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29. We thank Dr. A. C. Allison for stimulating this work; Prof. G. M. Edington and H. M. Gilles for encouragement; Dr. D. Schlessinger and Dr. D. Apirion for helpful suggestions; Professors A. O. Lucas, J. Beetlestone, and R. G. Hendrickse for reviewing the manuscript; and I. O. Olanipekun for technical assistance. Financial support was given by the World Health Organization.

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Digitoxin Poisoning: Prevention by Spironolactone

Abstract. *Spironolactone (Aldactone®) protects the rat against the production of myocardial necroses and other manifestations of digitoxin poisoning.*

In the rat, spironolactone, which has been found to block the hypertensive action of aldosterone by competitive inhibition (*1*), minimizes both the cardiovascular lesions characteristic of overdosage with desoxycorticosterone (*2*) and the infarct-like myocardial necroses produced by certain corticoids in combination with bisodium phosphate, excess fat intake, or stress (*3*). In agreement with expectations, spironolactone is also beneficial not only in primary aldosteronism but also in many of the common types of clinical hypertension (*4*).

Independently of these studies it was noted that heavy overdosage with digitoxin produces massive cardiac necroses in the rat and that concurrent oral administration of excess bisodium phosphate and fat facilitates the production of these lesions (*5*).

The aglycones of cardiac glycosides resemble the corticoids in that they possess a steroid nucleus; since, in addition, they have a lactone ring at C-17, they are structurally even more closely related to spironolactone than is desoxycorticosterone or aldosterone. In view of these considerations, it seemed promising to explore the possibility of counteracting digitalis toxicity by a competitive inhibitor of aldosterone such as spironolactone.

Forty female rats from Holtzman Farms (Madison, Wis.), with a mean initial body weight of 100 g (range 90 to 110 g), were divided into four equal groups and treated as outlined in Table 1. Digitoxin (0.5 or 0.25 mg) and bisodium phosphate (1 m*M*), dissolved together in 2 ml of water, and 1 ml of corn oil were given separately by stomach tube twice daily for the production of the cardiopathy. In addition, for prophylaxis, certain groups received spironolactone (10 mg), added to the solution of digitoxin and

Table 1. Prevention of digitoxin poisoning by spironolactone. In addition to the treatments listed, all groups received Na_2HPO_4 and oil as described in the text. The readings include both the animals that succumbed during the experiment and the survivors that were killed on the 5th day.

Group	Treatment		Motor disturbances	Cardiac necroses	Mortality (%)
	Digitoxin (mg)	Spironolactone (mg)			
1	0.5	0	+++	++	100
2	.5	10	+	0	30
3	.25	0	++	+	50
4	.25	10	0	0	0

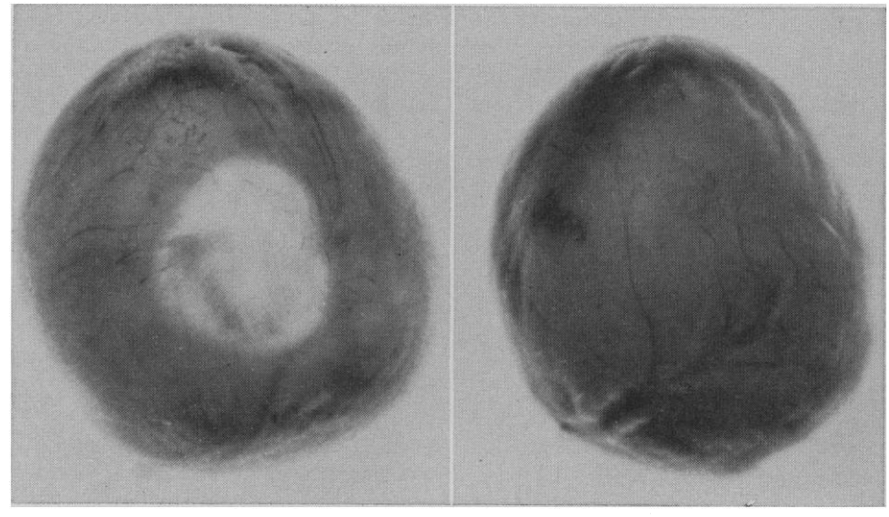

Fig. 1. Prevention of digitalis-induced myocardial necrosis by spironolactone. (Left) Sharply circumscribed circular apical necrosis in the heart of a rat treated with digitoxin (group 1). (Right) Prevention of the necrosis by additional treatment with spironolactone (both hearts are photographed from their apical aspect).

Na_2HPO_4 without an increase in the volume of water.

Throughout the experiments, the rats were maintained exclusively on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water. The cardiac necroses and the convulsive motor disturbances typical of digitoxin poisoning were assessed in terms of an arbitrary scale of + to +++ (*5*). For histologic studies, specimens were fixed in Susa solution saturated with picric acid and stained with the periodic acid–Schiff technique.

The first manifestations of digitoxin poisoning were the characteristic motor disturbances which became evident within 24 hours after initiation of the experiment in the rats receiving the higher dose of the glycoside (groups 1 and 2). During this early stage, the motor disturbances were almost as severe in the rats given spironolactone as in the corresponding controls. However, in the course of the next 2 days, the rats treated with spironolactone recovered despite continued treatment with digitoxin, whereas the controls became increasingly more affected, so that, toward the end of the experiment, they were almost continuously in convulsions and unable to stand up. The rats receiving the smaller dose (groups 3 and 4) were much less affected; here, convulsions did not appear in treated animals at any time and in the controls only toward the 3rd day.

Cardiac necrosis was sharply limited to the apex of the heart and easily visible to the naked eye as a disk-shaped whitish area (Fig. 1). Histologically, in the affected region, there was massive necrosis of muscle fibers with histiocytic infiltration, but without any evidence of vascular occlusion. The intensity of the cardiac necrosis was greater in rats receiving a high dose of digitoxin than in those receiving a low dose, but spironolactone completely prevented the development of lesions in both groups. Mortality of those receiving high doses was only diminished by treatment with spironolactone, but was totally abolished in those treated with a low dose.

Under the conditions of these experiments, spironolactone is a potent antidote for digitoxin; yet this effect cannot be ascribed with certainty to a competitive antagonism between the cardiac glycoside and the steroid inhibitor. The fact that both spironolactone and digitoxin are steroids possessing a lactone side chain merely suggests such an interpretation.

According to current opinion, the competitive antagonism between spironolactone and mineralocorticoids is limited to their effect upon potassium elimination by the renal tubules. In this respect the action of spironolactone can be duplicated by amiloride, an agent that inhibits renal elimination of potassium, but does not act by competitive inhibition of mineralocorticoids. However, amiloride is much more potent than spironolactone as a potassium-sparing agent and in protecting the rat against the cardiac necrosis produced by fluorocortisol in combination with bisodium phosphate (*6*); yet, even at optimum potassium-sparing doses, amiloride fails to protect the rat against the manifestations of digitoxin poisoning. The same is true of KCl given at the near-toxic dose (1 m*M*) twice daily by stomach tube.

Although additional treatment with phosphate and fat aggravates the cardiac lesions produced by digitoxin, it has no effect on the associated motor disturbances. Thus, these two manifestations of digitalis intoxication are separable, yet both can be prevented by spironolactone. Furthermore, if, under otherwise identical conditions, digitoxin alone is given subcutaneously and spironolactone is given by mouth, the motor disturbances are also prevented; hence, apparently, the prophylactic effect is not due to a direct chemical interaction between the two compounds in the gastrointestinal tract nor to an inhibition of digitoxin absorption from the intestine.

These findings suggest that spironolactone interferes specifically with digitoxin actions not only in the heart but also at various receptor sites and that its protective effect is not due merely to the restriction of potassium elimination. The mechanism of this interaction cannot yet be appraised, and indeed, we have no proof that the digitalis antagonizing effect would occur under clinical conditions. However, since digitalis and spironolactone are frequently administered conjointly to cardiac patients, we wanted to call attention to the possible influence of antimineralocorticoid diuretics upon digitalization.

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Membrane Alterations in Hemolysis: Internalization of Plasmalemma Induced by Primaquine

Abstract. *Incubation of normal human erythrocytes with primaquine, a derivative of 8-aminoquinoline, results in internalization of the cell membrane and the formation of intracellular vacuoles. These changes are similar to those observed in other types of cells in pinocytosis. The reduction in surface cell membrane which accompanies internalization of plasmalemma may be generally significant in the destruction of red cells.*

The destruction of erythrocytes in physiological or pathological processes is often related to alterations in the integrity of the membrane (*1*). Thus, mechanisms of hemolysis in normal extracellular fluid include changes in effective cation permeability, changes in macromolecular permeability, or fragmentation of the membrane (symmetrical lipid loss). In the latter case, the loss of membrane lipid is accompanied by a decrease in the surface area and an increase in spheroidicity which results in a change in the cell from a deformable body to a more rigid one. Fragmentation may occur repeatedly without loss of cell contents to the surrounding medium (*2*). Such an occurrence attests to the ability of the membrane to repair itself. We now present