TABLE III.
Mean and S.E. of Level of Sulfonamides in Blood of Ducks of Table II.

Drug	% in diet	8 A.M. No. of detms.	8 A.M. Free	8 A.M. Total	2 P.M. No. of detms.	2 P.M. Free	2 P.M. Total
			mg%	mg%		mg%	mg%
Sulfanilamide	1.0	13	20.9 ± 1.6	31.9 ± 1.5	13	19.0 ± 1.9	29.9 ± 2.0
Sulfathiazole	0.65	15	4.9 ± 0.6	5.6 ± 0.7	15	5.2 ± 0.5	6.0 ± 0.5
Sulfadiazine	0.65	14	25.3 ± 1.2	27.6 ± 1.3	13	20.2 ± 1.4	21.9 ± 1.4

quite uniform because of the presence of heating lamps. The blood levels and the degree of conjugation were surprisingly uniform for a given drug (Table III). The average percentages of total drug conjugated were: sulfanilamide, 35%; sulfathiazole, 13%; and sulfadiazine, 8%. The metabolism of the 3 sulfonamides used, so far as this can be inferred from the blood levels, resembles that in mammals. The experiments suggest that in terms of the blood levels of drugs, sulfathiazole is the most effective and sulfanilamide is the least effective.

Summary. Malarial infection *(P. lophurae)* of the young Peking duck usually progresses with rapid multiplication of the parasites in the blood and death after 5-16 days[10] (7-10 days in our series). If sulfonamides are administered by incorporation in the food, the multiplication of parasites is checked after a few days and the birds survive at least several weeks with relatively few or, occasionally, no parasites in the blood (sulfathiazole or sulfadiazine). Sulfanilamide appears to be less effective.

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Comparison Between Destruction of Angiotonin, Hydroxytyramine and Tyramine by Renal Extracts.

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According to Page and Helmer,[1] the arteriolar constriction occurring in experimental renal hypertension is caused by angiotonin,

[10] Hegner, R., West, E., Ray, M., and Dobler, M., *Am. J. Hyg.*, 1941, **33**, Sec. C, 101.

[1] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

a dialysable substance of unknown chemical constitution. Bing and Zucker,[2, 3, 4] on the other hand, found that experimental hypertension can be caused by pressor amines formed from amino acids through the action of decarboxylating enzymes contained in the renal cortex. Both angiotonin and pressor amines appear to be inactivated by substances contained in the normal kidney.[5, 6, 7] To investigate whether the angiotonin inhibitor was identical with the renal deaminase, which destroys the pressor amines, experiments were performed to ascertain (a) whether both aerobic and anaerobic incubation of angiotonin with renal extracts lead to its destruction; (b) whether fractions of renal extracts which destroy angiotonin also inactivate hydroxytyramine, (an amine formed in the ischemic kidney from the amino acid, *l*-dihydroxyphenylalanine); and (c) whether octyl alcohol, which inhibits deaminization, prevents the destruction of angiotonin by renal extracts.

Methods. Either 0.3-0.5 cc of angiotonin,* 2-3 cc of hydroxytyramine solution,† or 3 cc of 0.1% tyramine solution were added to 2.6-5.0 cc of kidney extract in 500 cc flasks. The volume of fluid was brought to 20 cc with M/15 phosphate buffer (pH 7.4). The flasks were filled with N_2, O_2, or air, and shaken for 3 hours in a constant temperature bath (40°C).‡ The proteins were then precipitated with 5 cc of 50% trichloracetic acid. After filtering, the solutions were extracted 3 times with ether and distilled to dryness *in vacuo*. The residues were redissolved in 6 cc of distilled water and neutralized with 1 N NaOH. To determine their pressor activity, 1-3 cc of the solutions were injected intravenously into cats anesthetized with nembutal (39 mg per kilo of body weight intraperitoneally).

The kidney extract employed in these incubations consisted of either 3-5 cc of a crude renal extract prepared by the method of Bing,[2] or 2.6-5.0 cc (equivalent to 13.5 g of pork kidney) of a more purified extract prepared by the procedure described by Page in which inert proteins were precipitated from a saline extract of pork

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

3 Bing, R. J., and Zucker, M. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 343.

4 Bing, R. J., and Zucker, M. B., *J. Exp. Med.*, 1941, **74**, 235.

5 Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. J., and Kempf, G. F., *J. Exp. Med.*, 1941, **73**, 7.

6 Blaschko, H., Richter, D., and Schlossmann, H., *Biochem. J.*, 1937, **31**, 2187.

7 Holtz, P., and Heise, R., *Arch. Exp. Path. und Pharm.*, 1939, **191**, 87.

* We take this opportunity to thank Dr. I. H. Page for a generous supply of angiotonin.

† Prepared by the anaerobic incubation of 10 mg of *l*-dopa (*l*-dihydroxyphenylalanine, Hoffman-La Roche) with renal cortical extracts.[2]

‡ We wish to express our thanks to Dr. Victor Ross for the use of his apparatus.

TABLE I.
Effect of Kidney Extracts on Angiotonin, Hydroxytyramine and Tyramine.

Exp.	Substrate: Hydroxy-tyramine	Substrate: Angio-tonin	Kidney extract		Flask filled with	Octyl alcohol	Effect on B.P.
	cc	cc	cc			cc	mm Hg
1	1		3	whole kidney	O_2		—20
		.5	3	" "	O_2		0
		.5	3	" "	N_2		—10
		.5			O_2		+35
2		.5	3	" "	N_2		0
		.5	3	" "	O_2		0
3	3		4	cortical	N_2		+30
	3		4	"	O_2		0
		.5	4	"	N_2		0
		.5	4	"	O_2		0
4	3		5	Page's*	O_2		+40
	3		5	"	N_2		+40
		.5	5	"	O_2		0
		.5	5	"	N_2		0
5	2.5		5	"	O_2		+65
	2.5		5	"	N_2		+80
		.5	5	"	O_2		0
		.5	5	"	N_2		0
6		.3	5	"	Air		—60
		.3	5	" (heated)	"		+30
		.3			"		+10
7		.3	2.6	"	"		—30
		.3			"		+40
8		.3			"		+30
		.3			"	.3	+30
		.3			"		+30
		.3			"	.3	+30
9		.3	5	"	"		—20
		.3	5	"	"	.3	—20
		.3			"	.3	—15 followed by +30
10		.4	5	"	"		0
		.4	5	"	"	.2	0
11		.3	2.6	"	"		0
		.3	2.6	"	"	.3	0
		.3	2.6	" (heated)	"		+25
12	2		5	cortical	O_2		0
	2		5	"	O_2	.3	0
13	2		5	"	O_2		0
	1.8		5	"	O_2	.3	0
14	3†		5	"	O_2		—20
	3†		5	"	O_2	.3	+60
	3†				O_2		+70
	3†				O_2	.3	+50
15	3†		5	"	O_2		0
	3†		5	"	O_2	.3	+90
	3†				O_2		+100
	3†				O_2	.3	+100

*Extract prepared according to method of Page.[5]
†Tyramine, 1 mg per cc.

kidney at pH 4.7.[5] In control experiments the kidney extract was omitted.

Discussion. Experiments in which hydroxytyramine was incubated with crude kidney extracts under aerobic and anaerobic conditions demonstrated that this amine was destroyed only when oxygen was present. Angiotonin, however, lost its pressor effect after both aerobic and anaerobic incubation. Since oxygen is a necessary factor for the action of deaminating enzymes, this result suggests that the principle responsible for the inactivation of angiotonin is not identical with the amine oxydase contained in the kidney.

In 5 instances hydroxytyramine or angiotonin was incubated with a purified renal extract prepared according to the method of Page. Angiotonin was inactivated in every instance, whereas no loss in the pressor effect of hydroxytyramine was noticeable. These results give further proof that the breakdown of angiotonin and that of hydroxytyramine are caused by different factors.

From these experiments it was to be expected that octyl alcohol, which poisons the mono-amine oxydase, would prevent the enzymatic breakdown of hydroxytyramine but would not affect that of angiotonin. In 6 experiments, however, it was shown that both pressor substances were destroyed by kidney extracts even in the presence of the alcohol. On the other hand, tyramine was not destroyed by kidney extract when octyl alcohol was present. Since the work of Blaschko makes it clear that octyl alcohol effectively blocks the oxydative deaminization of both tyramine and hydroxytyramine, these results can only be interpreted by postulating the existence of another aerobic enzyme in kidney extracts capable of destroying hydroxytyramine, but differing from the renal deaminase in its resistance towards octyl alcohol. It is possible that this enzyme is a polyphenolase, since Keilin and Hartree[8] have shown that renal tissue contains an enzyme which oxidizes d- and l-dopa but not d- or l-tyrosine.

Summary. (1) Angiotonin is inactivated following incubation with renal extracts. This destruction does not occur by oxidative deaminization since (a) oxygen is not necessary for the reaction, (b) a fraction of kidney extract can be prepared which destroys angiotonin but fails to inactivate hydroxytyramine, and (c) angiotonin is destroyed in the presence of octyl alcohol. (2) Evidence is presented for the existence of an aerobic polyphenolase in kidney extracts, capable of destroying specific pressor amines.

[8] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.*, Series B, 1935-6, **119**, 114.