plained by the fact that activated C1 can destroy C4 in the fluid phase (*14*), thereby decreasing the amount of C4 that can bind to the virus. In vivo, fluid phase destruction of C4 normally does not occur because C1 exists in the precursor nonactivated state. Thus, in vivo, IgM-sensitized virus should be effectively neutralized by C4.

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8. The complement components were produced under PHS contract PH 43-66-936 by the Cordis Laboratories (Miami, Florida). The nomenclature of complement conforms to that recommended in *World Health Organ. Bull.* **39**, 935 (1968). The purified preparations of $C\bar{1}$, C4, C2, and C3 contained $7.0 \times 10^{12}$, $6.0 \times 10^{11}$, $6.0 \times 10^{11}$, and $3.0 \times 10^{11}$ hemolytically effective molecules per milliliter, respectively (*9*). At the concentration used in our experiments, as determined by "effective molecular titration" (*9*), $C\bar{1}$ contained no measurable amounts of C4, C2, C3, C5, C6, and C7 and less than 1 percent of C8 and C9; C2 contained no measurable amounts of $C\bar{1}$, C4, C3, C5, C6, C8, and C9 and less than 2 percent of C7; C4 contained no measurable amounts of $C\bar{1}$, C2, C3, C5, C6, C7, and C9 and less than 1 percent of C8; C3 contained no measurable amounts of $C\bar{1}$, C4, C5, C7, and C9 and less than 1 percent of C2, C6, and C8.
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## Ventricular Arrhythmias Related to Antibiotic Usage in Dogs

Abstract. *In dogs, pretreatment with the macrolide antibiotic tylosin (5 milligrams per day per kilogram of body weight) increased the incidence of ventricular tachycardia and fibrillation during acute myocardial ischemia. Another group received a dose of acetyl strophanthidin which was nontoxic in controls, but which resulted in a ventricular arrhythmia in six of seven animals on antibiotic treatment. Enhancement of loss of potassium ion from the myocardium by the antibiotic was presumed to be related to the altered cardiac rhythm.*

Despite the widespread use of antibiotics their effects upon the cardiovascular system are undefined. Certain antibiotics are known to influence ion transport in vitro, and may exhibit a selective effect on potassium (*1*, *2*). During in vivo studies the net movement of this ion in the myocardium has been shown to be closely related to the onset of ventricular arrhythmias and restoration of normal sinus rhythm by antiarrhythmic agents (*3*, *4*).

Preliminary observations which suggest a cardiac effect were made in dogs receiving a macrolide antibiotic, tylosin, used in veterinary medicine for control of upper respiratory infection, prior to the induction of myocardial ischemia. The following study was designed to compare animals undergoing ischemia or receiving a digitalis compound, acetyl strophanthidin, either with or without antibiotic.

An electrode catheter was placed in the left anterior descending coronary artery in intact anesthetized dogs as previously described (*4*). All animals were healthy, male mongrels weighing between 19 to 24 kg, and all received morphine sulfate, 3 mg/kg subcutaneously, and Nembutal, 15 mg/kg intravenously. The production of a thrombus that completely occludes the anterior descending artery by this method is associated with an acute, persistent elevation of the ST segment in the pre-

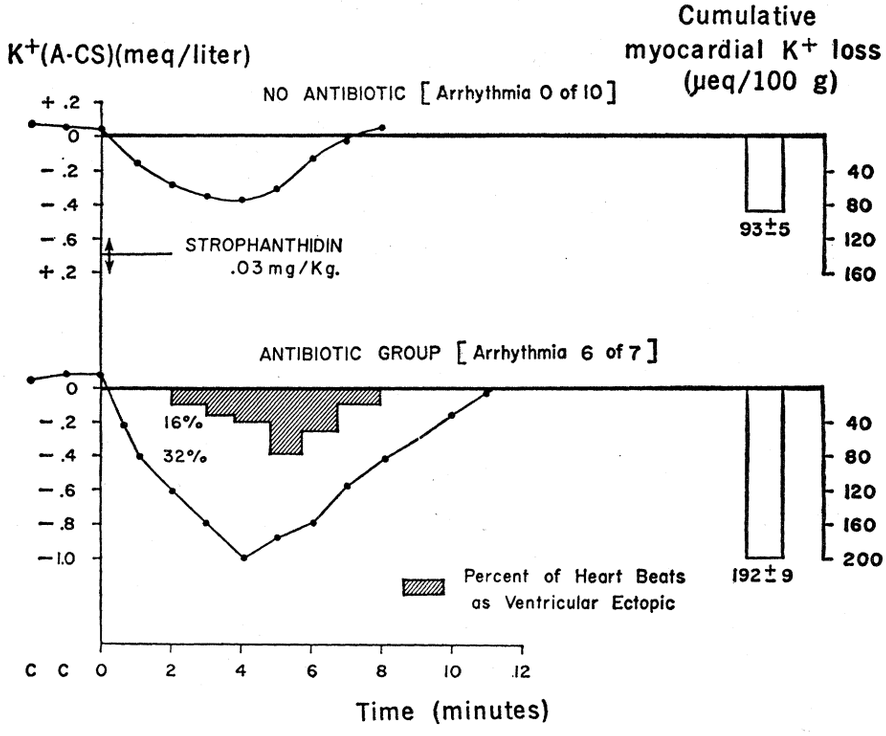


Fig. 1. The upper panel depicts the negative arterial-coronary sinus (*A-CS*) difference of potassium, reflecting myocardial loss of this ion after strophanthidin in the group receiving no antibiotic. The lower panel shows the corresponding changes in the tylosin-treated group, with a larger $K^+$ loss, and a maximum ventricular ectopic beat incidence that averaged 32 percent of all beats. The cumulative ion changes (columns) were calculated from the product of coronary blood flow ($^{85}Kr$ method) and the arterial-coronary sinus difference. *C*, controls at −10 and −5.

Table 1. Incidence of ventricular fibrillation during acute myocardial ischemia, expressed as the number positive of the number tested.

| Tylosin pretreated (mg/kg) | Procaine amide for arrhythmia (mg/kg) | Fibrillation incidence |
|---|---|---|
| | *Group 1* | |
| 0 | 0 | 13/15 |
| | *Group 2* | |
| 0 | 19 ± 1.5* | 3/11 |
| | *Group 3* | |
| 5 per day, for 2 days | 22 ± 1.8* | 12/12 |

* Standard errors of mean.

cordial electrocardiogram followed by ventricular ectopic beats (*4*). Within 4 hours of the onset of ischemia, approximately 90 percent of untreated animals had a ventricular tachycardia and fibrillation (group 1, Table 1). Dogs in group 2 received the antiarrhythmic agent procaine amide, intravenously, at the onset of ventricular ectopic beats, and this resulted in a significant reduction of the 4-hour mortality rate to less than 30 percent. Group 3 animals were pretreated with tylosin (5 mg/day per kilogram of body weight) for 2 days prior to ischemia. Since significant enhancement of the incidence of ventricular fibrillation would be difficult under the circumstances of the group 1 study, the animals pretreated with antibiotic were compared by the method of study in group 2. All 12 animals of group 3 succumbed, usually relatively soon after the onset of the initial ventricular ectopic beat, despite receiving an amount of antiarrhythmic equivalent to that in group 2.

To investigate the role of potassium ion in this process, an experimental design was selected in which analysis of ion transfer is more readily performed. Seven intact anesthetized animals, pretreated with tylosin, received a therapeutic nontoxic dose of acetyl strophanthidin, 0.03 mg/kg, and the net movement of $K^+$ in the myocardium was studied by serial sampling of arterial and coronary sinus blood (*4*). Strophanthidin (*5*) was infused in the femoral vein over a 30-second period. In nine controls not receiving antibiotic, there was a modest egress of potassium ion during the first several minutes, which terminated by 6 minutes (Fig. 1). Normal sinus rhythm was maintained and was associated with a positive inotropic response, represented by an increase in the rate of rise of the ventricular pressure (*6*). In the animals treated with tylosin, no abnormality of rhythm was present before injection of the digitalis compound. The same nontoxic dose of strophanthidin produced a significantly larger loss of potassium from the myocardium. Concurrently, ventricular ectopic beats appeared in six out of seven animals, persisting for an average of 6 minutes. The average peak incidence of ectopics was 32 percent of all beats. In addition, five animals received a toxic dose of strophanthidin, 0.05 mg/kg; they exhibited a ventricular tachycardia that had a greater frequency than occurred in eight animals without antibiotic, which also persisted approximately twice as long.

In vitro data have indicated that macrolide antibiotics may produce selective uptake of potassium ion in isolated mitochondria. Alterations of cell metabolism, such as reduced energy production, may reverse this process, resulting in selective loss of potassium ion (*1, 2*). While the locus from which potassium ion is lost has not been identified, it would appear reasonable to assume that strophanthidin has altered $K^+$ metabolism in a manner that permits the relatively small loss of cation from heart muscle after a nontoxic dose of strophanthidin to be enhanced in the presence of the macrolide antibiotic. Whether this represents an additive effect of the two lactones remains to be evaluated. The results of the studies during myocardial ischemia indicate that the macrolide compound does not require another pharmacologic intervention to affect cardiac rhythm.

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## Crystalline L-Asparaginase from Escherichia coli B

Abstract. *L-Asparaginase has been crystallized from a partially purified extract of* Escherichia coli *B. The crystalline enzyme is homogeneous, as judged by analytical polyacrylamide-gel electrophoresis and sedimentation behavior. This enzyme preparation is active in preventing lymphoma in mice and also has low glutaminase activity.*

L-Asparaginase has been prepared by column chromatography on DEAE-cellulose and hydroxylapatite or by preparative gel electrophoresis (*1*). We have developed a process for purification of L-asparaginase by simple precipitation steps followed by crystallization. The crystalline enzyme is nearly homogeneous. It has a specific activity of 300 ± 15 international units per milligram (I.U./mg) based on the dry weight of the crystals and the assay procedure of Campbell *et al.* (*2*) modified by the incorporation of 0.1 percent bovine serum albumin (BSA) in the buffer. The crystalline enzyme is active in preventing lymphomas in mice. Our purification process should lead to the availability of crystalline L-asparaginase for further clinical evaluation of its effectiveness in cancer chemotherapy.

The enzyme was extracted from a paste of *Escherichia coli* B cells (*3*). The solution was filtered through Hyflo, and the *p*H of the effluent was adjusted to 5.0 by the addition of 2*M* acetic acid. The suspension was clarified by filtration, and the filtrate was adjusted to *p*H 8.0. The protein fraction precipitating in $(NH_4)_2SO_4$ (between concentrations of 2 and 4*M*) was collected and dissolved in $10^{-3}M$ $NH_4HCO_3$; it was dialyzed against $10^{-4}M$ $NH_4HCO_3$ overnight. Ethanol (one volume) was added to the dialyzed solution, and the resulting precipitate was discarded. Another volume of ethanol was then added to the supernatant solution. The resulting precipitate was dissolved in $10^{-3}M$ $NH_4HCO_3$ and lyophilized. The enzyme powder was then suspended in distilled water and dialyzed overnight against $10^{-4}M$ $NH_4HCO_3$. Insoluble material was removed, and one volume of ethanol was added to the supernatant. After standing at 4°C for 1 hour, the solution was made 0.02*M* in $(NH_4)_2SO_4$. Upon standing at −20°C for 4 hours, a precipitate formed. The