sion could account for the marked effect of the antibiotics at low levels.

The antibiotics do not act specifically on glutamate oxidation, for in all cases an equal or greater action on the toxicity and infectivity for mice occurred. The earlier finding that glutamate oxidation is intimately associated with viability(1) is here confirmed, and the depression of this oxidation by aureomycin and terramycin is most likely due to their rickettsicidal action.

It is of interest to note that aureomycin, at concentrations corresponding to those used in the present experiments, specifically depressed phosphorylation in mitochondria without affecting their respiration(8). This may suggest the mode of action of the antibiotic to be an interference of energy transfer. Observations by Hahn and Wisseman(9) on the effect of aureomycin, terramycin, and chloramphenicol on the formation of adaptive enzymes by *E. coli* support this hypothesis. Our present lack of knowledge of the energy metabolism and synthetic reactions of rickettsiae precludes speculation on a similar mechanism for the action of these antibiotics on rickettsiae.

Summary. Aureomycin and terramycin in concentrations of 100-300 μg per ml were found to inhibit markedly the respiration of purified murine and epidemic typhus rickettsiae *in vitro*. Chloramphenicol in concentrations up to 300 μg per ml, aureomycin and terramycin in concentrations up to 30 μg per ml produced only slight inhibition. The inhibition of respiration was correlated with a decrease in the number of viable rickettsiae as determined by toxicity and infectivity for white mice. These experiments demonstrate the rickettsicidal action of aureomycin and terramycin *in vitro*.

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Citrovorum Factor, Vitamin B_{12}, and Folic Acid Activity of Whole Blood of Several Species.* (19328)

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In the course of a series of investigations concerning the release of *Leuconostoc citrovorum* factor (LCF) from tissues(1) and the conversion of folic acid (PGA) to LCF(2), we became interested in the LCF content of blood. This paper reports studies on the LCF, vit. B_{12}, and PGA content of the blood of rats (in various stages of vit. B_{12} depletion or repletion), chicks, sheep, and cattle.

Experimental. Blood was obtained from rats under Nembutal† anesthesia by hypo-

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† Pentobarbitol sodium, Abbott.

TABLE I. Free Vitamin Content and Effect of pH of Autolysis on Release of *S. faecalis* and *L. citrovorum* Activity from Whole Blood and Plasma.*

	Mature rat, non fasted					Mature rat, fasted 24 hr					Veal calf				
	CF		PGA		B_{12}	CF		PGA		B_{12}	CF		PGA		B_{12}
pH	Free	Total	Free	Total	Free	Free	Total	Free	Total	Free	Free	Total	Free	Total	Free
4.5		5.2		9			1		3.8			2.6		4.5	
5		11.3					1		3.9			2.5		4.5	
5.5		12.3		20			2		4			2.1		4.5	
6		5.4					2.2		4.2			2.4		5.5	
6.5		7.8		10			2.3		3.5			2.4		6	
7	<.2	11.6	2	19.5	.70	<.2	3.2	3.3	4.3	.50	<.1	1	.5	9	.97
7.5		8.2		12			2.8		4.2			.9			
8		6		12			1.8		2.8						

	Chicken					Sheep					Ox plasma				
4.5		3		<4			.1					2.9		6.5	
5		7.5		19			.9		1.5			2.1		7.5	
5.5		16.2		23.1			1.1		2.5						
6		18		37.5			1.2		3.5			3		4	
6.5		16.5		51.5			1.2		4.2			1.3		5.5	
7	<2	13.5	6.1	21	1.25	<.2	1.4	.50	6	.80		1.8		8.5	
7.5							.5		5.4			1.6		3.5	
8		4		12			.5								

* All values are mγ/ml.

dermic syringe from the portal vein, or from the post cava after the rat had been stunned by a blow on the head. The blood from the chick, sheep, and ox was obtained at slaughter. In all cases blood was collected into a chilled container containing double oxalate(3). For the determination of the free vitamin, 1 ml of blood was diluted with 5 ml water, adjusted to pH 7 and autoclaved for 10 minutes at 120°C. The resulting precipitate was homogenized and 4 ml of water was used to rinse the homogenizer. The particulate matter was centrifuged down and the supernatant liquid poured off and frozen at –4°C until ready for analysis.

In the study of the release of bound LCF and PGA, samples of blood were placed in appropriate buffers (citrate-phosphate, or bicarbonate) and incubated at 37°C, under toluene, for 20 hours. After incubation they were adjusted to pH 7, autoclaved for 10 minutes at 120°C, homogenized, and centrifuged. The supernatant solution was removed, and frozen until ready for analysis. Samples for the determination of vit. B_{12} activity were incubated with trypsin (50 mg Difco trypsin/ml of heated blood) for 18 hours and treated as outlined above. The vitamin content of the samples was determined by microbiological assay. When large amounts of blood were available, turbidimetric assays with an 18-22-hour incubation at 37°C were employed. When the amount of sample was limited as in the case of individual rats, 72-hour acidimetric assays were run. Vit. B_{12} was determined with the medium of Thompson *et al.*(4) using *Lactobacillus leichmannii* 4797 as the test organism. Total *Streptococcus faecalis* R activity was determined with the medium of Luckey *et al.*(5) and *Leuconostoc citrovorum* 8081 activity was determined using the procedure of Sauberlich and Baumann(6).

Results and discussion. It was found that concentrations greater than 1 ml of blood in 5 ml total volume interfered with the assay of LCF and PGA regardless of whether acidimetric or turbidimetric procedures were used. Therefore, the reported values are for 1 ml of blood in 10 or more ml total volume. In a few cases a 1:20 or 1:30 dilution interfered with the turbidimetric assay. However, such cases were the exception and occurred only with the turbidimetric assay.

Whole blood was found to contain low levels of free LCF and PGA as is shown in Table I. A considerable portion of the vit. B_{12} activity was free or else was released by hemolysis and autoclaving. However, treatment with trypsin at pH 8.2 increased the level of measurable B_{12} slightly. The PGA values are in fair agreement with those reported by Simpson and Schweigert(7). The

TABLE II. The Effect of Vit. B_{12} on the Level of B_{12}, LCF and PGA in the Blood of Vit. B_{12} Deficient Hyperthyroid Rats.

Treatment	Vitamin activity, mγ/ml whole blood		
	LCF	PGA	B_{12}
Control (1)*	.10	14	.30
" (pooled 3)	.10	14	.21
3 γ vit. B_{12} inj./day (1)	2	14	1.50
" " " " " (pooled 3)	2	10	1.25
" " " oral/day (1)	2.9	11	.90
" " " " " (pooled 3)	2.8	14	1.00

LCF and PGA activity determined after autolysis at 37°C, pH 7 for 18 hr. B_{12} activity determined after incubation of 1 ml heated blood with 50 mg trypsin for 24 hr at pH 8.2. There was no free vit. B_{12} in the blood of group III.

* Figures in parentheses represent number of animals used.

vit. B_{12} values were in the range of those reported by Couch *et al.*(8).

The amount of LCF and PGA activity released by autolysis varied with the pH of the reaction medium (Table I). The optimum pH for the release of LCF and PGA by whole blood enzymes was 7.0 and 7.0 for the rat, 4.5 and 6.5 for the calf, 6.0 and 6.5 for the chicken and 7.0 and 7.0 for the sheep. Ox plasma appeared to have 2 points of release at pH 4.5 and 6.0 for LCF and 4.5 and 7.0 for PGA. Simpson and Schweigert(7) reported an optimum pH of 6.0 for the conjugase of the whole blood of rabbits, of 8.0 for turkey blood and 7.0 for turkey plasma. These data suggest that there are at least 3 factors active in the release of PGA and LCF activity from blood. Simpson and Schweigert (7) reported a large increase of PGA activity following treatment with chick pancreas. Hence, the values reported here may not represent total activity present, but merely that released by blood enzymes.

The blood of rats on a vit. B_{12} deficient diet containing 0.15% iodinated casein was very low in LCF (Table II). Blood from similar rats receiving 3 μg of vit. B_{12} per day, orally or by injection, contained appreciable LCF. There was no detectable change in the PGA activity upon the administration of vit. B_{12}. The B_{12} level increased significantly following vit. B_{12} administration.

Summary. (1) The whole blood of the rat, chick, ox, or sheep was found to contain little or no free *L. citrovorum* active material. However, demonstrable amounts of free *S. faecalis* activity were found. (2) The release of LCF and PGA during autolysis is dependent on the pH of the reaction mixture. Optimum values for LCF and PGA were obtained at pH 4.5 or 6.5-7.0 depending on the species involved. (3) Blood from rats on a B_{12} deficient diet containing iodinated casein had little LCF activity. However, blood from hyperthyroid rats receiving ample vit. B_{12} contained LCF levels similar to those of normal rats after fasting.

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