$MgSO_4$, and $CaCl_2$. Trisodium citrate had no effect. Cyanide, fluoride, physostigmine and sulfhydryl compounds inhibited the enzyme. Compounds capable of eliminating sulfhydryl groups present in the enzyme preparation had no influence, nor did Congo red or methylene blue.

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Inactivation of Pressor Amines by Quinones and Related Diketones.

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In the metabolism of certain aromatic l-amino acids in the kidney pressor amines are formed by decarboxylases as intermediary products. In the normal kidney the enzyme amine oxidase deaminizes these amines to inert substances. This oxidase is not effective in the ischemic kidney, and the process is arrested at the stage of the pressor amine. This mechanism is thought to be one of the causes of experimental hypertension (Holtz,[1] Bing[2]).

It was believed that an approach to the problem of hypertension could be effected by causing the oxidative destruction of pressor amines circulating in the blood stream. Kisch[3] has shown that certain quinones act as catalysts in the oxidative deamination of amino acids and polypeptides.

Since catechol derivatives and aliphatic enediols form quinones and diketones respectively on aeration in the presence of certain metallic ions as catalysts, it was of interest to examine their effect on various pressor amines *in vitro*. The quinones (or diketones) formed could act either by deaminating the amines or by forming compounds of the azophenine type.

Experimental. Solutions containing tyramine, indolethylamine,

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1 Holtz, P., Heise, C., and Luedtke, C., *Arch. exp. Path. u. Pharm.*, 1938, **191**, 87.

2 Bing, R. J., *Am. J. Physiol.*, 1941, **132**, 497.

3 Kisch, B., *Oppenheimer's Handb. der Biochem.*, Jena, 1933, Ergw. **1**, 563.

4 Beyer, K. H., *J. Pharm. Exp. Ther.*, 1941, **71**, 394.

ephedrine, benzedrine and paredrine were aerated in the presence of catechol, dihydroxyphenyl-alanine (dopa), reductone, gallic acid, *l*-ascorbic acid, *d*-glucoascorbic acid, and *p*-benzoquinone. Copper, cobalt, nickel and iron salts were used as catalysts. At the same time blanks composed of the pressor amines and the metal catalysts but without the quinone precursor or the aliphatic enediols were similarly aerated.

After aeration the solutions were filtered and 1 cc was injected intravenously into nembutalized cats whose femoral artery was cannulated for blood pressure measurements. The controls gave the immediate response typical of the pressor amine used, but aeration

TABLE I.

Destruction of Pressor Amines During Autoxidation of Quinone Precursors and by Quinones.

Quinone precursors	Bivalent metal ion	Pressor substance used: Tyramine or Indol-ethylamine	Ephedrine	Benzedrine or Paredrine	Renin*
		% destruction of pressor amines inferred by diminished response			
None		0	0	0	S
d,l-Dopa	Cu, Co or Ni	100	100	30	100
d,l-Dopa	Fe	80	0		
Catechol	Cu, Co or Ni	100		20	100
Catechol	Fe	80			
Gallic Acid	Cu	20			
l-Ascorbic Acid†	Cu	30			
d-Glucoascorbic Acid‡	Cu	30	30		
Reductone†	Cu	100		30	
Quinones					
p-Benzoquinone					
Non-aerated		30	70		
Aerated		100	20	100	
"Dopa" Quinone					
Non-aerated		20			0
Aerated		60			100

S = slight.

* = See legend below.

† = 20 mg per cc of incubated mixture.

‡ = 40 mg per cc of incubated mixture.

A solution was made up from 2 cc of a 0.5% solution of quinone precursor, 2 cc of a 0.05% solution of pressor amines and 1 cc of a 0.02% solution of the metal catalysts. (In case of *l*-ascorbic acid and reductone of 5% solution and in case of *d*-glucoascorbic acid a 10% solution was taken.) These solutions were aerated at room temperature for about 16 hours. 1 cc of each solution, thus containing 0.5 mg of pressor amine and 0.2 mg of the sulfate of the respective metal ion plus 2 mg of quinone precursor, was injected intravenously into nembutalized cats. In the case of renin 3.0 cc of a bicarbonate extract of acetone dried hog kidney were aerated for 3 hours with the oxidation catalysts and quinone precursors. 1 cc containing 0.5 cc of the original extract was injected. The results given represent the average value of single observations on 3 different animals.

of the solutions in the presence of dihydroxyphenyl-alanine, catechol or reductone had completely destroyed the pressor effect of tyramine and indolethylamine. Aeration in the presence of gallic, *l*-ascorbic or *d*-gluco-ascorbic acids caused partial destruction of the pressor effect of these amines. Renin prepared from acetone-dried hog kidney was likewise inactivated by the quinone precursors and other diketones tried. Ephedrine, benzedrine and paredrine were only partially destroyed in most cases (Table I).

It is interesting to note that when tyramine or indolethylamine were added to *p*-quinone, only partial destruction of the amine took place if the solution was not aerated, whereas total destruction took place during aeration. A similar effect was noticed with "dopa" quinone obtained from dihydroxyphenyl-alanine by oxidation with potassium ferricyanide at pH 8.

The same system may destroy the pressor substances produced in the ischemic kidney. The daily injection of a mixture of copper sulfate and dihydroxyphenyl-alanine into hypertensive rats, made hypertensive by perinephric scar and observed for over 3 months, gave significant drops in blood pressure, averaging about 34 mm mercury.‡

The same amounts of copper sulfate or dihydroxyphenyl-alanine, each injected alone, had no effect on the blood pressure of the hypertensive animals. The same mixture, as above, injected during a 6-day period into normal rats did not produce any change of blood pressure. No toxic effects were observed after any of these injections (Table II).

Discussion. Schroeder[6] has described the reduction of blood pressure in hypertensive animals by the injection of mushroom tyrosinase. The above observations may indicate that the suggested oxidative destruction of pressor amines by tyrosinase is achieved through the intermediate oxidation of catechol derivatives such as dopa and epinephrine, resulting in the formation of ortho-quinones as hydrogen acceptors. Bing[7] has observed that the pressor activity of dihydroxyphenylethylamine is destroyed by oxygen in the presence of kidney extract, whereas tyramine is not so destroyed. This effect can also be explained by the intermediate formation of an ortho-quinone.

‡ A drop in blood pressure of 24 mm mercury as measured by arterial puncture was also obtained in a "Goldblatt" dog by intramuscular injection on two successive days of 300 mg "dopa" and 30 mg copper sulfate.

6 Schroeder, H. A., and Adams, M. H., *J. Exp. Med.*, 1941, **73**, 531.

7 Bing, R., Zucker, M. B., and Perkins, W., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 372.

TABLE II.
Blood Pressure Responses of a Group of Hypertensive and Normal Rats to injection of Metal Catalysts and Quinone Precursors.

Group	No. of rats in group	Treatment	Avg blood pressure for group, successive days								
			1	2	3	4	5	6	7	8	9
	Hypertensive Rats										
A	4	0	226	224	220	216	218	226			
B	2	1 mg $CuSO_4$	206	214	206	212	208	212			
C	2	10 mg Dopa	238	232	230	238	242	228			
D	6	1 mg $FeSO_4$ + 10 mg Dopa	194	190	186	186	182	*	(178)		
D	6	Same rats; 1 mg $CuSO_4$ + 10 mg Dopa	178	172	168	166	160	*	(164)		
D	6	Same rats without treatment	164	166	170	168	170	172	189	—	190
	Normal Rats										
E	4	1 mg $CuSO_4$ + 10 mg Dopa	128	134	134	134	136	136	130	132	

*No injection this day.

Blood pressure was measured in the rats' tails according to Williams, Harrison, and Grollman.[5] Each rat in group B received 1 cc of a 0.1% $CuSO_4$ solution, each in group C 1 cc of a 1% dopa solution by daily intramuscular injection for a 6-day period. The 6 rats of group D and E received a course of daily intramuscular injections of 1 cc per day and animal of a solution containing 1 mg of the metal sulfate and 10 mg of dopa.

Summary. 1. The pressor activity of certain aromatic amines is destroyed by aeration *in vitro* in the presence of quinones. 2. Simultaneous injection of dihydroxyphenyl-alanine and small amounts of copper salts in hypertensive rats causes a considerable lowering of blood pressure. 3. It is suggested that tyrosinase reduces the blood pressure by its oxidative effect on acceptor substances of the catechol type such as dopa and epinephrine, which in turn, upon oxidation to ortho-quinones, destroy the pressor amines *in vivo*.

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[5] Williams, J. R., Jr., Harrison, T. R., and Grollman, A., *J. Clin. Invest.*, 1939, **18**, 373.