyacetophenone) and arterenone ( $\omega$ -amino-3,4 dihydroxyacetophenone) are inhibitors  $\footnotesize$ ion.<br>  $\footnotesize\begin{array}{r} \text{Adrenalone} \quad (\omega\text{-}\text{[N-methylamino]} - 3 \text{ , } 4 \quad \text{dihy-}\quad \text{if} \text{drosyacetophenone)} \text{ and} \text{ arterenone} \quad (\omega\text{-amino-}\quad \text{g} \text{3,4} \quad \text{dihydroxyacetophenone)} \text{ are} \text{ inhibitors} \text{ of} \quad \text{c} \text{OMT} \quad (\text{Udenfried}, \text{ personal communication}) \quad \text{in} \end{array}$  $\Lambda$ drenalone ( $\omega$ -[N-methylamino]-3, 4 dih<br>droxyacetophenone) and arterenone ( $\omega$ -amin<br>3,4 dihydroxyacetophenone) are inhibitors<br>OMT (Udenfriend, personal communication<br>and it is interesting to note that under in vit Antenatone ( $\omega$ -[14-metalylamino]-5,  $\pm$  uniy-<br>droxyacetophenone) and arterenone ( $\omega$ -amino-<br>3,4 dihydroxyacetophenone) are inhibitors of note<br>OMIT (Udenfriend, personal communication) nand it is interesting to note th  $\sigma$ , a dinyaroxyaeetophenone) are inhibitors of normal communication) and it is interesting to note that under *in vitro* the conditions these ketone analogs of epinephrine and norepinephrine had a much greater affinity for the entirely personal communicational it is interesting to note that under  $\dot{n}$   $v$  conditions these ketone analogs of epinephiand norepinephrine had a much greater affir for the enzyme than did their hydroxy counte For the interesting to how that the two differential conditions these ketone analogs of epinephrine and norepinephrine had a much greater affinity in for the enzyme than did their hydroxy counterparts. While catechol was for the enzyme than did their hydroxy counter-<br>parts. While catechol was indicated by Axelrod<br>TABLE 1

 $S \times 10^{-4}$  M epinephrine as substrate parts. While catechol was indicated by Axelrod<br>it<br>*OMT inhibitory activities of polyphenols using*<br> $3 \times 10^{-4}$  *M epinephrine as substrate* **3** *TABLE 1*<br>*3 X 10<sup>-4</sup> <i>M* epinephrine as substrate<br><sup>3</sup> X <sup>10-4</sup> *M* epinephrine as substrate

Inhibitor	Epinephrine I50 Molar Conc. Estimated	Relative $ActivityCatechol = 1$
Catechol	$5 \times 10^{-4}$	
Adrenalone	$5 \times 10^{-5}$	10
Arterenone	$6 \times 10^{-5}$	8
Pyrogallol	$3 \times 10^{-5}$	16
Gallic acid	$5 \times 10^{-5}$	10
Cocaine	Inactive	

nd Tomchick (1958) to be methylated somewhat<br>Ind Tomchick (1958) to be methylated somewhat<br>Independent of the may be see more readily than epinephrine, it may be seen<br>in than epinephrine, it may be seen<br>from table 1 that the trihydroxy phenols, pyrofrom table 1958) to be methylated somewhat<br>more readily than epinephrine, it may be see<br>from table 1 that the trihydroxy phenols, pyro-<br>gallol and gallic acid, appeared to be considerable nd Tomchick (1958) to be methylated somewhat<br>more readily than epinephrine, it may be seen<br>from table 1 that the trihydroxy phenols, pyro-<br>gallol and gallic acid, appeared to be considerably<br>more sensitive to methylation t more readily than epinephrine, it may be seen<br>from table 1 that the trihydroxy phenols, pyro-<br>gallol and gallic acid, appeared to be considerably<br>more sensitive to methylation than was epi-<br>nephrine, since they markedly in more readily than ephiephrine, it may be seen<br>from table 1 that the trihydroxy phenols, pyro-<br>gallol and gallic acid, appeared to be considerably<br>more sensitive to methylation than was epi-<br>nephrine, since they markedly in point cable 1 that the thinyan<br>gallol and gallic acid, appeared<br>more sensitive to methylati<br>nephrine, since they marked<br>the formation of metanephrin<br>Luduena *et al.* (1959) repo not and gaint acid, appeared to be consider<br>ore sensitive to methylation than was ophrine, since they markedly interfered we<br>formation of metanephrine.<br>Luduena *et al.* (1959) reported that intra<br>us epinephrine, in doses o

more sensitive to inethymation than was epherephrine, since they markedly interfered with<br>the formation of metanephrine.<br>Luduena *et al.* (1959) reported that intrave-<br>nous epinephrine, in doses of 50 to 100  $\mu$ g/kg,<br>cau depiring, since they marketily interfered with<br>the formation of metanephrine.<br>Luduena *et al.* (1959) reported that intrave-<br>nous epinephrine, in doses of 50 to 100  $\mu$ g/kg,<br>caused pulmonary edema and death in rats. The<br> Luduena *et al.* (1959) reported that intra<br>nous epinephrine, in doses of 50 to 100  $\mu$ g/<br>caused pulmonary edema and death in rats.<br>data in table 2 show that simultaneous adminitration of polyphenols augmented this toxic Luduena et  $u$ . (1999) reported that intrave-<br>nous epinephrine, in doses of 50 to 100  $\mu$ g/kg,<br>caused pulmonary edema and death in rats. The<br>data in table 2 show that simultaneous adminis-<br>tration of polyphenols augmente considerably. data in table 2 show that simultaneous administration of polyphenols augmented this toxicity considerably.<br>Intravenous norepinephrine also caused pul-<br>monary edema and death in rats, but it was not

give of adjappear to appear to infinite appear to  $\frac{1}{2}$ <br>TABLE 2 did not alter the toxicity of norepinephrine; mata in table 2 show that simultaneous animis-<br>tration of polyphenols augmented this toxicity<br>considerably.<br>Intravenous norepinephrine also caused pul-<br>monary edema and death in rats, but it was not<br>as toxic as epinephrine ration of polyphenois augmented this toxicity<br>considerably.<br>Intravenous norepinephrine also caused pul-<br>monary edema and death in rats, but it was not<br>as toxic as epinephrine. Pyrogallol, at twice the<br>dose which augmented donsiderably.<br>Intravenous norepinephrine also caused pu<br>monary edema and death in rats, but it was no<br>as toxic as epinephrine. Pyrogallol, at twice th<br>dose which augmented the toxicity of epinephrine<br>did not alter the toxi monary edema and death in rats, but it was not as toxic as epinephrine. Pyrogallol, at twice the dose which augmented the toxicity of epinephrine;<br>did not alter the toxicity of norepinephrine;<br>catechol, adrenalone and arte monary euchia and death in rais, but it was not<br>as toxic as epinephrine. Pyrogallol, at twice the<br>dose which augmented the toxicity of epinephrine,<br>did not alter the toxicity of norepinephrine;<br>catechol, adrenalone and art as toxic as epinepinne. I yroganot, at twice the<br>dose which augmented the toxicity of epinephrine,<br>did not alter the toxicity of norepinephrine;<br>catechol, adrenalone and arterenone showed po-<br>tentiating activity but less t did not alter the toxicity of pomepineme,<br>did not alter the toxicity of norepinephrine;<br>catechol, adrenalone and arterenone showed po-<br>tentiating activity but less than was seen with<br>epinephrine (table 2). Thus the differe did not aller the tworthy of notephepmine,<br>catechol, adrenalone and arterenone showed po-<br>tentiating activity but less than was seen with<br>epinephrine (table 2). Thus the differences in<br>LD50's of the two amines and their di eatecho, adveration and arte-enone showed po-<br>tentiating activity but less than was seen with<br>epinephrine (table 2). Thus the differences in<br>LD50's of the two amines and their differing de-<br>gree of augmentation by OMT inhi dentiating activity out less than was seen w<br>epinephrine (table 2). Thus the differences<br>LD50's of the two amines and their differing<br>gree of augmentation by OMT inhibitors do i<br>appear to be related to differences in their epmepirme (cause 2). Thus the differing c<br>LD50's of the two amines and their differing c<br>gree of augmentation by OMT inhibitors do n<br>appear to be related to differences in their mech<br>nisms of action in causing death. Furth



Inhibitor	Dose Inhibitor $mg/kg$ i.v.	Effect of Inhibitor on Toxicity of Catecholamines i.v. LD50 $\pm$ S.E. $\mu$ g/kg		i.v. $LD50 \pm S.E.$ Inhibitor mg/kg
		Epinephrine	Norepinephrine	
		$45.0 + 7.5$	$95.0 \pm 12.3$	
Catechol 5.0 10.0 20.0		$27.5 \pm 4.1$		$95.0 \pm 15.4$
		$19.5 \pm 2.1$	$98.4 \pm 16.0$	
	$19.5 \pm 2.1$	$48.0 \pm 11.2$		
Adrenalone	0.5	$21.0 \pm 7.0$	$63.0 \pm 7.0$	$42.5 \pm 5.4$
Arterenone	0.5	$22.0 \pm 6.2$	$63.0 \pm 7.2$	$100.0 + 23.0$
Pyrogallol 20.0 40.0 80.0	$46.0 \pm 10.3$		$565 \pm 59.0$	
		$22.0 \pm 3.4$		
		$100.0 \pm 3.2$		
Gallic acid	400.0	$25.5 \pm 7.7$		>2000
Cocaine 0.1 0.2		$29.0 \pm 6.5$		$12.5 \pm 1.1$
		$18.8 \pm 5.8$		

*Effect of polyphenols on intravenous toxicity of epinephrine and norepinephrine in rats\** 

\* Twenty rats were used at each dose level and a minimum of three dose levels were tested for each agent or combination of agents.

the data in table 3 show that the toxicity of epinephrine, when augmented by an OMT inhibitor, was still adrenergic in mechanism, as the same dose of chlorpromazine protected the animals from epinephrine plus inhibitor as from epinephrine alone. These results should be expected as the same amount of epinephrine was administered when given alone and when given with inhibitor; doubling the dose of epinephrine made it necessary to increase the dose of adrenolytic.

The relative activities of the polyphenols as inhibitors of the methylation of epinephrine were different in the rat experiments from those obtained in the *in vitro* studies. Adrenalone and arterenone possess  $\frac{15}{20}$  to  $\frac{1}{100}$  of the activity of

TABLE 3 Adrenolytic activity of chlorpromazine against i.v. epinephrine alone and with catechol (OMT inhibitor) Adrenolytic Potency\* Dose Epinephrine Dose of Cate-

$\mu$ g kg i.v.	chol mg kg i.v.	$ED50 \pm S.E., \mu g$ kg
200		$40.5 \pm 3.8$
40O		$108.0 \pm 23.5$
20O	20	$42.5 \pm 7.3$

\* To determine adrenolytic potency the drugs were administered simultaneously. Four doses of chlorpromazine were used in each test, 10 rats per dose (Luduena, 1959).

their respective hydroxylated amines, so that augmentation may be partially the effects of summation of sympathomimetic effects. The adrenergic action of the ketones was further confirmed by the finding that they caused death in a manner similar to epinephrine and that rats could be protected from  $3 \times$  LD50 dose of adrenalone by the adrenolytic chlorpromazine in a manner similar to the protection from epinephrine toxicity by chlorpromazine. The doses of adrenalone and arterenone used to augment the toxicity of epinephrine were well below their respective LD50's, and, thus, in view of the enzyme results, their action would appear to be synergistic rather than additive.

The other polyphenols alone, unlike adrenalone and arterenone, did not cause epinephrine-like effects and the animals were not protected from their various actions by adrenolytic agents. Catechol when given alone caused marked twitching, hyperreflexia and tonic convulsions followed by death. The appearance was identical to that produced by strychnine. Twitching and hyperreflexia were also noted in the spinal cat, suggesting action at the spinal level. These responses were unaffected by chlorpromazine given either simultaneously with the catechol at 16  $mg/kg$  i.v. or 40 minutes before the catechol at 64 mg/kg s.c. Chlorpromazine is centrally active and has adrenolytic properties; it was therefore unlikely that the central effects of catechol described above were due to an accumulation of



FIG. 1. Effects of catechol and pyrogallol on the duration of pressor effects of intravenous epinephrine.

sympathomimetic amines due to inhibition of OMT.

In the spinal cat preparation, we found that all of the polyphenols in table 1 prolonged the pressor effects of epinephrine (fig. 1) in agreement with the data presented by Bacq (1936a).

Pyrogallol and catechol, when given simultaneously with epinephrine, caused increased duration of action. Larger doses (5.0 mg) had pressor effects but if the administration of the epinephrine was delayed until the blood pressure had returned to normal the effects of the epinephrine were still prolonged.

All of the OMT inhibitors tested caused some pressor effects when given alone, as will be discussed later, but the effects were small or not apparent at the dose levels used to augment

epinephrine activity. However, adrenalone and arterenone had sufficient pressor activity to make interpretation of the results difficult.

Augmentation of the pressor effects of norepinephrine was not readily produced by pyrogallol and catechol. A total of 7 cats have been used and the results indicate that in no instance was it possible to prolong the pressor effects of norepinephrine in as effective a manner as was possible with epinephrine (fig. 2).

Large doses of pyrogallol increased the duration of contraction of the cat nictitating membrane in response to supramaximal stimulation of the preganglionic sympathetic nerve (fig. 3). This has been repeated in 12 cats. In every case the contraction following the intravenous administration of 160 mg pyrogallol was of longer



FIG. 2. Augmentation of epinephrine and norepinephrine by pyrogallol in the same cat.



FIG. 3. Effect of pyrogallol on the contraction of the nictitating membrane following stimulation of the preganglionic sympathetic nerve. (Duration of stimulation 30 secs. Frequency  $24/\text{sec} V = 5$ .)

duration than the controls, and for 30 minutes following the medication subsequent responses to stimulation were also prolonged. After the second administration of pyrogallol, however, augmentation of the contraction was not so marked.

Burn (1958) showed that the tonus of the blood vessels was probably maintained by constant production of sympathomimetic amines at the nerve endings. Maintenance of a sufficient blood level of an OMT inhibitor, therefore, should cause some pressor effect and it can be seen from fig. 1 that a single injection of pyrogallol had slight pressor effects. Following repeated single administrations of pyrogallol, the general level of the blood pressure rose at first followed by tachyphylaxis to further administrations.

DISCUSSION. Various polyphenols are methylated at the 3-hydroxy position in the same way as epinephrine, and it is probable that the polyphenols reported here have reduced methylation of epinephrine by acting as competitive substrates for the enzyme system.

The data we have obtained suggest that the augmentation of epinephrine effects by polyphenols is probably due to an increased life-span of the epinephrine because of this reduced rate of methylation.

The effects of circulating norepinephrine in rats and cats were not augmented by pyrogallol or catechol as effectively as the epinephrine responses, while under in vitro conditions these polyphenols inhibited methylation of both amines.

A possible explanation for this difference between epinephrine and norepinephrine could be that norepinephrine has a greater affinity for O-methyl transferase than does epinephrine and was thus more difficult to displace by a competitive inhibitor, especially since both catecholamines and the polyphenols have very short durations of action. However, under in vitro conditions no differences in affinities of the amines for the enzyme system could be detected, the Km for epinephrine was found to be  $2.3 \times 10^{-4}$  M and that for no<br>repinephrine was 3.5  $\times$  10<sup>-4</sup> M.

All of the blood pressure experiments involved intravenously administered catecholamines. However, our experiments using the cat metitating membrane preparation, showed that OMT inhibitors were also able to delay methylation of

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differed in velocity; there was a phase of rapid methylation in which more than half (60 to 70%) pro<br>
of the injected amin mepurine *in vivo* occurred in two phases which<br>differed in velocity; there was a phase of rapid<br>methylation in which more than half (60 to 70%)<br>of the injected amine was methylated within 10<br>minutes, the remaining methyla tended in velocity, there was a phase of rapid in<br>
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minutes, the remaining methylation being ex-<br>
tended over 3 or more h from the data that, although 0-methylation in which mine was methylated within 10<br>minutes, the remaining methylation being ex-<br>tended over 3 or more hours. Axelrod theorized<br>from his data that, although 0-methylation<br>start of the injected anime was incluy aded whall to examinates, the remaining methylation being extended over 3 or more hours. Axelrod theorized from his data that, although O-methylation parted immediately, some of the epineph minutes, the remaining methylation being ex-<br>tended over 3 or more hours. Axelrod theorized<br>from his data that, although O-methylation<br>started immediately, some of the epinephrine was<br>also bound rapidly onto tissues from w subsequently released and methylation presstarted immediately, some of the epinephrine was but also bound rapidly onto tissues from which it was nore subsequently released and methylated. Such an immediate dual mechanism o if the mechanism of the epinephrine was busined inmediately, some of the epinephrine was business bound rapidly onto tissues from which it was no subsequently released and methylated. Such an immediate dual mechanism of me **biarced immediately**, some of the epinephrine was<br>also bound rapidly onto tissues from which it was<br>subsequently released and methylated. Such an<br>immediate dual mechanism of methylation and<br>binding would help account for also bount raphtly onto ussues from which it was<br>subsequently released and methylated. Such an<br>immediate dual mechanism of methylation and<br>binding would help account for the findings that<br>the physiological response to inje **is consequently** released and methylated. Such an immediate dual mechanism of methylation and binding would help account for the findings that the physiological response to injected epinephrine is completed even when unch immediate dual incentionship of metalyiation and<br>binding would help account for the findings that<br>the physiological response to injected epinephrine<br>is completed even when unchanged epinephrine<br>is still detectable in the a the physiological response to injected epinephrine Pyrogallol prolonged<br>is completed even when unchanged epinephrine endogenously released<br>is still detectable in the animal. Our data sug-<br>nictitating membrane,<br>gested that is completed even when unchanged epinephrine is still detectable in the animal. Our data suggested that inasmuch as there is no difference in the affinities of the two amines for the enzyme, the difficulty in augmenting circulating norepi-<br>nephrine by pyrogallol coul gested that inasmuch as there is no difference in gested that mashinen as there is no difference in<br>the affinities of the two amines for the enzyme,<br>the difficulty in augmenting circulating norepi-<br>nephrine by pyrogallol could possibly be ac-<br>counted for by the fact that the difficulty in augmenting circulating norepi<br>the difficulty in augmenting circulating norepi<br>nephrine by pyrogallol could possibly be ac<br>counted for by the fact that norepinephrine may<br>be more readily bound and thus phy ine amicuty in augmenting circulating intep-<br>nephrine by pyrogallol could possibly be accounted for by the fact that norepinephrine may<br>be more readily bound and thus physiologically<br>inactivated than is epinephrine. Axelro meparine by pyroganor count possibly be ac-<br>counted for by the fact that norepinephrine may leir<br>be more readily bound and thus physiologically for<br>inactivated than is epinephrine. Axelrod found<br>independently (personal co be more readily bound and thus physiologically<br>inactivated than is epinephrine. Axelrod found<br>independently (personal communication) that in<br>the case of norepinephrine less than half was im-<br>mediately methylated, the major be more readily bound and thus physiologically to<br>inactivated than is epinephrine. Axelrod found<br>independently (personal communication) that in<br>the case of norepinephrine less than half was im-<br>mediately methylated, the ma macuvated than is epinephrine. Axerod found<br>independently (personal communication) that in<br>the case of norepinephrine less than half was im-<br>mediately methylated, the major portion being<br>bound and slowly liberated and meth maependently (personal communication) that in<br>the case of norepinephrine less than half was im-<br>mediately methylated, the major portion being<br>bound and slowly liberated and methylated. If<br>binding is also a method of physio Interest of norepinepirme less than han was im-<br>
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binding is also a method of physiological inactivation, the differences in affi mediately metry ated, the major portion being<br>bound and slowly liberated and methylated. If<br>binding is also a method of physiological inacti-<br>vation, the differences in affinities of the two<br>amines for binding sites might bound and slowly liberated and methylated. If 153, 1960.<br>
binding is also a method of physiological inacti-<br> **AXELROD**, J.: Science **126:** 400, 1957.<br>
vation, the differences in affinities of the two<br> **AXELROD**, J.: Physio vation, the differences in affinities of the two amines for binding sites might explain the dif-<br>ferences obtained in our experiments. It could be<br>further postulated that adrenalone and artere-<br>none might owe their greater ability to potentiate<br>both amines to the fact th ferences obtained in our experiments. It could be further postulated that adrenalone and arterence might owe their greater ability to potentiate **both** amines to the fact that they chemically resemble the amines and may co Further postulated in our experiments. It could be<br>further postulated that adrenalone and artere-<br>none might owe their greater ability to potentiate 233:<br>both amines to the fact that they chemically re-<br>BACQ,<br>semble the am further posturated that advenatione and<br>none might owe their greater ability to po<br>both amines to the fact that they chemic<br>semble the amines and may compete with<br>for both the enzyme and binding sites.<br>Inhibition of O-meth Inhibition of O-methyl transferase is obviously<br>
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Inhibition of O-methyl transferase is obviously<br>
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latter property to a high degree but is in not the only means whereby epinephrin<br>augmented, as cocaine, for instance,<br>latter property to a high degree but is<br>as an inhibitor of this enzyme system.

### SUMMARY

A series of olyphenols have been found to the series of polyphenols have been found to  $\frac{1}{M_1}$ <br>duce methylation of epinephrine and norepireduce methylation of this enzyme system.<br>SUMMARY<br>A series of polyphenols have been found<br>reduce methylation of epinephrine and nore

**nephrine** by 0-methyl transferase *in vitro,* Fr AL. Vol. 1<br> **probably** by acting as competitive substrates.<br>
These substances augmented the toxicity

**These substances augmented the toxicity of the toxic substances augmented the toxicity of travenous epinephrine in the rat, the aug**nephrine by O-methyl transferase *in vitro* probably by acting as competitive substrates.<br>These substances augmented the toxicity of intravenous epinephrine in the rat, the augmented toxicity being adrenergic in nature and meputine by O-inctifyt transferase in viro,<br>probably by acting as competitive substrates.<br>These substances augmented the toxicity of<br>intravenous epinephrine in the rat, the aug-<br>mented toxicity being adrenergic in nature a probably by acting as competitive substrates.<br>These substances augmented the toxicity of<br>intravenous epinephrine in the rat, the aug-<br>mented toxicity being adrenergic in nature and<br>probably due to an increase in the life-s These substances augmented the toxicity of<br>intravenous epinephrine in the rat, the augmented toxicity being adrenergic in nature and<br>probably due to an increase in the life-span of<br>epinephrine. The effects with norepinephr mtravenous epinepr<br>mented toxicity bei<br>probably due to an<br>epinephrine. The were less pronounced<br>The inhibitors pr The inhibitors pering autentify it in the durative and<br>bably due to an increase in the life-span of<br>inephrine. The effects with norepinephrine<br>re less pronounced.<br>The inhibitors prolonged the duration of the<br>essor effects epinephrine. The effects with norepinephrine<br>were less pronounced.<br>The inhibitors prolonged the duration of the<br>pressor effects of epinephrine in the spinal cat,

but were less active **in prolonging the effect of** norepinephrine. The inhibitors protonged the duration of the<br>essor effects of epinephrine in the spinal cat,<br>t were less active in prolonging the effect of<br>repinephrine.<br>Pyrogallol, when given repeatedly, caused a<br>e in blood pressure, but

pressor enects of epinephrine in the spinal cat,<br>but were less active in prolonging the effect of<br>norepinephrine.<br>Pyrogallol, when given repeatedly, caused a<br>rise in blood pressure, but this was soon followed<br>by what appea but were less active in prolonging the<br>norepinephrine.<br>Pyrogallol, when given repeatedly,<br>rise in blood pressure, but this was soc<br>by what appeared to be tachyphylax<br>Pyrogallol prolonged the duration o Pyrogallol, when given repeatedly, caused a<br>e in blood pressure, but this was soon followed<br>what appeared to be tachyphylaxis.<br>Pyrogallol prolonged the duration of action of<br>dogenously released catecholamines at the rise in blood pressure, but this was soon followed<br>by what appeared to be tachyphylaxis.

ryroganol, when given repeatedly, caused a<br>rise in blood pressure, but this was soon followed<br>by what appeared to be tachyphylaxis.<br>Pyrogallol prolonged the duration of action of<br>endogenously released catecholamines at the Pyrogallol prolonged the duration of action of

I yioganoi prolonged the duration of action of<br>endogenously released catecholamines at the<br>inicitiating membrane.<br>Possible explanations for the difference be-<br>tween epinephrine and norepinephrine are dis-<br>cussed. cussed. Possible explanations for the difference be-<br>reen epinephrine and norepinephrine are dis-<br>ssed.<br>ACKNOWLEDGMENTS. We wish to thank Made-

tween epinephrine and norepinephrine are dis-<br>cussed.<br>ACKNOWLEDGMENTS. We wish to thank Made-<br>leine Hart, John P. McAuliff and Robert Familiar<br>for their able assistance. leine Hart, John P. McAuliff and Robert Familiar

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