neighborhood of Pittsburgh. Hu 39 was not recognized among the 209 virus strains isolated. Attempt was made to demonstrate presence of antibodies in the blood of people residing in Allegheny County (or Western Pennsylvania). Of 277 adult sera tested, antibody against the virus was found in only one at the screening dilution level of 1:8. The serum exhibited a neutralizing titer of 1:64 against 175 $TCID_{50}$. These data indicate a very limited prevalence of the virus in the population of Pittsburgh during the last few years. Human gamma globulin diluted 1:20 failed to neutralize the virus. The presence of specific antibodies in human serum in one instance and the fact that the virus was successfully reisolated from the original specimen in 1962 indicate that the agent is very likely a human enterovirus. The possible relationship of the virus to the clinical disease observed in the patient from whom the agent was isolated could not be investigated in the absence of acute and convalescent serum specimens.

Summary. A virus was isolated in human amnion FL cell cultures on April 14, 1959 from the rectal swab obtained from a 9-month-old male child admitted to the Children's Hospital, University of Pittsburgh, with an acute respiratory illness of undetermined etiology. The virus exhibits the properties of a typical enterovirus. It is not pathogenic for suckling mice, and multiplies and produces CPE only in human cells. It was not isolated in subsequent years from 2275 rectal swabs obtained from 516 children. Serological studies in a normal population group in the Pittsburgh area revealed antibodies against the virus in one serum among 277 adult sera tested. This fact and the re-isolation of the virus from the original specimen suggest that it is a human enterovirus. No conclusion can be drawn concerning its pathogenicity for man.

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Effect of Ouabain on Cardiac Output of Anesthetized Rat.* (28159)

TERESA PINAKATT, ALFRED W. RICHARDSON AND THEODORE COOPER
Department of Physiology and Center for Cardiovascular Research, St. Louis University, St. Louis, Mo.

Albino rats are considered resistant to ouabain. Large doses are required to produce electrocardiographic changes indicative of toxicity in this species(1). The relationship

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between toxic dose and the dose capable of inducing inotropic or other hemodynamic responses has not been established. The availability of a reliable technic for the serial measurement of cardiac output in the rat(2) has permitted us to study the hemodynamic responses of the anesthetized intact rat to intravenously administered ouabain over a wide dose range. These hemodynamic responses are the subject of this paper.

Methods. Eighty-four male albino rats weighing from 330 to 500 g were studied. Each animal served as its own control. The rats were divided into 12 groups; each group of 6 to 10 animals received a different dose of ouabain. Doses ranged from 0.01 mg/kg to 0.5 mg/kg. Control animals were injected with equivalent amounts of vehicle and saline flush in order to eliminate these solutions as possible causes for responses which were observed.

The rats were anesthetized with intraperitoneal injections of pentobarbital sodium (30 mg/kg). They were placed in the supine position after which the right jugular vein, right carotid artery and a femoral artery were exposed through small incisions in the skin. A calibrated catheter was introduced into the superior vena cava for administration of drugs and of cold saline injectate for measurement of cardiac output by the thermodilution method(2). A thermistor was inserted into the aorta by way of the right carotid artery for measurement of arterial blood temperature changes. Arterial blood pressure was measured with a Statham P 23D transducer through a PE 50 catheter inserted into the femoral artery. Blood pressure, electrocardiograms and thermal dilution curves were recorded on a direct writing oscillograph. The procedure and instrumentation for measurement of cardiac output by the thermodilution method is detailed elsewhere(2).

Control determinations of heart rate, blood pressure and cardiac output were performed in triplicate, after which ouabain was administered intravenously in predetermined doses. Measurements were repeated in triplicate within 10 to 30 minutes after injection. Stroke volume and peripheral resistances before and after drug administration were calculated by standard formulae. Statistical significance of the responses as compared to control were calculated by the t-Test.

Results. The data are summarized in Table I. There was no statistically significant alteration in heart rate, mean arterial pressure or pulse pressure at any of the doses employed. Cardiac output and stroke volume showed significant increases at doses of 0.05, 0.06, 0.07, 0.08, and 0.09 mg/kg. Calculated peripheral resistance fell at these doses.

Electrocardiograms showed no signs of toxicity at any dose employed. At the higher doses respiratory stimulation was apparent on occasion. Animals which were permitted to recuperate following the procedure survived without apparent ill effect regardless of dose employed. The intravenous injection of vehicle for the ouabain or saline produced no significant changes in heart rate, blood pressure or cardiac output.

Discussion. These data indicate that ouabain exerts significant effect on the cardiac output of the pentobarbital anesthetized rat in doses at which no evidence of toxicity was observed. The slight decrease in cardiac output seen at doses from 0.01 to 0.04 mg/kg are similar to the responses observed in the dog following administration of cardiac glycosides in comparable doses based on body weight(3). The significant increase in cardiac output observed at doses 0.05 to 0.09 mg/kg is probably due to the positive inotropic effect of the ouabain. Since cardiac rate does not change, the increase in output is mediated by an increase in stroke volume.

This demonstration supports the contention that cardiac glycosides are capable of exerting positive inotropic action in the nonfailing heart(4), although one might take the view that the heart of the rat anesthetized with barbiturate is modified from the normal physiological state(5).

The absence of an increase in cardiac output at dose levels above 0.09 mg/kg suggests that under these experimental conditions, the effects of ouabain on other portions of the vascular system(6,7) may exert a greater contribution to the hemodynamic changes than the direct cardiac effects.

TABLE I. Hemodynamic Responses of Rat to Ouabain.

Dose, mg/kg	No. Rats	Wt, g		Cardiac output, ml/min	Heart rate per min	Stroke vol, ml	Mean arterial blood pressure, mmHg	Peripheral resistance unit
Vehicle	4	415	before	120.8 ± 1.4	322 ± 14	.38 ± .01	122 ± 5.5	1.01 ± .04
Na_2PO_4			after	115.0 ± 2.1	330 ± 9	.35 ± .07	120 ± 3.9	1.05 ± .03
NaCl			% change	−4.8	+2.4	−7.4	−1.6	+3.4
K_2PO_4			P	NSD	NSD	NSD	NSD	NSD
.01	6	420	before	119.6 ± 4.1	333 ± 17	.37 ± .02	123 ± 3.0	1.04 ± .10
			after	109.7 ± 7.0	350 ± 11	.32 ± .01	125 ± 8.0	1.14 ± .07
			% change	−8.4	+6	−14	+1.1	+9.8
			P	NSD	NSD	NSD	NSD	NSD
.02	6	410	before	127.3 ± 5.2	362 ± 27	.36 ± .02	122.5 ± 2.6	.97 ± .05
			after	118.0 ± 5.7	364 ± 9	.33 ± .03	121.6 ± 2.0	1.04 ± .05
			% change	−7	+.6	−6	−.5	+7
			P	NSD	NSD	NSD	NSD	NSD
.03	6	400	before	123.6 ± 8.1	340 ± 26	.38 ± .09	122 ± 2.4	1.01 ± .12
			after	124.0 ± 9.0	340 ± 28	.38 ± .05	128.6 ± 5.8	1.09 ± .07
			% change	+ .3	0	0	+6	+7
			P	NSD	NSD	NSD	NSD	NSD
.04	6	400	before	118.9 ± 5.7	361.6 ± 23	.34 ± .03	120 ± 1.1	1.02 ± .1
			after	118.7 ± 7.5	361.6 ± 23	.33 ± .01	117 ± 5.7	1.00 ± .1
			% change	0	0	−3	−2.5	−2
			P	NSD	NSD	NSD	NSD	NSD
.05	10	432	before	121.4 ± 6.7	378 ± 13.4	.32 ± .02	121.7 ± 2.2	1.02 ± .05
			after	148.2 ± 7.7	376 ± 14	.40 ± .03	119.9 ± 11.8	.83 ± .06
			% change	+22	−.5	+23	−1.5	−19
			P	.001	NSD	.01	NSD	.001
.06	10	441	before	136.0 ± 3.8	368 ± 17	.38 ± .02	130 ± 2.8	.96 ± .04
			after	163.5 ± 7.2	349 ± 20	.48 ± .03	131 ± 3.4	.82 ± .04
			% change	+20	−5.4	+25	+.8	−15.4
			P	.02	NSD	.01	NSD	.001
.07	10	403	before	125.0 ± 2.0	375 ± 17	.34 ± .02	131 ± 6.0	1.05 ± .03
			after	149.0 ± 3.2	346 ± 24	.44 ± .03	130 ± 6.6	.87 ± .04
			% change	+19.2	−8	+31.8	−.75	−16.8
			P	.01	NSD	.025	NSD	.01
.08	6	416	before	121.5 ± 4.2	398 ± 9	.31 ± .01	132.5 ± 4.3	1.10 ± .04
			after	143.4 ± 4.7	370 ± 14	.37 ± .01	130.8 ± 4.0	.94 ± .05
			% change	+18	−7	+20.9	−1.2	−16.7
			P	.01	NSD	.001	NSD	.025
.09	6	400	before	113.5 ± 4	345 ± 11	.33 ± .001	121 ± 2.1	1.07 ± .02
			after	132.2 ± 5	345 ± 9	.39 ± .02	131.6 ± 3.0	1.00 ± .04
			% change	+17	0	+16.3	+9	−6.5
			P	.05	NSD	.05	NSD	.05
.1	6	430	before	127.0 ± 4.8	370 ± 14	.40 ± .02	136.6 ± 4.0	1.09 ± .07
			after	146.0 ± 5.0	348 ± 17	.43 ± .03	143 ± 5.0	.99 ± .05
			% change	+15	−6	+5.7	+4	−10
			P	NSD	NSD	NSD	NSD	NSD
.5	6	446	before	124.0 ± 1.5	330 ± 20	.38 ± .02	129 ± 5.0	1.04 ± .05
			after	139.6 ± 4.1	333 ± 11	.41 ± .03	133.3 ± 6.2	.96 ± .05
			% change	+12.5	+1	+7	+3	−8
			P	NSD	NSD	NSD	NSD	NSD

Values presented represent mean and standard errors of means for each group.
P = Level of significance of differences between means.
NSD = No significant difference.

Digitalis glycosides are said to increase calculated total peripheral resistance in the intact dog(6) and in man(8) by virtue of its ability to stimulate constriction of arteriolar smooth muscle. This effect was not obvious in the anesthetized rat, particularly in those doses wherein cardiac output was augmented. Calculated total peripheral resistance decreased. No direct evidence of actual dilatation of peripheral vasculature was obtained in these studies however.

The tolerance of the rat for ouabain in large doses may be due in part to the fact that in this species a large percentage of the agent is excreted unchanged by the liver into the bile and is not reabsorbed by the intestine(9). The metabolic pathway and hence the dose-response relationship for other car-

diac glycosides may be different. Gold and associates have described large differences in convulsant activities of a variety of cardiac glycosides in the rat. They suggested that the variations were due to differences in metabolism or fixation of the glycosides. Ouabain had relatively little convulsant action (10).

The volume of injectate used in each animal for each thermodilution curve was 0.1 ml and total volume for each animal did not exceed 0.8 ml physiological saline. This volume, even if given in one injection, could not explain the responses observed. The vehicle for the ouabain was similarly excluded as the explanation of the cardiac responses.

The thermodilution method in our hands is reproducible within ± 6% (2). The significant changes in cardiac output after administration of ouabain (0.05 to 0.09 mg/kg) were far in excess of this variability. This technic offers the possibility of further study of the mechanism of action of cardiotonic drugs in small animals. It is simple, inexpensive and provides a reliable, experimentally repeatable measurement of cardiac output in the same small animal.

Summary. The cardiac output of anesthetized albino rats increased following administration of ouabain in doses of from 0.05 to 0.09 mg/kg i.v. Mean arterial blood pressure and heart rate were unchanged; calculated total peripheral resistance decreased. Signs of toxicity were not observed over a dose range of from 0.01 to 0.05 mg/kg despite the appearance of the positive inotropic effects.

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Action of Parathyroidectomy and Dihydrotachysterol on Myocardial Calcification Due to Coronary Vein Obstruction.* (28160)

E. BAJUSZ AND G. JASMIN†

Department of Pathology, University of Montreal, Montreal, Canada

Extensive investigations during the past few years on various experimental cardiopathies(1) have not provided a satisfactory answer as to why some structural alterations in the myocardium and other tissues constitute a prerequisite for calcification. It is nevertheless true that among the various forms of necrotizing cardiopathies induced in animals, some (*e.g.,* those produced by proteolytic enzymes, neomycin, polymyxin, sulfonamides, nephrectomy) calcify more consistently than others(2). As yet, we are not justified in classifying cardiopathies as calcifying and non-calcifying since the same agent (Plasmocid or paraphenylenediamine), according to the mode of administration and other undetermined factors, elicits degenerative changes within the cardiac or skeletal musculature that may or may not be accompanied by calcific deposits(3,4). A new approach to the problem was provided by the observation that while the massive cardiac infarct that follows coronary artery ligature never calcifies, the less extensive but still

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† Research Associate of Medical Research Council of Canada.