to characterize the contribution of individual processes to the over-all discrimination. Double tracer studies of Ca* and Sr* ingestion over a 20-day period gave the same OR values as methods based on lifetime feeding. Absorption from the tract and urinary excretion were the major processes by means of which the rat discriminated against dietary strontium in favor of calcium. There is evidence of slight strontium preference in movement of the two elements from blood to bone. Excretion into the gastrointestinal tract did not contribute significantly to the over-all discrimination. Rats on a nonmilk diet ($OR_{bone\text{-}diet} = 0.27$) discriminated more against strontium than did those on a milk diet ($OR_{bone\text{-}diet} = 0.57$). This behavior was related to the absorptive process.

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Effect of Digoxin on Cardiac Glycogen of the Rat.* (22637)

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Non-toxic doses of cardiac glycosides have been reported to increase cardiac glycogen (1,2,3). On the other hand, the data of Bomskov and Kaulla(4) and Liebig(5) indicate non-toxic doses decreased the glycogen level of the heart, while the results of Kimura (6) showed digotoxin to be without effect. Toxic doses of the cardiac glycosides have been reported to cause increased glycogen in the heart of the pigeon(7) and in the rat doses of 3.4 mg of digitoxin per kilo body weight resulted in an increase of the cardiac glycogen(4). Nevertheless, depletion of cardiac glycogen in animals manifesting signs of cardiac glycoside poisoning has been reported by Cherkes(2) and Haendel and Munilla(8).

The recent report of Bloom, Lewis, Schumpert, and Shen(9) that the glycogen of tissues can be divided into acid soluble and acid insoluble fractions which maintain a reasonably constant ratio to each other and the report of Russell and Bloom(10) that most physiological changes in the tissue glycogen take place in the acid soluble (labile) fraction while the acid insoluble (protein bound) remains relatively constant suggested that some of the equivocal results of the digitalis studies could be resolved by a consideration of the effects of cardiac glycosides on each of the two glycogen fractions.

Methods. Female rats, of various ages, were injected subcutaneously with 0.2 mg/kg of digoxin suspended in distilled water. With the exception of the newborn animals, the rats were injected daily for 5 days and sacrificed 5 hours after the last injection on the 5th day. The newborn animals received one

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injection and were sacrificed 5 hours later. Animals receiving a toxic dose of digoxin, were given 100 mg of the drug and sacrificed 5 hours later. All animals were non-fasted and had been fed Purina Laboratory chow supplemented by fresh greens once a week up to the time of sacrifice. At the time of the sacrifice the animals were given an intraperitoneal injection of sodium pentobarbital (40 mg kg). The newborn and 10-day-old rats were decapitated. With a sharp scalpel, a single incision was made through the rib cage, the heart grasped, the ventricle snipped and quickly placed between 2 slabs of dry ice. The frozen button of tissue was removed and divided into 2 parts. It was necessary to pool from 3 to 5 hearts of animals in the newborn and 10-day age groups in order to obtain sufficient tissue for analysis. One part of the frozen tissue was weighed and placed in 2 cc of 30% KOH. After tissue solution, the glycogen was precipitated by adding 1.1 to 1.2 vol. of 95% alcohol(11). The other part was weighed and placed in 5 cc of ice cold 10% trichloroacetic acid contained in a glass tissue homogenizer set in cracked ice. After homogenization in the cold room, the content of the homogenizer tube was transferred to a centrifuge tube, the homogenizer washed with an additional 5 cc of ice cold 10% trichloroacetic acid and the wash added to the centrifuge tube. After centrifugation, the clear supernatant was decanted and the glycogen precipitated from the supernatant with 2 vol. of ice cold 95% alcohol. The glycogen was allowed to precipitate in the cold over night, and then centrifuged. The glycogen from both procedures was hydrolyzed by addition of 2 cc of 0.6 N HCl and heating in a boiling water bath for 2½ hours. The acid hydrolysate was neutralized to phenolphthalein, made up to an appropriate volume with water and 2 cc taken for glucose determination by the method of Benedict(12).

Results. The results presented in Table I show that in the normal animal the trichloroacetic acid soluble (labile) fraction of cardiac glycogen, remains reasonably stable from birth through 360 days of age with a rather abrupt decrease in labile glycogen in the 100-

TABLE I. Cardiac Glycogen.

Age in days	Controls					Digoxin treated, .2 mg/kg subcut. daily for 5 days*				
			Glycogen in mg/100 g fresh ventricular tissue					Glycogen in mg/100 g fresh ventricular tissue		
	No. animals	Body wt, g	Total	Acid sol.	Acid insol.	No. animals	Body wt, g	Total	Acid sol.	Acid insol.
Birth	84	6.5	221 ± 7‡	173 ± 7	49 ± 8	84	6.5	249 ± 9	183 ± 6	66 ± 8
10	54	16 ± 2	259 ± 5	159 ± 3	100 ± 5	54	14 ± 1	289 ± 5	160 ± 5	129 ± 7
30	8	92 ± 2	367 ± 17	153 ± 11	214 ± 20	8	92 ± 2	367 ± 13	144 ± 7	223 ± 12
100	11	177 ± 10	420 ± 12	93 ± 7	327 ± 15	10	169 ± 9	320 ± 21	41 ± 4	279 ± 18
360	11	241 ± 7	462 ± 21	149 ± 9	313 ± 23	12	240 ± 5	296 ± 27	86 ± 16	210 ± 30
630	7	411 ± 14	363 ± 16	115 ± 6	249 ± 12	7	414 ± 12	378 ± 10	42 ± 7	336 ± 14
								100 mg subcut.§		
						5	170 ± 11	501 ± 22	101 ± 11	400 ± 19‖
						5	247 ± 7	700 ± 20	256 ± 14	444 ± 22¶

* Rats at birth given 1 inj. and sacrificed 5 hr later. ‡ No. of pooled samples. $\pm \sqrt{\frac{E(F^2)}{N(N-1)}}$. § Single inj. and sacrificed 5 hr later.

‖ Controls for this group are the 100-day-old. ¶ Controls for this group are the 360-day-old.

day age group, and a significant fall in the 630-day-old rats. The trichloroacetic acid insoluble (bound) fraction shows a steady rise from birth through 100 days of age, a stable level from the 100 through the 360-day age group and a decline in the older 630-day age group.

Effect of Digoxin on cardiac glycogen. Table I shows that digoxin has a varied effect on the cardiac glycogen of the rat when injected before 30 days of age. When rats at birth are treated with the drug, there is a slight increase in total glycogen. The significance of this rise becomes questionable when it is seen to be due to the additive effect of a small non-significant increase in both the labile and bound fractions. In the 10-day age group a 12% increase in total glycogen resulting from the digoxin injection was due entirely to the 29% increase which occurred in bound glycogen fraction. The labile fraction showed no change from the control. In 30-day-old rats digoxin gave no evidence of effect on cardiac glycogen.

At 100 days the heart responded to digoxin with a 24% decrease in total glycogen. This fall, when considered in terms of absolute amounts, occurred in both the labile (52 mg depression) and bound (48 mg decrease) fraction. The labile fraction fell 56% from the control value. The 15% change in the bound fraction, although statistically of doubtful significance ($P = 0.10\text{-}0.05$), is suggestive of the onset of bound glycogen depression evident in the next age group studied. In 360-day-old rats, digoxin depressed the total cardiac glycogen, which fell from a control level of 462 mg per 100 g tissue to 296 or a decrease of 36%. Both glycogen fractions contributed to the decrease. The acid soluble (labile) fraction was decreased 43% while the acid insoluble (bound) fraction fell 33%. In animals at 630 days of age, digoxin caused no change in total glycogen. However, this negative effect was due to a 55% increase in bound glycogen, accompanied by a 64% decrease in labile glycogen.

Effect of toxic dose of digoxin on cardiac glycogen. The 100- and 360-day age groups were selected to note the toxic effect of 100 mg of digoxin on cardiac glycogen. The drug was administered in one dose and the animals sacrificed 5 hours later. Both age groups showed a rise in total and bound glycogen with this toxic dose. The labile fraction rose 72% in the 360-day age group while the 100-day age group showed no change from the control.

Discussion. These results suggest that the age of the animal has an influence on response of the cardiac glycogen stores to digoxin treatment. Rats of 30 days of age or less did not respond to digoxin or in some cases as the bound fraction of the 10-day age group, with a slight increase. Digoxin consistently caused a decrease in the acid soluble fraction of glycogen in the hearts of all rats of 100 days or older. Russell and Bloom(10) in commenting upon previous work and adding results of their own have suggested that the labile glycogen is the more mobile portion physiologically and seems to be associated with the immediate functional demands made upon the tissue. This is of interest, in view of Wollenberger's(13) comments that the effect of the cardiac glycosides do not appear to be related to the energy yielding processes of muscle. The age groups represented by 100- and 360-day-old rats respond to digoxin with a decrease in total glycogen. In the 100-day age group the fall in total glycogen is due to an equal, in absolute amounts, decrease in the labile and bound fraction The digoxin depression in the 360 age group is predominantly due to a decrease in the bound fraction of glycogen. The ages 100 and 360 days fall within the period of sexual maturity and activity which implies that the sex hormones may alter the digoxin response of labile and bound glycogen. This problem is under investigation.

The values on cardiac glycogen here reported are higher than the results reported elsewhere(10,14,15) This may be attributable in part to a sex difference; preliminary observations in our laboratory suggest that this may be true at least in regard to estrogens. Leonard(16) reported that estradiol increased glycogen content of skeletal muscle in spayed females. The higher values re-

ported here may be due to the Benedict method for glucose which is sensitive to nonfermentable reducing substances occurring in acid hydrolysates of tissues. However, values obtained in our laboratory for liver glycogen using the Benedict method for glucose agree with those reported by Russell and Bloom (15), using the anthrone method. In the instance where approximate ages are mentioned for the male rats used by Bloom and Russell (14) age (from 3-4 mo. to 6-8 mo.) increased both the labile and bound glycogen whereas in our series of females the labile fraction remained relatively constant from birth through 360 days of age with a considerable fall occurring in the 100-day age group. Nevertheless, results are consistent with the rise due to age reported by Bloom and Russell(14) if their 3-4 mo. old series is considered comparable to our 100-day age group.

Several observations may have a bearing on the increase in cardiac glycogen observed with toxic doses of digoxin. It is known that high concentrations of the digitalis glycosides have a central vasomotor effect(17) which implies stimulation of sympathetics and release of epinephrine. Bloom and Russell(14) show that epinephrine increases the total and labile glycogen in the heart. However, their results do not indicate an increase in bound glycogen which does occur with toxic doses of digoxin.

Summary. Digoxin in a dose of 0.2 mg/kg caused either no change or a slight increase in the cardiac glycogen fractions when administered to rats of 30 days of age or less, but caused a decrease in all fractions of cardiac glycogen when given to rats of 100 and 360 days of age. At 630 days of age, digoxin caused a fall in the trichloroacetic acid soluble fraction and an increase in the acid insoluble fraction with no change in total cardiac glycogen. When administered to 100- and 360-day-old rats toxic doses of digoxin increased all fractions of cardiac glycogen except the acid soluble fraction of the 100-day-old rats which showed no change.

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