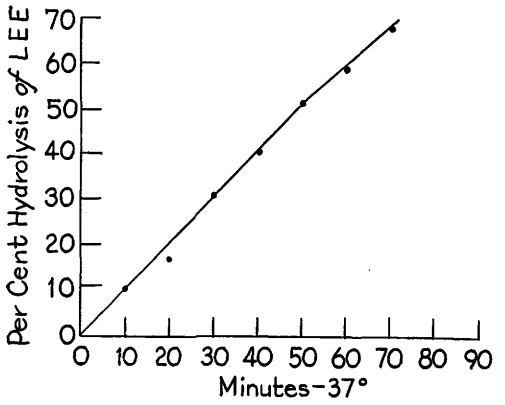

FIG. 3. Hydrolysis of LEE by trypsin at pH 7.

7.8, 0.4M sodium phosphate buffer to yield a 0.168M solution. The reagents were incubated separately at 37°C for 5 minutes with the exception of LEE which was prepared just prior to starting the test. The reaction mixture was prepared by combining 5 ml of enzyme solution, 10 ml of water and 5 ml of LEE solution. The substrate control contained 5 ml of 0.0025N HCl in place of the trypsin and the enzyme blank contained 5 ml of pH 7.0 0.4M phosphate buffer in place of the LEE. The final pH's of solution in all tubes was 7.0. After the addition of the LEE a timer was started and as incubation proceeded 2 ml aliquots were removed and analyzed. The colorimeter readings of the substrate control and enzyme blanks were subtracted from the colorimeter readings of reaction mixture and the corrected value was converted to per cent hydrolysis of LEE. The results are shown in Fig. 3. The data demonstrate that enzymatic breakdown of LEE may be conveniently followed by complexing the liberated lysine with copper.

The present studies employed trypsin as the lysine ethyl esterase but any lysine ethyl esterase may be used. The breakdown of LEE by plasmin (serum proteolytic enzyme) has also been studied by the above technic. Furthermore, by substituting the appropriate amino acid ester as substrate in the procedure described above, it has been possible to study the action of other amino acid esterases(4).

Summary. 1. The determination of lysine by reaction with copper phosphate has been described. 2. Within the range of concentrations studied the reproducibility of the method was found to be ± 3.5%. 3. The rate of hydrolysis of lysine ethyl ester by trypsin was determined by measurement of liberated lysine. 4. Application of the method to studies with lysine ethyl esterases and other amino-acid esterases was briefly discussed.

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Effect of Digitoxin and Quinidine on Intracellular Electrolytes of the Rabbit Heart.* (22566)

M. M. GERTLER,† J. KREAM, J. W. HYLIN, H. ROBINSON, AND E. G. NEIDLE.

Department of Cardiology, Francis Delafield Hospital, Columbia-Presbyterian Medical Center, N.Y.C.

Digitalis and quinidine preparations are much employed in the treatment of cardiac disorders(1). The beneficial effects of these drugs are ofttimes nullified by toxic phenomena which occasionally cause death(2,3). Although the exact mechanism for either toxicity or death is unknown, it has been suggested that electrolyte imbalance in the cardiac muscle results in establishing condi-

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† Present address: Co-ordinator of Medicine, Metropolitan Division, New York Medical College-Metropolitan Medical Center, N.Y.C.

tions which are favorable to the development of the toxic phenomena(4). The evidence for digitalis toxicity, albeit indirect, is fairly well established but far less is known concerning the concomitant electrolyte disturbances during quinidine therapy. In the main, studies concerning digitalis toxicity have established that there is a depletion of intracellular cardiac potassium with sequential changes of toxicity leading to death(4,5). Sherrod(6) has presented evidence which demonstrates potassium loss, sodium gain and calcium gain in following the use of digitalis dog ventricular muscle. Aikawa and Rhoades(7) have shown that there is a cellular depletion of potassium in rabbit atrium and ventricle following a single dose injection of digitoxin, 0.2 mg per kg. This loss in intracellular potassium is not only limited to cardiac muscle but is also observed in rabbit skeletal muscle (7) and has been known to reproduce the symptoms of familial idiopathic paralysis in humans(8).

Materials and methods. Seventy-four male albino rabbits weighing from 2650 to 3400 g were divided into 4 groups: (1) Eleven as a control group for quinidine treated animals, (2) thirty-two animals as a quinidine gluconate treated group, (3) ten as a control group for digitaline animals and (4) twenty-one as a digitaline nativelle treated group. The quinidine control animals received 1.5 ml quinidine gluconate solvent intramuscularly while the quinidine treated animals received the same volume which contained 0.12 g of quinidine gluconate.‡ The quinidine animals, both control and treated, were injected twice daily for 5 days and were sacrificed one hour after the last injection on the morning of the sixth day. The digitaline nativelle control animals were each injected with 1.5 ml of solvent and the digitaline nativelle treated animals received 1.5 ml of solvent containing 0.3 mg digitaline nativelle. Each animal received a daily intramuscular injection for 10 days and was sacrificed on the morning of the tenth day 2 hours after the digitaline nativelle injection.§

Blood was drawn from each rabbit's ear into a tube which contained 0.1 ml heparin as an anti-coagulant. The heart was removed and the ventricle was dissected at the atrioventricular groove; the ventricle was opened, the fat was trimmed and the valves were removed. The entire ventricle was blotted free from blood. One aliquot was removed for nitrogen determination while the remainder was placed in a tared weighing bottle and put into a vacuum oven at 60°C for 24 hours and reheated for 24 hours until constant weight was established. Skeletal muscle tissue was treated similarly to the ventricular muscle. After constant dry weight was established, the fat content was determined according to the method of Hastings and Eichelberger(9). The extracted fat was returned to the tube and the solvents were evaporated. The contents of the tube were digested in a boiling water bath in a solution containing 3.0 ml of concentrated nitric acid to which silver nitrate, 50% in excess of anticipated chlorides, was added. Chlorides were determined by the standard technic(10) after the solution was brought to known volume. Sodium and potassium were determined on two 1 ml aliquots of the digested solution in an internally standardized flame photometer after volume adjustments and lithium sulfate was added in the proper amount.

Derived values. The basic principle of Hastings and Eichelberger was used throughout(9). Serum water was determined by correcting serum concentrations after drying to a constant weight. Extra-cellular water (E.C.W.) was calculated as chloride space using a Gibbs-Donnan factor of 95 for striated muscle and 98 for heart muscle. In addition, the striated muscle and heart muscle were considered to contain 1% and 5% solids respectively. Intracellular water (I.C.W.) was obtained by subtracting the E.C.W. values from the tissue water values. Electro-

‡ Kindly furnished by Eli Lilly and Co., Indiana.

§ Kindly furnished by Varick Pharmacal Co., New York City. Solvent system contains:

	cc
Polyethylene glycol 300	28.6
Glycerin	43.8
Benzyl alcohol	4.
Ethyl alcohol	5.
Water for injection Q.S. A.D.	100.

TABLE I. Water, Electrolyte and Fat Values, Both Determined and Calculated, in Heart, Muscle and Serum in Normal Rabbits and Rabbits Treated with Digitaline Nativelle and Quinidine Gluconate.

Tissue	Sample	Drug	No.	Muscle composition: Cl (mEq per kg fat free tissue)	Muscle composition: Na (mEq per kg fat free tissue)	Muscle composition: K (mEq per kg fat free tissue)	Muscle composition: H_2O, g	Muscle composition: Fat, % fresh tissue	Serum values: Cl (mEq/l)	Serum values: Na (mEq/l)	Serum values: K (mEq/l)	Serum values: H_2O %	Calculated values: E.C.W. I.C.W. ml/kg fat free tissue	Calculated values: Na mEq/l of I.C.W.	Calculated values: K mEq/l of I.C.W.
Muscle	Control		11	16.0	22.8	93.7	768	1.05	126	149	4.5	92.5	114	646	9.1	143
	Exp.	Quinidine	31	16.4	23.4	94.0	768	.74	122	159	4.7	92.8	119	649	5.9	144
"	Control		11	16.8	25.8	94.9	772	.53	125	157	4.8	92.9	119	653	10.8	144
	Exp.	Digitoxin	21	15.0	23.1	94.4	767	.75	136	153	5.3	92.9	106	661	10.4	142
Heart	Control		11	20.7	31.3	68.5	772	1.69	126	149	4.5	92.5	147	625	13.6	108
	Exp.	Quinidine	32	22.3	31.3	76.2	778	1.23	122	159	4.7	92.8	162	616	6.8	124
"	Control		10	20.9	32.0	67.5	789	.26	125	157	4.8	92.9	149	640	12.0	106
	Exp.	Digitoxin	19	20.4	32.7	70.2	775	.96	136	153	5.3	92.9	134	641	17.5	109

lytes were expressed in terms of a liter of I.C.W.

Results. The results of the experiments are summarized in Table I. The single important result here is a statistically significant increase in ventricular muscle of the intracellular potassium following quinidine injection from 106 ± 3.8 to 124 ± 2.1 (mean ± standard error) mEq per liter of intracellular fluids. There is also a statistically significant decrease in ventricular muscle of intracellular sodium in the same animals from 13.6 ± .9 to 6.8 ± .4 mEq per liter of intracellular fluids.

Discussion. The rabbits receiving digitaline nativelle intramuscularly did not evidence a potassium depletion as has been reported by other investigators(7). There is no obvious explanation for this observation except perhaps that the dose of digitoxin which was employed in this study was far less than that employed in the other studies mentioned. (Aikawa and Rhoades(7) used 0.2 mg/kg of rabbit in contrast to 0.1 mg/kg daily for 10 days in this study.) The dose of quinidine employed here was considered large in spite of the lack of comparisons.

There are several interesting implications of this study. It has been observed that the electrocardiographic signs of quinidine toxicity, *i.e.* sinus arrest, incomplete heart block, complete heart block and intraventricular block(11), are also seen in the early and terminal stages of uremia(12,13). It appears from this study and the other studies(12) that quinidine toxicity and uremia have a common denominator in that there is intracellular potassium retention in the heart ventricular muscle. One must not, however, infer from these observations that intracellular hyperpotassemia is the sole cause of these changes in both instances. The hypothesis that a decrease in intracellular cardiac potassium is accompanied by, and not necessarily causally related to, ventricular arrhythmias, and that an increase in intracellular cardiac potassium is accompanied by, and again not necessarily causally related to, a depression of cardiac arrhythmias is further strengthened from the observations of Harris *et al.*(4).

It is reasonable to suggest a possible mechanism of quinidine action in converting cardiac arrhythmias. It is now generally accepted that digitaline nativelle causes a depletion of intracellular cardiac potassium, and alters the concentration of other intracellular electrolytes such as sodium and calcium(6). The results of this study reveal that intramuscular quinidine gluconate produces in the rabbit intracellular hyperpotassemia, and possibly intracellular hyponatremia. (Other electrolytes such as Mg, Ca and Mn were not studied.) Therefore, quinidine gluconate may effect its action via intracellular enzyme systems which are either stimulated or inhibited by alteration in sodium, potassium or other trace metals. This possibility is being studied actively at present.

Conclusions. *1.* 32 rabbits were injected intramuscularly in the gluteal region with 0.12 g of quinidine gluconate twice daily and 21 rabbits were similarly injected daily with 0.3 mg digitaline nativelle for 5 days and 10 days respectively. *2.* A gain in intracellular cardiac potassium as expressed in liters of intracellular water was observed in the quinidine treated animals (from 108 to 124 mEq). A loss in intracellular cardiac sodium from 13.6 to 6.8 was also observed. *3.* The mechanism of digitoxin toxicity and its reversal by quinidine is discussed. It is suggested that one of the actions of quinidine may be to reverse the ionic flow of sodium and potassium (and perhaps other ions) in cardiac muscle which occurs in digitoxin toxicity.

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Oxidation of Specifically Labeled Glucose by Rat Adipose Tissue.* (22567)

S. W. MILSTEIN. (Introduced by Jay S. Roth.)

William Goldman Isotope Laboratory, Division of Biological Chemistry, Hahnemann Medical College, Philadelphia, Pa.

Recent studies have indicated that the rate of glucose utilization *in vitro* by adipose tissue of the rat is greater than that of liver(1,2). The extensive lipogenic activity of adipose tissue implies the occurrence of rapid glycolysis(3), but does not exclude other pathways of hexose metabolism. Considerable evidence for the extraglycolytic breakdown of glucose in hepatic and other tissues is available, although accurate quantitation of its role in carbohydrate metabolism has been hampered by both technical and interpretative difficulties(4). Using one of the more direct experimental approaches to this problem (5), the oxidation of C^{14} glucose substrates, labeled in the first and sixth positions, has been studied in liver and testicular adipose

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