with respect to microbiological potency under the conditions studied.

Studies on modifications of the aqueous propylene glycol formulation indicate that pH and propylene glycol content are critical factors. If the propylene glycol content is at a level of 50% or greater at pH 8.0–8.5, there is little or no loss of microbiological potency after 9 weeks at 37°. If the pH ranges from 8.0 to 8.5, the formulation retains its potency after 5 weeks at 37°. Below this pH range, the formulation slowly loses activity, presumably through C.4 epimerization, the kinetics of which have recently been described (4).

SUMMARY

Stability studies indicated that stable preconstituted formulations suitable for parenteral administration can be prepared with DMCTC and TC-aluminum-calcium-gluconate complexes in 50–80% propylene glycol at pH 8.0–8.5. Both pH and propylene glycol content are important factors affecting the stability of the formulation. The degree of blood-level enhancement is not altered significantly by the percentage of propylene glycol in the formulation or by the molar ratios of the complexes within the range studied following intravenous and intramuscular administration. By either route, the DMCTC complexes in the aqueous propylene glycol formulation appeared to be well tolerated in dogs when administered at therapeutic levels.

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Some Pharmacological Properties of Polymyxin B Sulfate

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Polymyxin B sulfate produces diastolic standstill followed by contracture in the isolated perfused rabbit heart. No increase in cardiac output was noted in the perfused frog heart. Large doses inhibit splanchnic nerve innervation to the perfused superior mesenteric artery of the cat.

SYSTEMIC ADMINISTRATION of polymyxin B sulfate has a potent bactericidal effect on Gram-negative bacilli, particularly *Pseudomonas aeruginosa*. However, because of nephrotoxic and central nervous system effects, such as vertigo and paresthesias, it has generally been limited to topical application. Although it is generally realized that the systemic use of this antibiotic is hazardous, reports in the literature describe its use in this manner (1–4). Timmerman *et al.* (5) have demonstrated the neuromuscular blocking action of polymyxin in the rabbit.

The present work describes the effect of polymyxin B on the isolated rabbit heart, on cardiac output measured by the modified Howell and Clark (6) frog heart preparation, and on the vascular resistance of the perfused superior mesenteric artery of the cat.

METHODS

The effect of polymyxin B sulfate on the perfused isolated rabbit heart was studied using the preparation described by Langendorf (7). Dutch rabbits weighing 2–3 Kg. were sacrificed by a blow at the base of the skull, and the heart was removed *via* a midline chest incision. A glass cannula, with side arm tube, was placed in the aorta, and the coronary vessels were perfused with oxygenated Locke-Ringer's solution previously warmed to 37°. All drugs were administered through the side arm tube of the glass cannula. Isotonic recordings were made using an ink recorder on kymograph paper. The action of potassium chloride, calcium chloride, and magnesium chloride on the isolated rabbit heart was evaluated before and after the administration of polymyxin B sulfate. The results are expressed as per cent changes of the systolic contraction.

The effect of polymyxin B sulfate on cardiac output in the frog *in situ* was evaluated by a modification of the Howell and Clark technique (6). *Rana pipiens*, weighing 200–250 Gm., were double pithed and the hearts exposed. After ligation of the right aortic arch cannulae were placed in the inferior vena cava and cardiac output measured by collecting the perfusate in graduated cylinders for 1-minute periods. The heart rate was also recorded.

The action of polymyxin B sulfate on the perfusion of the cat gut was evaluated as follows. Anesthesia was induced by pentobarbital sodium (35 mg./Kg.), and a tracheotomy was performed. For intravenous administration of drugs, the inferior vena cava was cannulated below the renal vein. Heparin sodium (5 mg./Kg.) was administered intravenously. The abdominal aorta was cannulated with Tygon tubing and a Sigmamotor pump (model T-8) maintained a constant volume flow into the superior mesenteric artery. To measure changes in perfusion pressure, a pressure transducer was connected between the pump and the artery. The superior mesenteric plexus was isolated, and bipolar silver electrodes were placed on these nerves. Supramaximal stimulation with a frequency of 20/second for 30 seconds was employed. Systemic blood pressure was measured by cannulation of the common carotid and recorded using an Offner dynograph (type 542). The effect of intra-arterial doses of polymyxin B sulfate varying from 1 to 50 mg. (total dose) was evaluated in this preparation.

The data were evaluated statistically using a Student *t* test (8).

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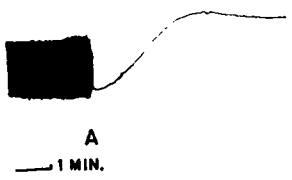

Fig. 1.—Effect of polymyxin B sulfate on the isolated rabbit heart. A, 10 mg. polymyxin B sulfate injected into the perfusing solution.

RESULTS

The effect of polymyxin B sulfate on the isolated rabbit heart was observed in 20 isolated heart preparations. Cardiac responses to polymyxin B sulfate are illustrated in Fig. 1. After the injection into the aortic cannula of 5 to 30 mg. of polymyxin B sulfate, a marked depression occurred within 30 seconds. The systolic contraction was 22 ±11 ($p < 0.01$) of the control contraction. After 3 to 8 minutes, a slowly increasing contracture occurred. The contracture was equal to 160 ±22 ($p < 0.01$) of the control systolic contraction.

After the administration of potassium chloride (0.1–0.3 ml. of a 30% solution) in a nonpolymyxin B sulfate treated heart, cardiac arrest was observed, and the heart became flaccid. Recovery was spontaneous in 20 experiments. The administration of 20 to 40 mg. of polymyxin B sulfate after recovery from potassium chloride produced cardiac arrest and contracture similar to that illustrated in Fig. 1.

At this time, repetition of the initial dose of potassium chloride produced an increase in contracture in 12 of 20 preparations and no change in the other eight. The average increase was 18%; this change was not significant ($p > 0.4$). In 10 experiments, 1 to 5 ml. of a 1:200 solution of magnesium chloride or 1 to 5 ml. of a 1:200 solution of calcium chloride also failed to produce relaxation or additional contraction after contracture had been produced by polymyxin B sulfate ($p > 0.5$).

In six isolated perfused frog hearts *in situ*, administration of increasing doses of polymyxin B sulfate (0.1–1.0%) produced only progressive depression, evidenced by the cardiac output. The average control heart rates were 52 ± 6.0, and the average cardiac output was 2.2 ± 0.5 ml./minute. After 5 minutes of infusion with polymyxin B sulfate prepared in the frog-ringer solution, the heart rates averaged 48 ± 8.0, and the average cardiac output was 0.7 ± 0.3 ml./minute. Difference in heart rate was not significant ($p > 0.5$), but the decrease in cardiac output was significant ($p < 0.01$). With continued infusion the hearts ceased to beat in diastole. These results suggest strongly that the mechanism of cardiac action is other than digitalis-like activity.

In 10 experiments using cats, when the superior mesenteric plexus was stimulated, an increase in perfusion pressure resulted. After the administration of 10 mg. of polymyxin B sulfate into the superior mesenteric artery, there was significant antagonism of the increase in vascular resistance produced by nerve stimulation. The increase in the mean blood pressure before polymyxin B was 109 ± 18 mm. Hg. After 10 mg. of the compound, nerve stimulation produced an elevation of 33 ± 14 mm. Hg. This reduction in response to nerve stimulation was significant ($p < 0.01$). The vascular response to 1 to 4 mcg. of norepinephrine was unaltered by this dose of polymyxin B sulfate.

DISCUSSION

The diastolic standstill and subsequent contracture produced by polymyxin B sulfate on the isolated rabbit heart may be due to the chelation of an essential ion by this polypeptide antibiotic. Loss of the ion may then account for the intracardiac block produced and subsequent cardiac arrest. However, the infusion of the calcium chloride, magnesium chloride, and potassium chloride failed to produce recovery after diastolic standstill and contracture had been produced by polymyxin B sulfate.

The possibility that polymyxin B sulfate may alter the permeability of the cellular membranes to sodium or other ions and thus prevent repolarization must also be considered. Finally, the contracture that takes place after diastolic standstill may be due to the direct action of polymyxin B sulfate on the contractile mechanism of the muscle cell.

The observation that polymyxin B sulfate did not antagonize the pressor response after the injection of norepinephrine into the superior mesenteric artery, but did depress the pressor response normally elicited by sympathetic nerve stimulation, suggests that polymyxin B sulfate may have an action at the presynaptic sympathetic nerve terminal. That the inhibition occurred within 1 minute after arterial injection of the antibiotic suggests that the mechanism is other than prevention of synthesis. A mechanism that prevents the release of norepinephrine from the nerve terminals would be consistent with the results reported above.

From the above discussion it is obvious that more experimental work must be done before any valid conclusion concerning the mechanism of action of polymyxin B on the isolated rabbit heart or on the sympathetic nerve endings to the cat vascular bed can be determined. However, these studies do illustrate that the polypeptide, polymyxin B sulfate, does possess various unusual cardiovascular properties.

SUMMARY

This report describes the effects of polymyxin B sulfate on the isolated rabbit heart, the perfused frog heart *in situ*, and the perfused superior mesenteric arterial bed. In the isolated rabbit heart, polymyxin B sulfate produces diastolic standstill and subsequent contracture; this is not reversed by the administration of potassium, calcium, or magnesium ions. Depression of cardiac output is noted *in situ* using perfused frog hearts. Vascular constriction induced by nerve stimulation was antagonized by polymyxin B sulfate.

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