sible to ascribe the changes to the effect of the infusion.

The authors wish to acknowledge the capable technical assistance of Miss Lillian Gong, Miss Lenore Safier, and Mr. Jack Newman.

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Received August 7, 1956. P.S.E.B.M., 1957, v94.

Cardiotonic Activity of 9-Alpha Fluorohydrocortisone.* (22914)

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Previous work from this laboratory(1) reported the positive inotropic action of 9-alpha fluorohydrocortisone‡ (9-a FF), and its effect upon Na^{22} distribution in the papillary muscle preparation. In the present paper evidence is presented showing the positive inotropic action of 9-a FF in low concentrations, and its negative inotropic action in higher concentrations. These findings are correlated with histological changes in cardiac muscle and intramuscular Na^{22} and K^{42} movement.

Methods. 1. *The cat papillary muscle preparation* first described by Cattell and Gold(2) was employed with certain modifications(1). Briefly, 2 muscles were isolated from the right ventricle without causing gross injury. A control and a treated muscle were stimulated in two identical chambers at 60 times per minute throughout the experiment. Krebs-Ringer bicarbonate solution enriched with dextrose was used as the bathing fluid maintained at 38°C and a mixture of 95% O_2 and 5% CO_2 bubbled through it. A minute amount of either Na^{22} or K^{42}§ was added to the bathing fluid, the amount of K^{42} added being taken into account when preparing the medium. 9-a FF in amounts indicated in Table I was then added to the bathing fluid of one of the muscles. The uptake of isotopic Na or K was expressed in counts/sec/mg and then in terms of the ratio of radioactivity of the samples treated with 9-a FF to that of control samples. 2. *The isolated heart preparation* as modified by Anderson and Craver (3) was employed using whole cat hearts and enriched Krebs-Ringer bicarbonate solution as the perfusing medium. Failure was induced by perfusing the preparation with fresh, unused perfusate(4). Since 9-a FF in a concentration of 0.5 μg/ml caused a positive inotropic action in the papillary muscle preparation, this concentration was added to the reservoir bottle and perfused through the heart after signs of cardiac failure appeared. 9-a FF 'toxicity' was induced by adding 10 μg/ml to the perfusate at 0 time, as indicated in Fig. 2. 3. *Histologic sections* were obtained from fresh papillary muscles or those stimulated at 60 times per minute for 6 hours in concentrations of 0, 0.5 or 20 μg/ml

* This project was partially supported by funds from the Continuing Research Fund of University of Colorado School of Medicine.

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‡ Acknowledgement is made to Merck & Co., Rahway, N. J., who provided us with 9-alpha fluorohydrocortisone.

§ Specific activity of Na^{22} = 0.75 μc/ml (and amounted to 0.48 mEq/l in 143 mEq/l of Na); specific activity to K^{42} = 0.047 μc/ml (and amounted to 0.4 mEq/l in 5.66 mEq/l of K).

TABLE I. Results Obtained with 9-Alpha Fluorohydrocortisone.

Exp.	Drug conc. (μg/ml)	Isotope	Ratio:* Exp./C†	Δ inotropic action (%)‡
1–11	.5–1.0	Na^{22}	>1	all positive§
12	C†	K^{42}		
	.5		.57	+439
13	C			
	.5		.89	+219
14	C			
	.5		.96	+ 87
15	C			
	.5		.95	+105
16	C	Na^{22}		
	10		.77	- 52
17	C			
	10		.53	- 57
18	C			
	20		.66	- 22
19	C			
	20		.82	- 91
20	C	K^{42}		
	10		1.06	- 89
21	C			
	20		1.44	- 23
22	C			
	20		1.60	- 37
23	C			
	20		1.15	- 68

* In counts/sec./mg.
† C = Control.
‡ Measured from just prior to administration of drug to highest or lowest point reached after administration.
§ See Tanz *et al.*(1).

of 9-a FF. Typical sections are compared in Fig. 3.

Results. Fig. 1 shows the effect of adding 9-a FF to the perfusing fluid of the papillary muscle preparation. Average values of these experiments, which were measured at 20 minute intervals, are plotted in terms of % height of the initial contraction. The upper dotted line represents results obtained on 7 muscles using a concentration of 0.5-1.0 μg/ml(1). The lower dotted line shows results obtained in a range of 10-20 μg/ml on 7 muscles. These results show a positive or negative inotropic action (depending on dosage), when compared to mean control values represented by the solid line(1).

Results from individual experiments are tabulated in Table I. In the 0.5-1.0 μg/ml range a positive inotropic action is found along with an increased uptake of Na^{22} and a loss of K^{42}, in comparison with its control from the other chamber. In the 10-20 μg/ml, or 'toxic' range, a negative inotropic action is obtained with a loss of Na^{22} and an increased uptake of K^{42}, in comparison with its control.

Addition of 0.5 μg/ml to the perfusate of the failing isolated heart preparation resulted in partial recovery within 40 minutes. Conversely, addition of a 'toxic' dose of 10 μg/ml (Fig. 2) brought about cardiac failure in about 15 minutes.

Histologically, sections of the fresh papillary tissue show good cellular integrity, lightly stained elongated nuclei and typical cross-striations of cardiac tissue. The tissues stimulated continuously for 6 hours in a concentration of 0.5 μg/ml, show fairly good cellular integrity, slightly darker elongated nuclei and an impression of cross-striations. Conversely, control muscles and those treated with 20 μg/ml, stimulated for the same period of time, are greatly swollen with pyknotic and warped nuclei, a foamy cytoplasm and loss of cross-striations; all signs of

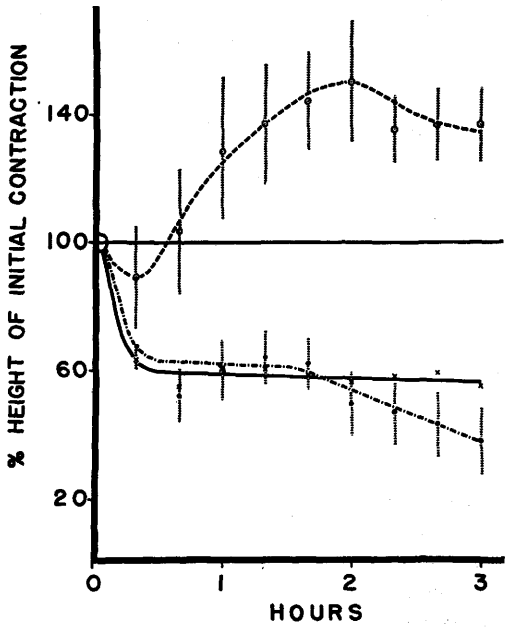

FIG. 1. Dotted curves show inotropic action when varying concentrations of 9-a FF are added. Upper curve = 0.5 – 1.0 μg/ml; lower curve = 10 – 20 μg/ml. Vertical lines represent the calculated stand. errors. Solid curve shows mean of 11 control experiments.

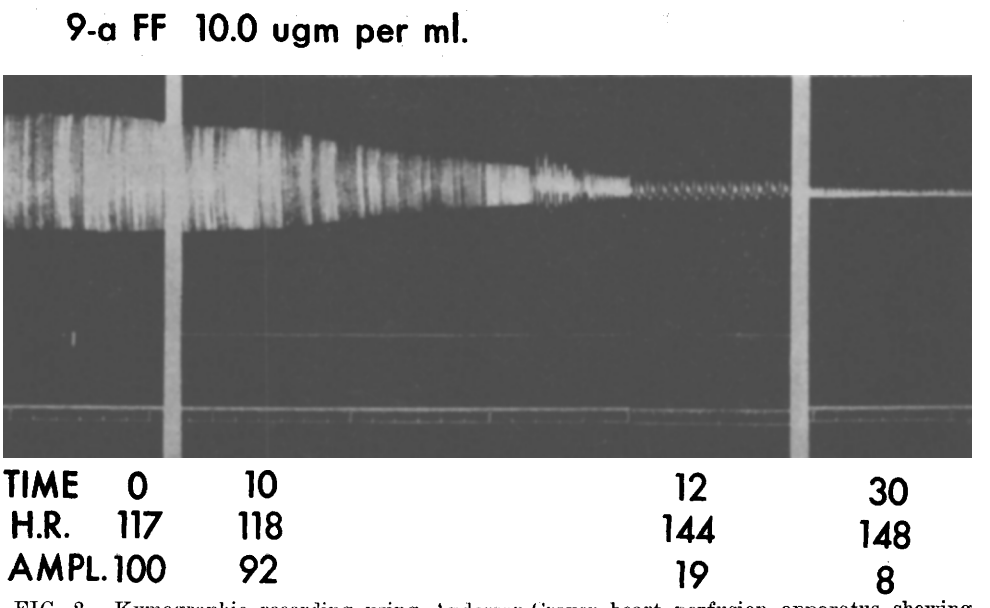

FIG. 2. Kymographic recording using Anderson-Craver heart perfusion apparatus showing 9-a FF 'toxicity.' 10 μg/ml of 9-a FF added at 0 time. (Time markings in 10 sec. and 1 min.; TIME in min.; AMPL. in %.)

cardiac degeneration.

Discussion. These results are presented as evidence of the cardiac glycoside-like action of 9-a FF on isolated heart tissue. The evidence that Gowdey *et al.* (1950) and previous workers(5-11) have presented using a wide variety of steroids, indicated to us that some might possess a direct glycoside-like action on cardiac tissue. The fact that the steroid nucleus is common to both cardiac glycosides and steroids lent further support to this belief.

Using the 8 K^{42} and 4 Na^{22} control experiments from Table I, it was calculated that there was about 9.5 times as much K^{42} as Na^{22} in the control muscles at the end of 3 hours. Although there were too few experiments for a quantitative measurement, it is certainly indicative that a positive K/Na ratio did exist in the controls. The fact that this ratio was not greater than 9.5 is in agreement with Fenn(12,13) and others(14,15) who have stated that muscles take up Na and extrude K during contraction.

When experimental muscles were given a 'therapeutic' dose of 9-a FF (0.5-1.0 μg/ml), and a positive inotropic action resulted, the ratios in Table I show that they took up Na^{22} and lost K^{42}. Conversely, a 'toxic' dose of 9-a FF (10-20 μg/ml), resulted in positive K^{42} and negative Na^{22} ratios, while showing a negative inotropic response. This is in agreement with Korol (1956), who showed that cardiac depression is associated with loss of Na and an uptake of K and Gertler *et al.*(16) who showed that quinidine toxicity produced intracellular hyperpotassemia, and possibly intracellular hyponatremia.

Hajdu and Szent-Gyorgyi(8) have shown that variations in physiological response of isolated cardiac tissue might be due to the washing out of some substance by the perfusion fluid. The addition of serum helped restore a normal physiological response, and partial identification of the responsible substance(s) in serum indicated a steroid type of compound. Furthermore, Leitch¶(4) has observed that the original perfusate must be recycled through the isolated heart preparation to prevent failure. On the basis of our results with the isolated heart perfusion apparatus, we believe that one or more naturally occurring steroids are necessary for normal cardiac function. The fact that such small amounts of 9-a FF are needed to elicit a re-

¶ Personal Communication.

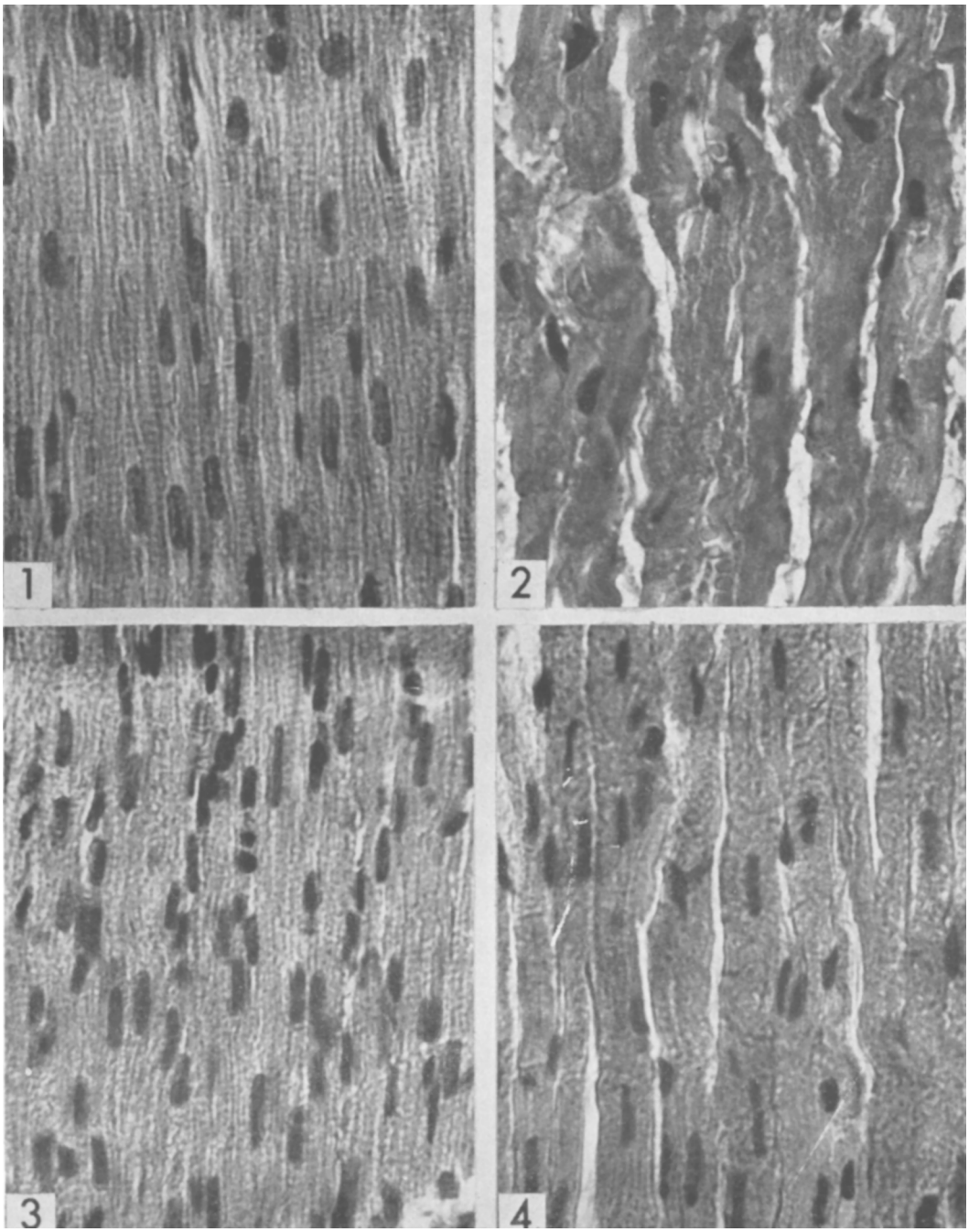

FIG. 3. Photomicrographs of papillary muscles. (1) Fresh tissue. (2) Control tissue stimulated 6 hr. (3) Tissue treated with 0.5 μg/ml of 9-a FF and stimulated 6 hr. (4) Tissue treated with 20 μg/ml of 9-a FF and stimulated 6 hr. (H & E × 1000.)

sponse is indicative of a specific action on cellular activity, probably exerting its influence by altering membrane permeability.

Summary. (1) Addition of 9-a FF in a concentration of 0.5-1.0 μg/ml results in a positive inotropic action, an increased uptake of intramuscular Na and loss of K. (2) In a concentration of 10-20 μg/ml, 9-a FF gives

rise to a negative inotropic action, loss of intramuscular Na and an increased uptake of K. (3) Employing the isolated heart preparation, addition of 0.5 μg/ml of 9-a FF to the failing heart results in a partial recovery. Conversely, 10 μg/ml added to the 'normal' heart brings about failure quite rapidly. (4) A histological correlation of the reported results is shown. (5) The mechanism of action and significance are discussed.

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Received October 3, 1956. P.S.E.B.M., 1957, v94.

A Cytopathogenic Agent Isolated from Naval Recruits with Mild Respiratory Illnesses.* (22915)

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A cytopathogenic agent has been isolated in monkey kidney cultures from nasopharyngeal washings obtained from Naval recruits with mild respiratory illnesses(1). In this paper, certain characteristics of the isolate are described which allow preliminary classification and identification. The relation of infection to clinical illness in 2 recruit companies and evidence suggestive of widespread infection in other populations are also presented.

Methods. Cultures were prepared with 0.25% trypsin (Difco, 1:250) dispersed kidney cells from adult Rhesus monkeys at a concentration of 500,000 cells per ml. These were incubated at 35°C in a stationary position in nutrient medium consisting of 90% mixture 199(2) (Difco Laboratories) or bovine amniotic fluid (Cudahy), 5% inactivated horse serum (56°C for 30 minutes), 5% bovine embryo extract(3), penicillin, 200 units and streptomycin, 200 μg per ml. The same media or mixture 199 alone plus antibiotics was used when the cultures were ready for inoculation. Monkey testis, cornea, human embryo kidney and chick embryo cultures were propagated similarly. HeLa, KB

* From Research Project, Bureau of Medicine and Surgery, Navy Dept., Washington, D.C. The opinions expressed herein are those of the authors and cannot be construed as indicating endorsement or approval by the Navy Dept. or of Naval Service at large. This work was also supported in part by Abbott Laboratories grant.

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