black rats following hypophysectomy, similar to the pigmentation occurring in black rats following adrenalectomy. The adrenals of the hypophysectomized rats were smaller than normal, with atrophic cortices. Excretion of ascorbic acid decreased to low levels following hypophysectomy. The increase in pigmentation is believed to be related to the derangement of the steroid hormones of the adrenal cortex of the hypophysectomized rat.

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In Vitro Effect of Aureomycin, Terramycin, and Chloramphenicol on Typhus Rickettsiae.* (19327)

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In a previous communication(1) from this laboratory it was reported that purified typhus rickettsiae oxidize glutamate, and that the rate of this oxidation is proportional to the concentration of viable rickettsiae, as determined by their toxicity for white mice. It was further shown(2) that the antibiotics effective in rickettsial infections, aureomycin, terramycin, and chloramphenicol, do not reduce the toxicity for white mice when tested *in vitro* for one hour at concentrations of 10 and 30 μg per ml. The present paper deals with the effect of these antibiotics in concentrations of 10-300 μg per ml on the rate of glutamate oxidation and the toxicity for mice of purified suspensions of epidemic and murine typhus rickettsiae.

Materials and methods. Antibiotics. Aureomycin hydrochloride (twice recrystallized, Lederle), terramycin hydrochloride (Pfizer), and chloramphenicol (synthetic, Parke, Davis)† were used. In some experiments, terramycin sterile base (Pfizer), and chloramphenicol (pure crystalline) were used; however, no differences between the two preparations of these drugs were observed. The antibiotics were dissolved in a salt solution of the following composition: KCl 0.126 M; NaCl 0.0018 M; KH_2PO_4 0.0012 M; Na_2HPO_4 0.0109 M (solution K 7.5)(3); the pH was adjusted to 7.5 by addition of KOH, and dilutions were made with solution K 7.5. *Rickettsial suspensions* (the Wilmington strain of *R. mooseri* and the Breinl strain of *R. prowazeki*) were prepared from frozen infected yolk sac pools as described previously (1,3). The precipitate from the final high speed centrifugation was resuspended in either sucrose-P (sucrose 0.225 M; KH_2PO_4 0.0016 M; K_2HPO_4 0.0086 M, pH 7.5)(3), or 6% bovine serum albumin powder, Armour's fraction V, in solution K 7.5, pH 7.5. In all experiments, 1 ml of the suspending fluid was used for each 2 g of original infected yolk sac. *The rate of oxygen uptake* was measured by the Warburg method at 34.5°C. Flasks of two capacities, 3 ml and 8 ml, both

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smaller than the conventional size, and manometers of appropriate size were used. In all cases the reaction mixture in the 3 ml flasks consisted of 0.25 ml of the 200% rickettsial suspension, 0.05 ml of 0.16 M glutamic acid neutralized with KOH, 0.05 ml of a mixture of 0.024 M $MgCl_2$ and 0.004 M $MnCl_2$, and 0.05 ml of antibiotic solution to give the indicated final concentration. The center well contained 0.05 ml 10% KOH. The 8 ml flasks were employed in those experiments in which the antibiotic was added from the side arm. The reaction mixture in these experiments consisted of 0.75 ml of the 200% rickettsial suspension, 0.1 ml of 0.16 M glutamic acid neutralized with KOH, 0.1 ml of a mixture of 0.024 M $MgCl_2$ and 0.004 M $MnCl_2$, 0.1 ml of solution K 7.5, and 0.15 ml of antibiotic solution. The center well contained 0.05 ml 10% KOH. *Toxicity for white mice* of the contents of the Warburg flasks at the end of the experiment was estimated by the intravenous injection of 0.25 ml of serial half log (3.16 fold) dilutions, using 4 mice for each dilution(4). The dilution required to kill 50% of the mice was calculated by the method of Reed and Muench(5). The mice surviving the toxic action of suspensions of murine rickettsiae were observed for 8 days, and deaths due to rickettsial infection as determined by autopsy were recorded.

Results. The rate of oxygen uptake with glutamate of the rickettsial suspensions used in these experiments ranged from 43 to 90 micro-liters (μl) O_2/hr/ml suspension; the oxygen uptake without added glutamate was negligible. With all three antibiotics at concentrations comparable to those effective *in vivo* (10-30 μg per ml), a slight but definite inhibition of the rate of oxygen uptake with glutamate was observed. This effect has been noted by Wisseman *et al.*(6). Higher levels of aureomycin and terramycin (100-300 μg per ml) caused increasingly stronger inhibition, while higher levels of chloramphenicol were without further effect. Typical experiments showing the progress and degree of inhibition are presented in Fig. 1. When aureomycin or terramycin was added to the respiring rickettsial suspensions, there was an immediate inhibition which increased rapidly for the first 30 minutes. In the case of aureomycin, this increase continued at a slower rate during the remaining 150 minutes. However, in the case of terramycin the levels of inhibition reached in the first 30 minutes remained constant throughout the period of observation. In the experiments presented here murine rickettsiae suspended in the 6% albumin solution were used. When the sucrose solution was used instead of the albumin one, the re-

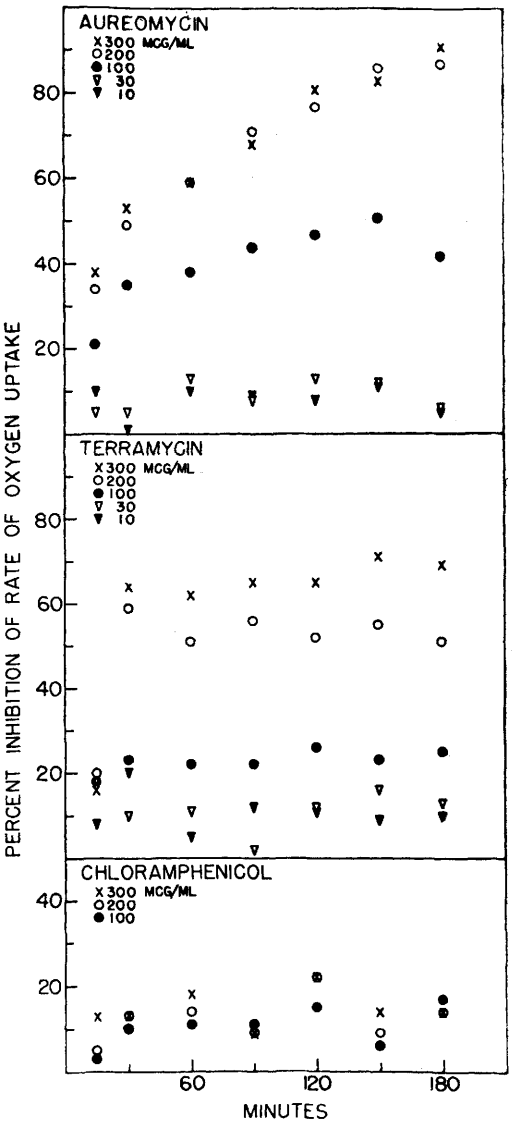

FIG. 1. Effect of aureomycin, terramycin and chloramphenicol on the rate of glutamate oxidation by murine typhus rickettsiae.

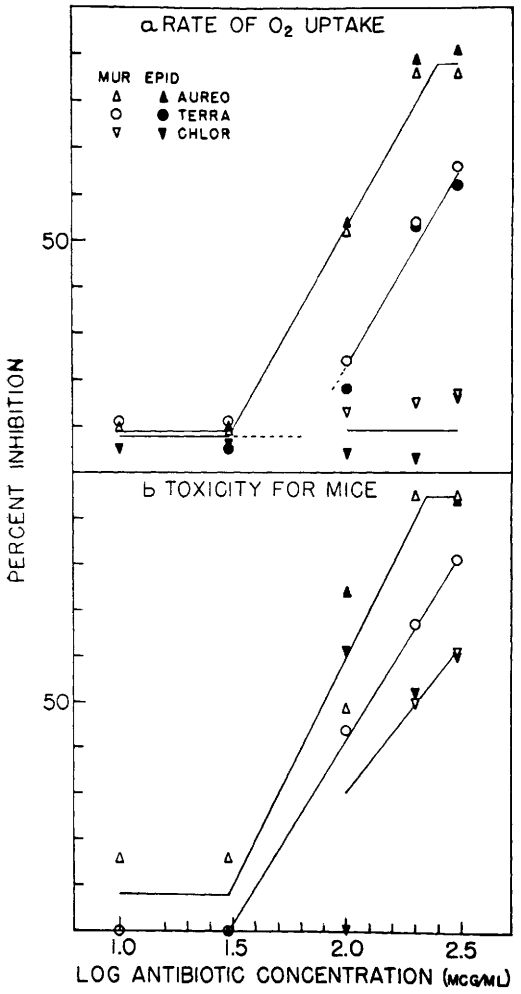

FIG. 2. Extent of inhibition of glutamate oxidation (a) and toxicity for mice (b) of epidemic and murine typhus rickettsiae with different levels of aureomycin, terramycin and chloramphenicol.

sults with aureomycin were essentially the same; with terramycin, although the same degree of inhibition as in albumin was attained (at concentrations of 300, 200, and 100 μg per ml), the progress of the inhibition was much more gradual. Thus, with 300 μg terramycin per ml, 50% inhibition was reached in albumin in about ½ hour, and in sucrose in about 2 hours. The respiration of epidemic typhus rickettsiae in albumin or in sucrose was affected by all three antibiotics in the same way as that of murine rickettsiae.

The relative effectiveness of the antibiotics as inhibitors of glutamate oxidation by both murine and epidemic typhus rickettsiae is shown in Fig. 2a. A linear relationship between the logarithm of the drug concentration and the degree of inhibition may be observed at concentrations above 30 μg per ml for aureomycin and above 70 μg per ml for terramycin. Thus, on a weight basis aureomycin appears to be about twice as effective as terramycin. The slight inhibition at lower levels of these two drugs and at all levels of chloramphenicol up to 300 μg per ml is independent of drug concentration. The inhibition of the toxicity for white mice of the rickettsial suspensions by aureomycin and terramycin paralelled rather closely the inhibition of respiration (Fig. 2b). In the case of chloramphenicol the toxicity for mice was inhibited more strongly than the glutamate oxidation.

The 3 antibiotics suppressed the infectivity of murine rickettsiae for white mice to approximately the same extent as the toxicity. However, the method used here to estimate infectivity is not sufficiently sensitive to allow accurate correlation.

Discussion. The concentration of the rickettsial cells used in the present *in vitro* experiments was approximately 10^4 times as high as in the *in vivo* chick embryo experiments(2). The influence of the concentration of bacterial cells on the bactericidal action of terramycin has been investigated by Hobby *et al.*(7). They showed that with *S. typhosa* and *P. pyocyaneus* the decrease in the number of organisms after contact with the antibiotic varied not only with the concentration of the antibiotic but also with the concentration of cells. The fact that marked inhibition of respiration and of toxicity for mice was observed only at levels considerably higher than those shown to be effective in chick embryos may be due to the much greater concentration of rickettsiae. Moreover, the rickettsial suspensions used here most likely consist of a mixed population of cells of varying susceptibility to the action of poisons. Young cells, *i.e.*, those having undergone recent cell division, are probably most sensitive, and the slight but definite inhibition observed at low levels of antibiotic may be due to the effect of the drugs on these cells. In the chick embryo or experimental animal continuing cell divi-

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.