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## Electrocardiographic and Electrolyte Abnormalities Caused by Amphotericin B in Dog and Man. (29391)

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Amphotericin B is the only antibiotic available for treatment of many systemic fungal diseases. Treatment with this drug is often complicated by untoward reactions, notably impairment of renal function(1), anemia(2) and hypokalemia(3-5). Electrocardiographic abnormalities suggesting electrolyte imbalance have been described previously by other investigators, either with (3,6) or without(7) concurrent hypokalemia.

In dogs, it has been observed that doses of amphotericin B greater than 5 mg/kg are followed by cardiac arrhythmias and sometimes by cardiac arrest. Therefore, in the present experiments serum values of sodium, potassium, calcium and magnesium were measured and compared with simultaneous electrocardiographic tracings. Similar studies were also performed on 5 patients receiving therapeutic doses of amphotericin B.

*Methods.* Acute experiments in adult male mongrel dogs were done under anesthesia with intravenous sodium pentobarbital (25 to 35 mg/kg). Precisely timed blood samples were collected through polyethylene tubing placed in a jugular vein. Drugs were given intravenously in a hind leg. Mean arterial blood pressures were obtained using a mercury manometer which was attached to tubing in the femoral artery. Electrocardiographic tracings included 6 standard leads and one mid-sternal chest lead. Long term experiments in unanesthesized dogs were carried out using methods described previously(8).

Experiments in 5 patients were performed

following a regular breakfast without tea or coffee which was given at 8 a. m. No further food was given until 3 p. m., when a dry snack was allowed. At 9 a. m., intravenous infusion of 5% dextrose in water was started at the rate of 100 ml/hour and continued throughout the experiment. Amphotericin B (0.4 to 1.0 mg/kg) was present in the infusions between 11 a. m. and 1 p. m. Venous blood samples were drawn at 9 and 10 a. m., 12 noon, 2, 4 and 6 p. m., and 9 a. m. the next morning. Specimens were allowed to clot and the serum was removed immediately and stored at —60°C. Urine specimens were collected hourly between 9 a. m. and 6 p. m. and were processed immediately after collection. Aliquots were frozen for chemical analyses. Control experiments were performed under identical conditions except that amphotericin B was not added to the infusion. Electrocardiographic tracings included 6 standard and 6 chest leads.

Determinations of sodium and potassium were performed by flame photometry, creatinine by alkaline picrate reaction, and urea nitrogen by diacetyl monoxime reaction, using an AutoAnalyzer (Technicon Co., Chauncey, N. Y.) and procedures described by the manufacturer. Calcium and magnesium values were determined by flame emission spectrophotometry(9,10). The chemical determinations for each experiment were performed in block.

The colloidal form of amphotericin B was used, prepared by addition of 5% dextrose in water to an ampoule containing "Fungizone for Infusion," a complex of amphotericin B, sodium desoxycholate and buffers. Lot 3C82333 was used in dog experiments, and lots 3A80431, 3A82334, and 3C82333 were used in the human studies. The technique of administration of the drug has been described(8,11). The volume of the drug infusion in the dog was 20 ml.

*Results.* I. *Canine studies.* A. Acute effects of large doses of amphotericin B. 1. *Electrocardiographic changes.* Electrocardiograms were monitored on 24 dogs before and after administration of amphotericin B. No abnormalities were observed following individual doses of 1.0 mg/kg or less. Doses between 2.0 and 5.0 mg/kg occasionally were followed by abnormalities, and doses between 5 and 15 mg/kg usually led to a sequence of abnormalities terminating in death within 15 minutes. Transient sinus bradycardia uniformly occurred during drug injection, the average heart rate decreasing from 100 to 66 per minute. This bradycardia was often associated with a transient increase of 10 to 20 mm Hg in blood pressure. The sequence of changes leading to death were tachycardia, elevation of T waves, prolongation of PR and shortening of QT intervals and elevation of ST segments. Terminally, aberrant ventricular complexes occurred followed by ventricular tachycardia and by fibrillation or ventricular standstill (Fig. 1).

In an attempt to modify the cardiac toxicity of high doses of amphotericin B, hydrocortisone (15 mg/kg) was given intravenously one hour before amphotericin B administration to 4 dogs. In these dogs, considerably larger doses of amphotericin B were required to produce the electrocardiographic changes described above. The average dose of drug causing death in the 4 dogs treated with hydrocortisone was 35.2 mg/kg (range: 22.5 to 53.0 mg/kg). The mean lethal dose of amphotericin B in 7 dogs which had not been pre-treated was 10.7 mg/kg (range: 5.0 to 15.0 mg/kg). Prior digitalization of one dog did not alter the toxic response to 10 mg/kg of amphotericin B.

2. *Electrolyte changes.* In 7 of the 24 dogs described above, amphotericin B was given intravenously over periods of 0.20 to 5.0 minutes, in doses of 5 to 10 mg/kg (Table I). Within 2 minutes after the drug was injected, the serum potassium concentration increased in all dogs. The average serum potassium value increased from 4.2 to 5.9 meq/l (t test; $p=<.01$). Serum sodium concentration either decreased or remained unchanged; the average value decreased from 152 to 146 meq/l ($p=<.02$). Two dogs (#4 and #7) died within 4 minutes after drug injection. The remaining 5 dogs were given a second identical injection 5 to 10 minutes after the first, and all died within 3 minutes. Serum potassium values in these 5 dogs immediately prior to death ranged

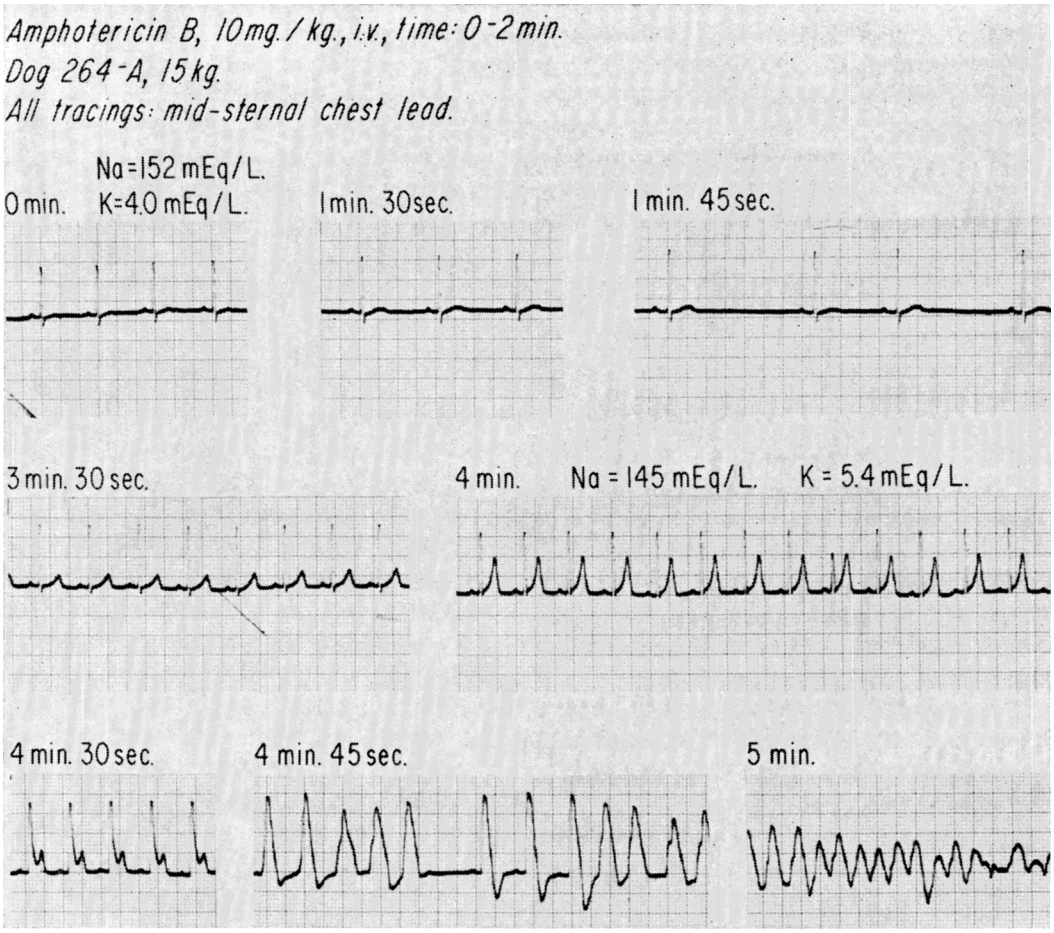


FIG. 1. Electrocardiographic sequence following intravenous amphotericin B.

TABLE I. Serum Potassium and Sodium Changes Following Rapid Intravenous Administration of Large Doses of Amphotericin B in the Dog.

| | Drug administration | | Serum potassium† | | | Serum sodium† | | |
|---|---|---|---|---|---|---|---|---|
| | | | | After initial dose of drug | | | After initial dose of drug | |
| Dog | Dose (mg/kg) | Duration (min) | Before drug | 2 minutes | Final value‡ | Before drug | 2 minutes | Final value‡ |
| | | | (mEq/l) | | | | | |
| 1 | 5.0* | .20 | 4.3 | 6.7 | 6.3 | 148 | 148 | 144 |
| 2 | 5.0* | 4.0 | 4.4 | 5.6 | 6.7 | 148 | 142 | 137 |
| 3 | 5.0* | 5.0 | 4.4 | 5.7 | 6.4 | 147 | 141 | 137 |
| 4 | 5.0 | .20 | 3.8 | 6.6 | — | 153 | 149 | — |
| 5 | 7.5* | 2.0 | 4.4 | 5.5 | 7.0 | 161 | 150 | 156 |
| 6 | 7.5* | 2.0 | 4.2 | — | 5.4 | 153 | — | 153 |
| 7 | 10.0 | 2.0 | 4.0 | 5.4 | — | 152 | 145 | — |
| Avg | | | 4.2 | 5.9 | 6.4 | 152 | 146 | 145 |

* Second identical dose given 5 to 10 min later.

† Normal values based on 23 determinations in 12 normal dogs were: Serum potassium, 4.2 mEq/l (range 3.5 to 4.8 mEq/l); serum sodium, 153 mEq/l (range 142 to 161 mEq/l).

‡ Avg time from end of second drug injection until final value was 2 min.

from 5.4 to 7.0 meq/l (average, 6.4 meq/l, $p=<.01$). Serum calcium and magnesium concentration showed no changes in these acute experiments. Previous administration of hydrocortisone did not alter the effect of amphotericin B on serum potassium concentration. Thus, although hydrocortisone pretreatment mitigated the electrocardiographic effects of amphotericin B, serum potassium concentration rose as promptly and as much as it had done in those dogs which were not pre-treated.

All serum samples drawn after administration of drug showed a small degree of hemolysis. The 2-minute samples showed an average hemolysis of 1.7% of the red cell volume, as determined by the cyanmethemoglobin method. This degree of hemolysis accounts for a maximum of 5% of the observed increase in serum potassium values, as calculated on the basis of previously reported values for the potassium content of dog erythrocytes(12).

The possible effects of sodium desoxycholate on serum potassium concentration were examined since this chemical is known to be active at surface interfaces, and since it is a component of "Fungizone for Infusion." Intravenous doses of sodium desoxycholate equivalent to those contained in the experimental injections of amphotericin B caused no changes in serum electrolyte values or electrocardiographic tracings.

B. Subacute and chronic effects of low doses of amphotericin B. Four daily doses of 0.5 mg/kg of amphotericin B were given intravenously to 5 dogs without producing any significant alteration in serum sodium, potassium, calcium or magnesium concentration. This course of amphotericin B previously had been found to depress effective renal blood flow and glomerular filtration rate and to produce transient azotemia(13).

Intravenous administration of 0.25 to 0.50 mg/kg doses over longer periods of time caused severe azotemia which was proportional to the dose administered(14). Four dogs received amphotericin B for one to 9 months at one to 2 day intervals. No significant changes occurred in serum sodium, potassium and calcium concentrations. The

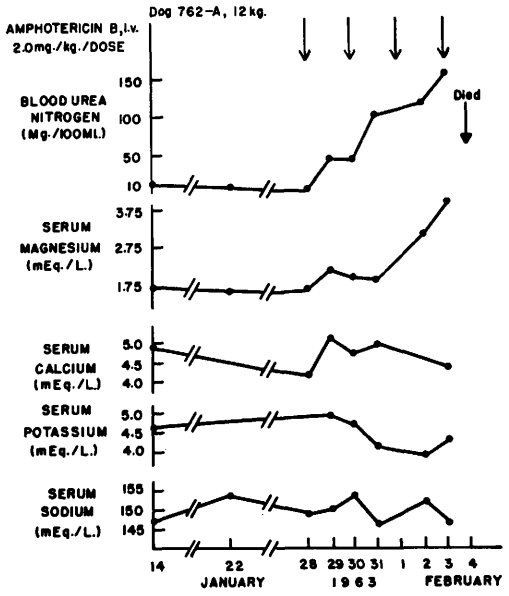


FIG. 2. Serum electrolyte and blood urea nitrogen values in the dog before and after 4 injections of amphotericin B (2.0 mg/kg each).

serum magnesium values increased in each dog. The mean value rose from 1.68 to 3.14 meq/l. In these 4 dogs and one other which was given 4 doses of 2.0 mg/kg on alternate days, increase in magnesium values paralleled development of azotemia (Fig. 2).

II. Human studies. Electrocardiographic and electrolyte studies were done on 5 adult patients with systemic cryptococcosis who previously had received one to 3 g of amphotericin B intravenously over a period of several months.

1. *Electrocardiographic changes.* Twenty-one electrocardiograms were taken on 5 patients before, during and after infusions of amphotericin B. Changes were seen in only 2 patients; prominent u-waves appeared during and after drug infusion. In both of these patients, questionable u-waves were present in mid-precordial leads before infusion and serum potassium values which were low before infusion decreased further after infusion, from a value of 3.2 meq/l to 2.5 meq/l in one patient and from 2.5 to 2.0 in the other.

2. *Electrolyte changes.* Concentrations of serum electrolytes were measured in 5 patients before, during and after infusion of

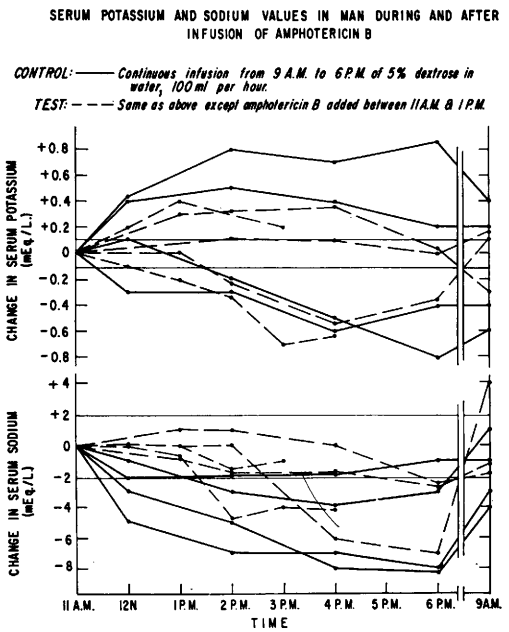


FIG. 3. Net changes in serum potassium and sodium values in 5 patients during and after intravenous infusion of amphotericin B and in 4 of the same patients during and after control infusion of 5% dextrose in water. Zero-value at 11 A.M. represents the control values obtained at 9 and/or 10 A.M. Mean zero-value for serum potassium was 3.8 mEq/l (range, 2.5 to 4.8 mEq/l) and for serum sodium was 143 mEq/l (range, 136 to 149 mEq/l). Area between horizontal parallel lines includes the 95% range of analytical variation of methods employed (±2 standard deviations).

amphotericin B, as detailed in *Methods*. Results are shown in Fig. 3 and 4. Although there was either a significant rise or fall in serum potassium concentration in each experiment except one, neither drug nor control infusions produced changes in a consistent direction. On the other hand, both drug and control infusions produced small but distinct decreases in serum sodium concentrations. Changes occurred gradually during a period of several hours, but values returned toward baseline by the next morning. Serum calcium values showed no significant trend. In the case of serum magnesium the values in the amphotericin B-treated group decreased to a greater extent than those of the control groups in 4 of the 5 cases. The maximum decrease was 34% (1.54 to 1.02 meq/l). Creatinine clearance values decreased in the majority of test and control patients, some to as much as 36% of baseline values. In most patients, renal clearance values of electrolytes tended to parallel changes in creatinine clearance, but to a lesser degree and were of doubtful significance. However, one patient, who had gradually developed a low serum potassium concentration of 2.5 meq/l during a 2-month course of treatment, showed a paradoxically high urinary potassium excretion of 30 to 40 meq/l at flow rates of one to 2 ml/min. Excretion increased even further to concentrations of 80 meq/l at the same flow rates on 2 occasions during and after drug infusion. At the same time, his serum magnesium decreased and his renal clearances of creatinine, sodium, calcium, and magnesium decreased.

*Discussion.* Within minutes following rapid intravenous injection of large doses of amphotericin B in dogs, serum potassium values increased significantly. The magnitude of the rise in serum potassium concentration was

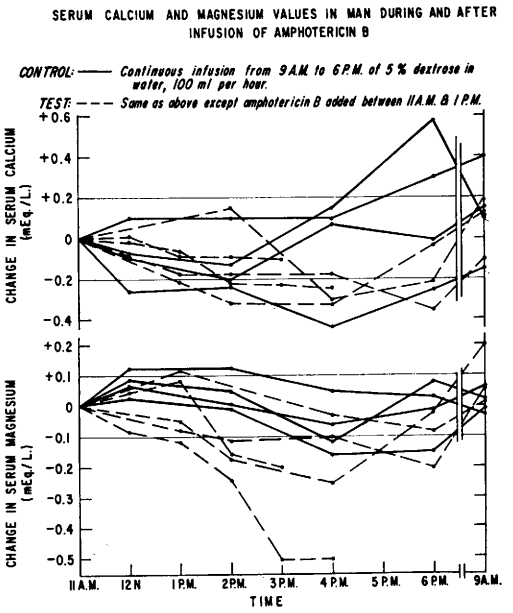


FIG. 4. Net changes in serum calcium and magnesium values in 5 patients during and after intravenous infusion of amphotericin B and in 4 of the same patients during and after control infusion of 5% dextrose in water. For further details see key to Fig. 3. Mean zero-value for serum calcium was 5.08 mEq/l (range, 4.47 to 5.22 mEq/l) and for serum magnesium was 1.60 mEq/l (range, 1.50 to 1.93 mEq/l).

much greater than can be accounted for by release of potassium from erythrocytes alone. A slight amount of hemolysis occurred, but at most can account for only one-twentieth of the observed rise in serum potassium. Plasma potassium concentration is known to increase when dog blood is incubated *in vitro* with amphotericin B(15). However, the rate of increase in serum potassium concentration which occurred *in vivo* was 7 times greater than that observed *in vitro* with comparable doses of drug. Furthermore, even if all circulating erythrocytes released potassium to equilibrium concentration with plasma and interstitial fluid, the rise in serum potassium concentration would be no more than one meq/l. This estimate is based on the fact that normal canine erythrocytes are of the low potassium type, containing about 10 meq of potassium per l of erythrocyte cell water, and that the whole body erythrocyte volume is about one-fifth of the extracellular fluid volume.

Based on *in vitro* results(15), the release of potassium from erythrocytes would account for a rise of only 0.2 meq/l compared with a rise of 1.7 meq/l in serum potassium concentration which was observed *in vivo*. The predominant source of potassium entering the serum therefore must be from body cells other than erythrocytes, which indicates a more general effect of the drug on cellular membranes. Further support for this hypothesis is derived from the electrocardiographic changes, which are more severe than those observed at a comparable degree of hyperkalemia produced by infusion of potassium chloride(16). Arora and Sinha(17) recently found somewhat similar electrocardiographic abnormalities in dogs following administration of the new antifungal antibiotic, hamycin, but electrolyte studies were not reported. Arora and Arora(18) reported, however, that the isolated frog heart exposed to hamycin released potassium into the perfusion fluid. The polyene structure of hamycin is similar to amphotericin B(19). It also has been reported recently that yeast cells begin to lose potassium soon after exposure to polyene antibiotics(20).

In the present experiments in dogs, there were no acute changes in serum calcium and magnesium values. As azotemia developed, serum magnesium values increased, a phenomenon which has been observed in renal failure in man(21). Serum phosphorus values also were elevated in dogs with azotemia(8).

In the human studies, an infusion of amphotericin B produced no consistent pattern of change in serum potassium concentration. However, in one patient who had developed hypokalemia following chronic administration of amphotericin B, infusions of drug on 2 occasions produced excessive potassium excretion, decreases in serum potassium and magnesium values, and slight electrocardiographic changes. Potassium depletion and hypokalemia following treatment with amphotericin B may be the result of cellular loss and increased excretion of potassium.

Reduction in serum magnesium occurred in 4 patients following drug infusion, without excessive magnesium excretion in the urine. The acute reduction in serum magnesium is of interest in view of an apparent association of severe hypomagnesemia and administration of amphotericin B to patients with leukemia(22). Experimental magnesium deficiency in rats has produced both potassium depletion and renal lesions(23). The renal lesions of magnesium deficiency and also of potassium deficiency(24) in some aspects resemble those seen following administration of amphotericin B (25).

*Summary.* In dogs, rapid intravenous administration of large doses of amphotericin B promptly caused significant increases in serum potassium and decreases in serum sodium concentrations. In dogs which died, electrocardiographic tracings demonstrated a characteristic sequence of changes leading to ventricular fibrillation. It was concluded that amphotericin B causes damage to cellular membranes with resultant shift of intracellular potassium into the plasma. Hydrocortisone mitigated the cardiac toxicity, but did not alter the hyperkalemic effect.

Dogs which received smaller doses of drug for longer periods of time showed no changes in serum sodium, potassium or calcium concentration. As azotemia developed, however,

serum magnesium concentrations increased significantly.

In patients, amphotericin B was given during infusions of 2 hours' duration to patients who previously had received drug for several months. In most cases the serum concentration and renal clearance of electrolytes were similar to control infusions without drug. However, the serum magnesium concentration tended to decrease after drug infusion more so than after control infusion. There was no measurable increase in urinary magnesium clearance. One patient with hypokalemia showed excessive urinary potassium excretion which increased further during infusion of amphotericin B.

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## Penetration of Herpes Simplex Virus into Human Epidermoid Cells.* (29392)

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There is general acceptance of the thesis that all viral infections are initiated by two sequential events: adsorption and penetration. The mechanisms by which virions attach to cell surfaces have been thoroughly studied and carefully reviewed(1,2,3). When cellular receptor sites greatly exceed numbers of virions, adsorption rates approximate first order kinetics. Temperatures between 0° and 37° influence the rate of virion attachment only by virtue of slight changes in viscosity of the suspending medium which, in turn, slightly alters the frequency of virion-cell collisions. DNA-containing bacterio-

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