CH-5 spectrometer using an electron beam potential of 70 eV and a sample inlet temperature of 190 °C. The elemental analyses were carried out by Micro-Tech Laboratories, Inc., Skokie, Ill.

The product of oxidized 6-OHDA and GSH was prepared and isolated in the following fashion. An equimolar ratio of the quinone (64 mg of free base) and the GSH (117 mg) was placed in a small vacuum flask. Deaerated, double distilled water (10 mL/100 mg) was added and the flask quickly attached to the vacuum line to prevent air oxidation. The flask was then placed in a warm water bath (35–40 °C) until the solution became completely colorless (ca. 20 min). The product was then precipitated by adding 3–4 vol of 0.01 M citric acid in acetone. The acidity retarded further air oxidation. The sticky residue was separated by decantation, washed with pure acetone, and dried in vacuo. About a 70% yield of gray-brown product was typically obtained in several preparations.

Animal Studies. Male Sprague–Dawley rats (300–350 g) were anesthetized with Nembutal and a stereotaxic injection of 6-OHDA was made via a Hamilton 50-μL syringe with a 30-gauge needle. The coordinates for hypothalamus (A, 6.0; L, 0.05; D, -3.7) were obtained from the atlas by Pellegrino and Cushman.[11] Experimental rats received 100 or 200 μg of 6-OHDA (free base) dissolved in a saline–ascorbate (1 mg/mL) vehicle. The injection volumes were 5 and 10 μL for the two doses, respectively. All injections were made slowly over a 1-min period. Following a 30-s wait, the needle was withdrawn, the hole closed with a jeweler's screw, and the animal placed back in its cage until time of sacrifice.

To check the validity of the injections, similar injections of crystal violet dye solution were made in several rats. One hour after injection the animals were sacrificed and the brains examined histologically. In all cases the dye had diffused into an area ca. 4 mm in diameter around the needle tip and very little appeared to back up the injection tract. Thus it was verified that the entire hypothalamus (HT) was reasonably bathed with this style of injection.

Rats were sacrificed at 1- and 3-h intervals after injection. The brains were rapidly removed and placed on a glass plate over dry ice. The HT was dissected out and immediately frozen until analyzed. The HT from three animals were pooled and weighed. The pooled HT was homogenzied by sonication in 300 μL of cold 0.2 M perchloric acid and then centrifuged at 10000 rpm for 15 min at 0 °C. A portion of the supernatant was used for GSH and GSSG analyses according to the method of Halprin[12] using glutathione reductase and NADPH. Other aliquots were injected directly into a LC column for detection of the reaction product of 6-OHDA and GSH.

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Correction of Prior Estimate of the Biological Activity of an *N*-Trifluoroacetyl Analogue of D-*threo*-Chloramphenicol Relative to Chloramphenicol

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The inhibitory rate constants, k, for the inhibition of the rate of *Escherichia coli* generation at various concentrations, C, of chloramphenicol and its *N*-trifluoroacetyl analogue were determined in vitro in accordance with $k_{app} = k_0 - kC$ where k_{app} is the apparent first-order generation rate constant at a drug concentration C and k_0 is that constant in the absence of drug. The activity of the analogue was one-tenth that of chloramphenicol in contrast to a previously reported value.

Previously[1] our laboratories provided rate constants, k_{app}, for the generation of *Escherichia coli* in peptone broth USP affected by various concentrations, C, of various analogues of chloramphenicol by monitoring the total number of organisms, N, with time, t, in accordance with

$$\ln N = k_{app}t + \ln N_o \quad (1)$$

where N_o was the total number of organisms at a time t_0 taken as zero. Linear plots were obtained when these k_{app} values were plotted against the concentration of the substituted chloramphenicol, C, in accordance with

$$k_{app} = k_0 - kC \quad (2)$$

where k_0 is the generation rate constant in the absence of drug and k is the inhibitory rate constant.

Recently,[2] it was brought to my attention that the action of the *N*-trifluoroacetyl analogue of D-*threo*-chloramphenicol (**1**) obtained from the same source[3] was not as inhibitory as chloramphenicol (**2**) in several systems. This was in contrast to the apparent results we had obtained. The compounds were restudied by the procedures given previously[1] and a systematic search was made of the original data to find a probable source of error.

Results and Discussion

Additional **1**, obtained from the same source,[3] and the original material used in our previous studies were eval-

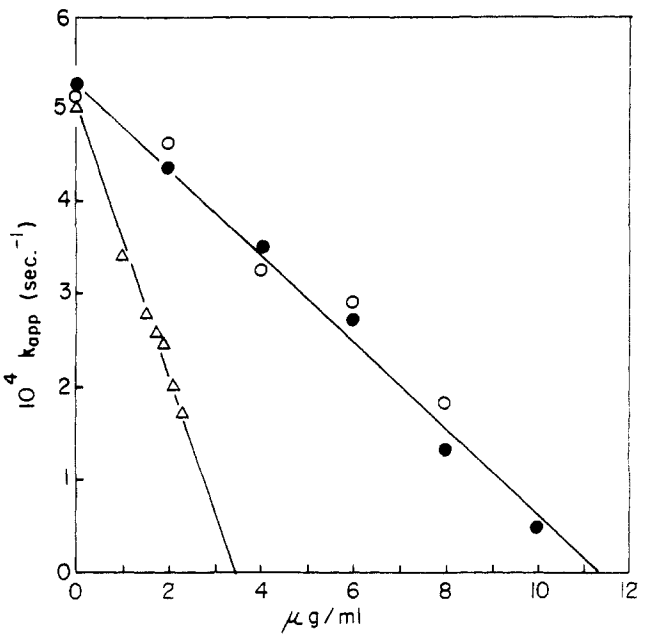

Figure 1. Plots of apparent microbial generation rate constants as a function of antibiotic concentration: chloramphenicol (△); *N*-trifluoroacetyl analogue of D-*threo*-chloramphenicol, old lot (○), new lot (●).

uated by microbial kinetics in the same manner[1] at pH 7 and compared to chloramphenicol. The results are given in Figure 1 and it can be seen that the inhibitory rate constant of 1, $k = 4.63 \times 10^{-5}$ mL/μg/s, was 0.32 the value for 2; $k = 1.45 \times 10^{-4}$ mL/μg/s. This value for 1 was tenfold less than that previously reported (see Figure 2 in ref 1) whereas the value for 2 was similar to that reported previously.

The data in the original laboratory notebooks were then carefully reviewed and a probable transcription error was observed. It was noted on one page that two stock solutions of 1 were prepared at 20.7 mg/100 mL and 51 mg/25 mL (0.207 and 2.04 mg/mL, respectively) and it was stated that dilutions of the former were used in the microbial kinetic studies where the results were tabulated on a different page for compound 1. It was apparent that the latter solution was actually used and it can be properly presumed that the concentrations used in the calculations were based on the premise that they were one-tenth the actual concentrations. Thus the inhibitory rate constant for 1 was erroneously reported as being tenfold its actual value.

Careful review of the original data in the laboratory notebook indicates that there is little probability of any other errors in the reported microbial kinetic data given in the published paper[1] for the 36 other chloramphenicols studied and it can be concluded that none of the analogues had greater potencies than chloramphenicol.

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Evidence for Separate Peptide Sequences Related to the Lipolytic and Magnesium-Accumulating Activities of ACTH. Analogy with Adrenergic Receptors

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Native adrenocorticotropin [ACTH-(1–39)] and ACTH-(1–24) stimulate both lipolysis and magnesium accumulation in rat adipocyte plasma membrane vesicles. ACTH-(1–20) retains full lipolytic activity but has a minimal effect on magnesium accumulation. In contrast ACTH-(11–24) stimulates magnesium accumulation but not lipolysis. These findings indicate that within the ACTH molecule the peptide sequence responsible for stimulation of magnesium accumulation is distinctly separate from the core sequence (residues 4–10) essential for stimulation of adenylyl cyclase activity and cAMP mediated lipolysis. Phentolamine, an α-adrenergic antagonist, blocks the bulk of magnesium accumulation stimulated by native ACTH and norepinephrine; propranolol, a β-adrenergic antagonist, blocks the earliest phase of Mg^{2+} uptake by these hormones but has little effect on net uptake. Isoproterenol, a β-adrenergic agonist, stimulates magnesium uptake only minimally. The pattern of uptake stimulated by methoxamine, an α-adrenergic agonist, or ACTH-(11–24) is quite similar to that produced by native ACTH in the presence of propranolol. The receptor through which ACTH mediates stimulation of the bulk of magnesium appears to be analogous to the α-adrenergic receptor through which norepinephrine stimulates this same process.

Epinephrine, norepinephrine, and ACTH have recently been reported to stimulate ATP-dependent magnesium accumulation in plasma membrane vesicles prepared from rat adipocytes.[1] Previous studies of ACTH have correlated its structure with steroidogenesis, binding to receptors, antigenicity, and lipolysis. Phylogenetic studies of ACTH have revealed an invariant region (residues 1–24) and a variable region (residues 25–39), which is more antigenic. The invariant region contains the core sequence (residues 4–10) identified with stimulation of adenylyl cyclase.[2] ACTH-(15–18) contains a sequence of basic residues important for binding to the target cell.[3,4] Residues 19–24 have not been investigated except insofar as they protect ACTH-(1–24) from degradation.[5]

In this study we compare the effects on Mg^{2+} accumulation of ACTH-(1–20), ACTH-(1–24), and ACTH-(11–14) with the parent peptide ACTH-(1–39). The importance of residues 21–24 for optimal stimulation of magnesium uptake has been indicated previously.[5] We are now able to show that the core sequence, necessary for the stimulation of adenylyl cyclase, is not required.

Some insight into the nature of the receptor involved in magnesium accumulation has been gained through the use of adrenergic antagonists.[5] These studies utilizing agonists as well as antagonists indicate that an α-adrenergic mechanism is involved not only in the action of nor-