has to be considered as a possible explanation for the capacity of the renal slice to accumulate the latter material 2½ hours after the administration of malonate. Busch and Potter (3) were able to recover only part of the malonate from the urine of animals receiving this compound. It is known that malonate can be oxidatively metabolized *in vivo*(8,9) and, therefore, may contribute to the pool of metabolites from which the excretory mechanism for PAH derives its energy. Furthermore, it is possible that malonic acid is metabolized or removed from the cell more rapidly than is succinate. Succinate (and other members of the Krebs cycle) which accumulate in the presence of malonate would be expected to be present in amounts greater than are usually found and supposedly would provide an additional source of energy for the transport of PAH. Whether or not either of these proposed mechanisms is correct remains to be determined.

Summary. The renal tubular excretion of PAH in the rat is inhibited by the *in vivo* administration of sodium malonate or antimycin A. The data here presented, when correlated with the results of other investigators, strongly suggest succinate oxidation can provide an *in vivo* source of energy for that renal mechanism which is responsible for the tubular excretion of PAH.

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Effects of Digitoxin on Exchangeable and Tissue Potassium Contents.* (22025)

JERRY K. AIKAWA† AND ELOISE L. RHOADES.

Department of Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.

The effect of digitalis on both normal and abnormal myocardium is well known. Although it is an established fact that the glycosides have a direct effect on the heart, controversy still exists as to whether they have an *extracardiac* effect as well. Because the chemical structure of digitalis is similar to that of other steroid compounds which are known to affect salt and water metabolism—for example, sex and adrenal cortical hormones, it might be suspected that the glycosides would act similarly. There are, in fact, experimental evidences to suggest that these agents, under certain circumstances, act in a fashion similar to the adrenal cortical hormones(1,2).

A previous study(3) suggested that the intravenous administration of 0.2 mg of digitoxin per kg of body weight results in reversible shifts in the distribution of body fluids. The availability of radioactive potassium (K^{42}) has made possible the direct measurement of the exchangeable potassium con-

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† Established Investigator of the American Heart Assn. Present address: Department of Medicine, University of Colorado School of Medicine, Denver.

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tent in intact animals. The purposes of the present study were (1) to determine the effects of large daily doses of digitoxin (0.1 mg/kg) on the exchangeable potassium content in normal rabbits, and (2) to determine the effect of a single large dose of digitoxin (0.2 mg/kg) on the tissue potassium content.

Material and methods. Sixteen normal male adult rabbits of mixed breeds were used; they were kept in individual metabolism cages and were fed a stock diet of compressed pellets. Tap water was given without restriction. In order to acclimate the animals to their new environment, they were placed in the cages one week prior to the initiation of the baseline studies. Commercial crystalline digitoxin dissolved in a 40% solution of alcohol was employed. *Experiment 1.* Direct measurements of the exchangeable potassium content (Ke) were made with K^{42} at weekly intervals in 8 animals. Starting on the 8th day and continuing every day for a week, 0.1 mg of digitoxin per kg body weight was given intravenously to each animal. No digitoxin was given during the third week. The initial Ke determination was made on the first day of the experiment; the second, on the 7th day. In the analysis of the data, the mean of these two determinations was used as the baseline value for each animal. Subsequent Ke determinations were made at weekly intervals. *Experiment 2.* Each of 4 animals was given an intravenous injection of 0.2 mg of digitoxin per kg of body weight through a marginal vein immediately prior to the administration of K^{42}. Another group of 4 animals were not given digitoxin and served as controls. Twenty-four hours after intravenous injection of the radiopotassium, the animals were killed by exsanguination and analyses for radiopotassium and for total water content were performed on 18 different tissues.

Measurement of the exchangeable potassium content (Ke). The preparation of the potassium solution for injection has been previously described(4). Five ml of the potassium solution, containing 30 microcuries of K^{42} in 0.5 meq, was injected intravenously. The urine was collected for 24 hours; a spot specimen was then obtained by catheterization and its specific activity was determined. The following formula was used to calculate the value for the exchangeable potassium content of the body: $Ke = \frac{Ki^{42} - Ko^{42}}{Ku^{42}/Ku^{39}}$, in which Ki^{42} = quantity of radiopotassium administered (arbitrary units), Ko^{42} = quantity of radiopotassium excreted in the urine in 24 hours, Ku^{42} = concentration of radioactivity in the spot specimen, Ku^{39} = concentration of total inorganic potassium in the spot specimen, and Ku^{42}/Ku^{39} = specific activity of the spot specimen. The potassium concentration in the urine and in the serum was determined by flame spectrophotometry. *Tissue analyses.* Three portions (1 to 3 g) of each tissue were obtained. They were kept in covered Petri dishes and were weighed as rapidly as possible on a Gramatic balance to an accuracy of 0.1 mg. Two portions were digested in concentrated sulfuric acid for determination of the radiopotassium concentration. The digest was made up to a volume of 20 ml and was counted with a dipping tube and a scaling circuit. The tissue content of K^{42} was expressed in counts per minute per kg of tissue (wet weight). In the calculation of the *total tissue content* of potassium, it was assumed that at 24 hr the K^{42} was in equilibrium with the non-radioactive potassium in the body, and the specific activity of the spot specimen obtained at 24 hr (K^{42} (cpm/1)/K^{39} (meq/1)) was used to calculate the total potassium content in each tissue. This value was expressed in meq/kg. *Tissue water content* was determined on the third aliquot which was dried to a constant weight at 110°C for 18 to 24 hours. *Statistical method.* The significance of the difference between the means was determined by the "t" test(5). In the first experiment, each animal served as its own control. The differences between the baseline values and the values at the end of the second and third week were calculated for each animal; the mean difference between the baseline values and each of the subsequent determinations was then obtained. The significance of the mean difference was then tested. In the analysis of the results of experiment 2, the "t" test was again used to determine the significance of the difference between the group

TABLE I. Effects of Digitoxin in Doses of 0.1 mg per kg Daily for 7 Days on Exchangeable Potassium and Serum Potassium in 8 Normal Rabbits.

	Wt (kg)	Exchangeable potassium (meq)	(meq/kg)	Serum potassium (meq/l)
Baseline values	2.578	207.4	78.7	5.35
Wk 1*	2.669‡	172.4‡	63.2‡	4.98‡
" 2†	2.769‡	232.9	83.7	5.29

* Mean values after 7 days of digitoxin.
† Mean values 7 days after discontinuation of digitoxin.
‡ Statistically significant difference when this figure is compared with the mean baseline value: P = less than 0.02.

means obtained in the test and control groups of animals. The calculated value for "t" was compared with the value at (n-1) degrees of freedom, as obtained from standard tables, and expressed as "P". A "P" value of 0.02 or less was considered significant.

Results. Experiment 1. (Table I). During the three-week period of observation, the mean body weight of the 8 animals in this group increased progressively from 2.578 to 2.769 kg. After 7 days of digitoxin the mean exchangeable potassium content decreased significantly from a baseline value of 207.4 meq (78.7 meq/kg) to 172.4 meq (63.2 meq/kg). The serum potassium concentration at this time was significantly lower than the baseline value, having fallen from 5.35 to 4.98 meq/1. One week after discontinuation of the drug, the exchangeable potassium content had risen to 232.9 meq.

Experiment 2. (Table II). All of the tissues in the rabbits given digitoxin contained less potassium than the tissues in the uninjected control animals; however, statistically significant differences in the mean potassium contents were observed only for the diaphragm, the abdominal muscle, and the subcutaneous connective tissue. No significant differences in the tissue water contents were found between the digitoxin-injected and the normal control animals and these values have not been included in the table.

Comment. The results of the first experiment indicate that the body store of potassium is considerably depleted within a week after the administration of digitoxin in daily doses of 0.1 mg/kg is begun. This reduction in the exchangeable potassium content was reflected in the slight, but significant, decrease in the serum potassium concentration. After discontinuation of the drug, the changes in exchangeable potassium content and in serum potassium concentration were promptly reversed.

The results of Experiment 2 suggest that the decrease in the total body content of potassium which follows the administration of a single dose of 0.2 mg of digitoxin per kg of body weight is due to a generalized loss of potassium from most of the tissues and organs of the body; the magnitude of this loss was greatest and most consistent in the abdominal and diaphragmatic muscles and in the subcutaneous connective tissue.

The results obtained in the present study are similar to those reported to occur during the administration of desoxycorticosterone acetate to normal animals(6,7). The prolonged administration of desoxycorticosterone may cause a decrease in the potassium content of muscle and necrosis of myocardial fibers. These lesions cannot be distinguished from those produced by the dietary restriction of potassium. A previous study in this laboratory has shown that the administration to nor-

TABLE II. Effects of a Single Dose of Digitoxin, 0.2 mg per kg, on Tissue Potassium Content in Normal Rabbits.

Tissues or organs	Potassium content (meq/kg) (mean ± S.E.) Uninjected control animals	Animals given digitoxin
Quadriceps muscles	66.77 ± 4.10	50.00 ± 6.06
Gastrocnemius muscle	66.42 ± 1.54	58.39 ± 4.75
Adrenal gland	63.33 ±24.60	37.42 ±22.80
Abdominal muscle	57.03 ± 4.21	33.34 ± 2.52*
Appendix	54.62 ± 4.10	42.28 ± 5.90
Liver	48.49 ± 3.33	38.69 ± 4.32
Spleen	45.63 ± 8.67	41.75 ± 5.77
Stomach	44.97 ± 5.18	34.79 ± 4.40
Diaphragm	44.19 ± 3.83	25.62 ± 3.76*
Heart, ventricle	41.63 ± 3.40	32.58 ± 3.34
Kidney	41.30 ± 3.30	31.91 ± 4.49
Lung	39.92 ± 2.07	29.97 ± 3.88
Heart, auricle	28.90 ± 3.41	19.59 ± 3.07
Subcut. connective tissue	28.09 ± 2.43	17.49 ± 1.86*
Skin	17.47 ± 2.99	11.07 ± 1.97

* Statistically significant difference when compared with the mean control value: "P" = 0.02 or less.

mal rabbits of large doses of cortisone acetate under experimental conditions closely resembling those employed in the present study also produces a significant decrease in the exchangeable potassium content(8). The results of the present study suggest that one of the effects of digitoxin, administered in the dosages and in the manner employed for these experiments, is to deplete the body store of potassium. This depletion is thought to be the result of an extracardiac effect of digitoxin.

Summary. 1. The daily intravenous administration of 0.1 mg of digitoxin per kg of body weight to normal rabbits resulted, within 7 days, in an increase in body weight and a reduction in the serum potassium concentration and the exchangeable potassium content. These changes in potassium metabolism were corrected within one week by discontinuing the glycoside. 2. The intravenous administration of a single large dose of digitoxin (0.2 mg per kg) to normal rabbits resulted within 24 hours in a decrease in the mean potassium content of all the tissues and organs. Statistically significant decreases in potassium content were noted in the abdominal and diaphragmatic muscles and in the subcutaneous connective tissue. The results suggest that one of the effects of the administration of digitoxin in these dosages is to deplete the body store of potassium.

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A Practical Method for Removal of Anticomplementary Properties from Human Serum. (22026)

FRED RAPP,*† GLADYS M. GNESH, AND IRVING GORDON.†

Division of Laboratories and Research, New York State Department of Health, Albany.

Methods for the removal of anticomplementary (AC) qualities from sera(1-11) are generally too complex or inconvenient for frequent use in complement-fixation (CF) tests. Since Coons and his colleagues have found that absorption of fluorochrome-labeled antibody with tissue powder reduces or removes nonspecific staining activity(12,13) the effect of tissue powder on the AC properties of human sera was investigated. Sera were freed of AC activity by the relatively simple procedure of treatment with mouse liver powder.

Materials and methods. To make tissue powder, livers from normal adult white mice (Albany strain) were cut into 10- x 10-mm pieces after the removal of fat and connective tissue, and homogenized in a Waring blendor with distilled water (30 ml/100 g). The suspension was centrifuged and the supernatant removed. The sediment was washed 3 times in distilled water, then in 0.85% NaCl solution, and mixed thoroughly with 4 volumes of acetone. The acetone was removed after centrifugation and one volume of acetone was added to the sediment. The acetone was

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† Present address: Department of Medical Microbiology, University of Southern California Medical School, Los Angeles, Calif.