plement-fixing antibodies were demonstrated 17 months after illness.

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Effect of Digitoxin On Creatinine and Histamine Output of the Isolated Heart.*

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In the course of some experiments on the isolated hearts of rabbits, cats and rats which were perfused with digitoxin to measure the amounts of fixation of the drug, some unexpected observations were made. In using the modified Baljet reaction[1,2,3] to determine the digitoxin colorimetrically, it was found that the intensity of the color in the perfusate was increased over that produced by the digitoxin in the perfusion fluid. This suggested the possibility that creatinine, which also gives the Baljet reaction, might be present in the perfusate. This was found to be the case. It was also observed that the perfusate contained a substance which caused a drop in the blood pressure of an anesthetized cat and a contraction of the isolated guinea pig intestine suspended in atropinized Tyrode solution. This led to the supposition that the perfusate contained histamine. Various investigators have found that the contracting heart muscle produces histamine;[4,5,6] however, this observation could not be confirmed by other workers.[7] Since the creatinine and histamine output of the heart muscle might be related to its physiological activity, it seemed worth while to perform experiments in which the release of these substances could be correlated with the action of cardiac glycosides.

Experimental. The effect of digitoxin in a final concentration of 1:40,000 was studied on isolated rat, cat, and rabbit heart preparations. The animals were sacrificed by a blow on the head, and the heart was immediately removed and washed in oxygenated Ringer-Locke solution. The hearts were perfused through the coronary arteries by means of a cannula inserted into the aorta. The temperature of the perfusion fluid was kept at 36-39°C. Samples of perfusate were collected at intervals before and after the introduction of digitoxin into the system. Digitoxin was dissolved in 95% alcohol. Five ml of this solution contained 25 mg which diluted to 1000 ml gives a final concentration of 1:40,000. Control measurements showed that this amount of alcohol did not affect the motility of the heart and did not influence the creatinine and histamine output. The volume of fluid perfused per unit of tissue was not affected by digitoxin. In the case

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TABLE I.
Creatinine Excretion of Isolated Hearts.

Animal		Samples (Creatinine gamma per 10 ml per g heart) 1	2	3	4*	5*	6*
Rabbit	Exper.	0.48	1.14	1.03	1.30	1.08	0.89
	Control	0.68	0.63	0.89	0.83	0.61	0.85
Cat	Exper.	0.76	0.52	0.43	0.73	0.82	0.74
	Control	1.23	0.92	0.76	0.47	0.84	0.62
Rat	Exper.	3.72	1.38	1.22	3.55	1.88	1.84
	Control	3.14	2.35	5.01	2.03	2.05	5.21

* After digitoxin (1:40,000) perfusion started.

of the rabbit and cat hearts, the time which elapsed between the introduction of digitoxin and complete arrest of the heart were noted. The more resistant rat hearts were allowed to beat for an hour with digitoxin perfusion before the experiment was discontinued. The hearts were weighed at the end of each experiment. Ten ml samples of perfusate were collected for creatinine and histamine analyses. The samples were kept at ice-box temperature and analyses were carried out afterwards.

The substance in the perfusate which gave the Baljet reaction was identified as creatinine by use of the NC bacterial enzyme suspension of Dubos and Miller.[8,9] The Jaffe alkaline picrate reaction was applied to samples of the perfusate before and after incubation for 90 minutes at 37°C with micro-organisms capable of destroying creatine and creatinine under aerobic conditions. The true creatinine was calculated from the difference in the color reaction before and after incubation. Two 4 ml aliquots of each perfusate sample were taken; one was subjected directly to colorimetric reaction with 2 ml of alkaline picrate, whereas the other was incubated for 90 minutes with 1 ml of cell suspension and 0.25 ml of M phosphate buffer, pH 7.0. Since the amount of protein present was less than could be precipitated by tungstate, the preparation of a tungstate filtrate was omitted. Care was taken to use a cell suspension of potency sufficient to metabolize all the creatinine present. After incubation, separation was carried out by centrifugation. To 4 ml of the supernatant liquid, 2 ml of alkaline picrate was added. The alkaline picrate solution was prepared from 1 volume of 10% NaOH in 5 volumes of freshly saturated picric acid. After the solution had been allowed to stand for 15 minutes, it was added to standard and unknown solutions in a ratio of 1 alkaline picrate to 2 of the other solution. The color was allowed to develop for 10 minutes and was then measured using a Coleman Universal Spectrophotometer, Model 11, with the PC-4 filter, and at a wave length of 525 mμ. A solution of 1 volume of alkaline picrate and 2 volumes of distilled water was used as the blank. The creatinine content of the perfusates was calculated from the readings on standard creatinine solutions and expressed in gamma per 10 ml of perfusate per gram of heart.

The histamine determinations were carried out as follows. Essentially the histamine extraction method of Barsoum and Gaddum[10] was applied. Ten ml samples of perfusion fluid were acidified with 1 ml concentrated HCl. The acidified perfusate was boiled for 2 hours, then evaporated to dryness, and extracted with 5 ml 95% ethyl alcohol saturated with NaCl. The alcohol was evaporated, and the residue taken up in 2 ml of water. This solution was tested on the atro-

[8] Miller, B. F., and Dubos, R., *J. Biol. Chem.*, 1937, **121**, 429, 447, and 457.

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TABLE II.
Statistical Analysis of Creatinine Results.

No. of animals	No. of samples		Mean values		T values*
	Before	After	Before	After	
10 Rabbits	21	22	0.97	0.90	1.4
10 Cats	21	20	0.69	0.73	1.5
7 Rats	14	16	4.54	3.84	1.9

* Significance of differences between the means; less than 3.0 is not considered statistically significant.

$$T = \frac{M_1 - M_2}{\sqrt{E_1^2 - E_2^2}}$$ (E = mean error, M = mean).[11]

TABLE III.
Histamine Excretion of Isolated Hearts.

Animal		Samples (Histamine, gamma per 10 ml per g heart)					
		1	2	3	4*	5*	6*
Rabbit	Exper.	.034	.036	.033	.015	.008	.000
	Control	.030	.026	.030	.028	.028	.030
Cat	Exper.	.019	.020	.017	.009	.007	.003
	Control	.014	.018	.010	.015	.013	.018
Rat	Exper.	.11	.11	.12	.11	.11	.11
	Control	.08	.07	.12	.11	.10	.10

* See Table I.

TABLE IV.
Statistical Analysis of Histamine Results.

No. of animals	No. of samples		Mean values		T values*
	Before	After	Before	After	
11 Rabbits	26	25	.033	.0095	6.71
10 Cats	16	18	.0135	.0053	7.52
10 Rats	22	23	.13	.13	0.00

* See Table II.

pinized guinea pig intestine in the usual way. The sensitivity of the intestine was frequently tested with known amounts of histamine. This extraction method proved to be quite useful for estimating histamine in perfusates containing digitoxin. The method eliminates digitoxin and potassium ions, which, if present in the original perfusate, cause contraction of the guinea pig ileum.

Results. The results are summarized in tables wherein typical experimental data and the statistical analyses of a large number of experiments are presented. The creatinine output in each of the 3 species investigated was fairly constant and not affected by digitoxin (Tables I and II). The highest creatinine output was observed in rat hearts. The creatinine outputs of rabbit and cat hearts were approximately the same.

On the other hand, the histamine output showed an interesting correlation with the action of digitoxin in rabbit and cat. In these two species there was a significant decrease in the histamine content of the perfusate, which in many cases reached zero in the last sample. However, the histamine output of the rat heart was not affected (Tables III and IV).

It is of interest to note that the time elapsing before complete arrest of auricles and ventricles of the isolated hearts varied with the species. The most resistant was

[11] Burn, J. H., *Biological Standardization*, 1937, Oxford University Press, London, p. 29.

TABLES V AND VI.
Comparison of the Sensitivity of Rabbit, Cat, and Rat Heart to Perfusion with Digitoxin, 1:40,000.

TABLE V.

	No. of exper. animal	Amt of digitoxin (1:40,000) ml/g heart to cause heart arrest	Survival time in min.
Rabbits	16	11.5	18.0
Avg heart wt equals 9.4 g	17	15.9	11.0
	18	13.2	12.5
	19	17.0	10.3
	20	20.2	11.0
	21	12.5	7.0
	23	—	11.0
	24	12.4	11.0
		M = 15.9*	M = 11.5*
Cats	1	11.4	10.5
Avg heart wt equals 14.9 g	2	21.7	16.0
	3	21.5	13.0
	4	11.3	7.0
	5	26.3	13.8
	8	29.6	10.0
	10	—	15.0
	11	23.6	13.0
	12	13.5	8.5
		M = 19.8*	M = 11.9*

* M = Mean values.

TABLE VI.
Rats—All hearts beating well at one hour after the perfusion started. Average heart weight equals 1.45 g.

No. of exper. animal	Amt of digitoxin perfused in one hour
1	42.0 ml g/hour
2	126.2 "
5	87.1 "
7	153.8 "
8	100.0 "
	M = 101.8* ml per gram per hour

* M = Mean values.

the rat heart, while the rabbit and cat showed essentially similar sensitivity (Tables V and VI).

Discussion. From these results, it is reasonable to assume that the toxic effect of digitoxin on the isolated heart results in decreased histamine output, while the appearance of creatinine in the perfusion fluid is not altered. It has been shown by Anrep and coworkers[6] that when the heart fails the histamine output diminishes. Our results with digitoxin-poisoned hearts corroborate these observations. It is interesting that the relative sensitivities of the 3 species to digitoxin are parallel to their relative sensitivities to histamine. The biochemical mechanism of these findings is being investigated in further detail in order to gain a better understanding of these phenomena.

Summary. The perfusion fluid from isolated hearts of cats, rabbits and rats contains creatinine and histamine. There is a species variation in the excretion of these substances. When digitoxin (1:40,000) is added to the perfusion fluid, the creatinine output of the heart is not significantly altered in the 3 species studied. However, the histamine output of the cat and the rabbit heart decreases, while that of the rat heart remains unchanged. The isolated rat heart is much more resistant toward digitoxin than the cat and rabbit hearts.