Influence of Digilanid on Creatine Reserve of Normal, Thyrotoxic and Hypothyroid Rat Ventricle.* (18410)

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Only a relatively few reports have appeared concerning the action of digitalis substances on the metabolism of hearts in failure. Since doses of digitalis which are toxic to normal hearts may actually improve the competency of insufficient hearts, it is likely that the chemical changes induced are different in each case. Clinically, it is not unusual to find heart failure in the presence of hyperthyroidism and hypothyroidism. Thyroxin injections and thyroparathyroidectomy offer easy means whereby these clinical states may be simulated in rats.

Several investigators(1-7) have established the fact that low cardiac creatine levels usually accompany myocardial failure in humans. Beltrametti(8) showed that during heart failure there is a marked creatinuria which gradually disappears as circulatory function is restored toward normal. Experimentally, Cowan(9), Bodansky(10) and Bodansky and Pilcher(11) succeeded in effecting a loss of creatine from rat heart by producing hyperthyroidism in their animals. Cowan(9) also reported that thyroparathyroidectomy produced a small but significant decrease in the creatine content of rat heart. Decherd, Herrman *et al.*(12,13) measured the effects of cardiac glycosides on the creatine reserve of hypertrophied rabbit hearts with sectioned aortic cusps. They found that digitalization alone had a tendency to increase the creatine content and seemed to offset the decrease in creatine percentage of great hypertrophy. These findings indicated that it might be of interest to measure the creatine reserves following the administration of Digilanid to rats in which an altered thyroid state had been induced.

Methods. The animals used were Sprague-Dawley rats ranging in weight from 230 to 300 g. For injection, synthetic thyroxin (Hoffman LaRoche) was dissolved to a concentration of 1 mg per cc in water made slightly alkaline with sodium hydroxide. The Digilanid solution contained 0.2 mg of Digilanid per cc, plus 7.5% alcohol and 15% glycerine by weight. Groups of 4 to 6 animals were injected intraperitoneally with one or both drugs given daily in 1 cc doses. Thyrotoxicosis was produced in all cases by injection of thyroxin for 21 days. Two sets of experiments were carried out on thyrotoxic animals. In the first set Digilanid was administered for 10 days to rats which had been pre-treated with thyroxin for 11 days. In the second set Digilanid was given for a total of 28 days, the animals to be thyroxinized being pre-treated with Digilanid for 7 days. Thyro-

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TABLE I. Creatine Reserve of Digitalized Normal, Hyperthyroid and Hypothyroid Rat Ventricle.

Exp.	Treatment*	No. of animals	Creatine, mg %	T‖ values	Initial† vent. wt, mg	Final‡ vent. wt, mg	Stand. dev.	VW/BW§ final	Initial† creatine content, mg	Final‡ creatine content, mg
I	Normal	13	188	—	653	890	35	2.61	1.23	1.67
	D-10	6	155	9.7	—	—	—	—	—	—
	T-21	4	114	17.5	—	—	—	—	—	—
	D-10, T-21	4	114	12.9	—	—	—	—	—	—
II	D-28	5	203	2.2	630	940	30	2.79	1.18	1.91
	T-21	5	136	16.2	642	1100	40	4.20	1.21	1.50
	D-28, T-21	5	133	10.3	631	1000	56	4.21	1.19	1.33
III	Normal	6	190	—	689	746	13	2.79	1.31	1.42
	D-28	4	191	0.08	714	759	49	2.83	1.36	1.45
	TP-21	4	213	3.04	717	650	15	2.34	1.36	1.52
	D-28, TP-21	5	222	8.88	714	578	21	2.21	1.36	1.28

* D, T, and TP refer to Digilanid (0.2 mg/day), Thyroxin (1 mg/day), and Thyroparathyroidectomy, respectively. Numerals represent number of days induced state was continued before animals were sacrificed.

† Calculated by use of the normal VW/BW.

‡ Observed values.

§ VW/BW equals mg of ventricular wt per g body wt.

‖ Significance of differences between means; less than 3 is not considered statistically significant(15).

$$T = \frac{M_1 - M_2}{\sqrt{E_1^2 - E_2^2}}$$ (E = mean error, M = mean).

parathyroidectomies were done on normal rats and rats pre-treated with Digilanid for 7 days. In the latter case, Digilanid injections were continued for an additional 21 days before the animals were sacrificed. Analyses were always carried out 21 days post-operatively. Litter mates served as controls. The animals were fasted for 24 hours before being sacrificed by decapitation. The thorax was opened at once and the ventricles were precisely separated from the auricles, divided into 2 approximately equal portions, blotted dry and weighed on a torsion balance. No attempt was made to distinguish right from left ventricle. One sample was used for creatine determination, the other for dry weight measurement or other analyses. The method of Rose, Helmer and Chanutin(14) as modified by Myers(7) was used for the estimation of total creatine in rat ventricle.

Results. The results of the creatine determinations are given in Table I. The normal controls for experimental Groups I and II showed an average creatine concentration of 188 mg %. In Exp. I it will be noted that injections of thyroxin (1 mg/day for 21 days) and Digilanid (0.2 mg/day for 10 days) effected a decrease in the creatine concentration of rat ventricle. Thyroxin produced the greatest decrease, amounting to about 39% below the normal value. Treatment of thyroxinized animals with Digilanid for 10 days had no effect whatsoever on the already lowered creatine percentage.

In Exp. II Digilanid was administered for 28 days to ascertain whether a longer period of digitalization would alter the percentage decrease of creatine in the hyperthyroid rat heart. The animals were weighed at the beginning and end of the experimental period in order to estimate changes in ventricular weight and total creatine content during the experimental period. Digilanid alone seemed to raise the creatine percentage slightly but this difference was found not to be statistically significant (T value less than 3.0). Other findings were essentially the same as in the previous experiment; thyroxin caused a drop of about 28% and this change was not affected by Digilanid. The theoretical average initial ventricular weight was calculated by multiplying the initial body weight by the ventricular weight/body weight ratio of normals. It was then possible to calculate the

14. Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, v75, 543.

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theoretical initial creatine content of each heart, using the normal value of 188 mg % as the creatine concentration. Final ventricular weight was measured directly and final content of creatine could be calculated from the creatine percentage as determined. Final V.W. B.W. ratios were measured directly. The animals receiving thyroxin lost weight during the experimental period and exhibited cardiac hypertrophy which was apparent grossly and in the final V.W. B.W. ratio, which was 4.20 as compared to the normal of 2.61. Digilanid decreased this hypertrophy somewhat but a final ratio of 4.21 showed that there was no relative change. The actual increase in total creatine content of thyroxinized ventricle during the experimental period was about 65% of the normal increase, and in the case of animals receiving Digilanid as well, only 35%. Thus, the effect of thyroxin in causing a relative loss of creatine from the hearts was increased by Digilanid. In spite of the diminished concentration of creatine in hyperthyroid ventricle the cardiac hypertrophy more than compensated for the difference to keep the total amount from decreasing.

In Exp. III similar studies were done with thyroparathyroidectomized rats. Again, Digilanid for 28 days did not significantly alter the creatine percentage. Thyroparathyroidectomy was followed by an increase of questionable significance, while the operated animals which were given Digilanid for 28 days showed a definite increase in creatine concentration. There was a clear cut loss of ventricular mass in those animals which were thyroparathyroidectomized, and this cardiac atrophy was increased by Digilanid. These changes are reflected in the lowered V.W./B.W. ratios, 2.34 and 2.21 as compared to the normal of 2.79. Digilanid produced an actual loss of creatine in operated animals but had no detectable effect on normal animals in this regard.

Measurements of dry weight of ventricles from drug-treated animals agreed closely with the normal values. Thus, where atrophy or hypertrophy occurred it was real and not due to changes in water content.

Discussion. The purpose of these experiments was to measure the action of Digilanid on the creatine reserve of normal rat hearts and to compare these results with any effects which might be produced in animals with an altered thyroid state. Digilanid administered to normal rats for 10 days effected a decrease in creatine concentration of about 18%. Following 28 days of Digilanid treatment, the creatine concentration, ventricular weight and total creatine content were found to be within normal limits. Digilanid had no influence on the percentage decrease in creatine produced by thyroxin injections, but did diminish the hypertrophy of the thyrotoxic ventricle and as a consequence increased the relative loss of total creatine. Although thyroparathyroidectomy elevated the creatine concentration slightly, concomitant cardiac atrophy accounted for an essentially normal total creatine content. The only statistically significant effect of Digilanid in the operated animals was to enhance the cardiac atrophy, thus leading to an actual loss of creatine from the hearts.

It is apparent then that Digilanid did not change appreciably the cardiac creatine concentration of normal, thyroxinized and thyroparathyroidectomized rats. Alterations in total creatine content were a direct consequence of diminished heart muscle mass. It is not known whether the cardiodynamic state of the animals used was affected by Digilanid but presumably the changes in heart size indicate that such was the case. Nonetheless, no correlation of such effects with the creatine concentration of the heart muscle can be made at this time.

Summary. Under the conditions of these experiments Digilanid (0.2 mg per day for 28 days) exerted no direct effect on the concentration of creatine in rat ventricle. However, in the animals with altered thyroid states, Digilanid brought about an actual decrease in ventricular mass which was reflected in a relative or absolute fall in the total creatine content.

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