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DRUG THERAPY OF ACUTE PULMONARY INSUFFICIENCY

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Contract No. DADA-17-71-C-1060 was initiated for the purpose of developing new drugs for the treatment of acute pulmonary insufficiency. Two compounds have been selected for further investigation. Eriodictyol administered orally to rhesus monkeys prevented or reduced the pulmonary congestion and edema induced by iodoacetamide. Other compounds known to provoke pulmonary edema in the dog (alloxan and ANTU) did not induce similar effect in the monkey. The naphthoquinone compound previously demonstrated to protect rodents from pulmonary edema was less effective in monkeys. In the dog heart, eriodictyol is non-toxic and potentiates the coronary vasodilator action of adenosine, a substance known to be released by hemolysis.

Two water-soluble benzoylcarbinols were tested in mice. Both were effective in preventing carbon dioxide-induced pulmonary edema. However, benzoylcarbinoltrimetyl acetate produced pulmonary hemorrhages. Benzoylcarbonolmorpholine acetate was selected for future studies to determine if intravenous injection would be effective in treating or reversing acute pulmonary insufficiency.

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REPORT NUMBER 2

DRUG THERAPY OF ACUTE PULMONARY INSUFFICIENCY

Annual Summary Report
(1 April 1972 to 1 January 1973)

Domingo M. Aviado, MD and Miroslaw A. Belej, Ph. D. and Leonardo V. Bacalzo, Jr., MD

Department of Pharmacology, University of Pennsylvania Medical School

Philadelphia, Pennsylvania 19174

1 January 1973

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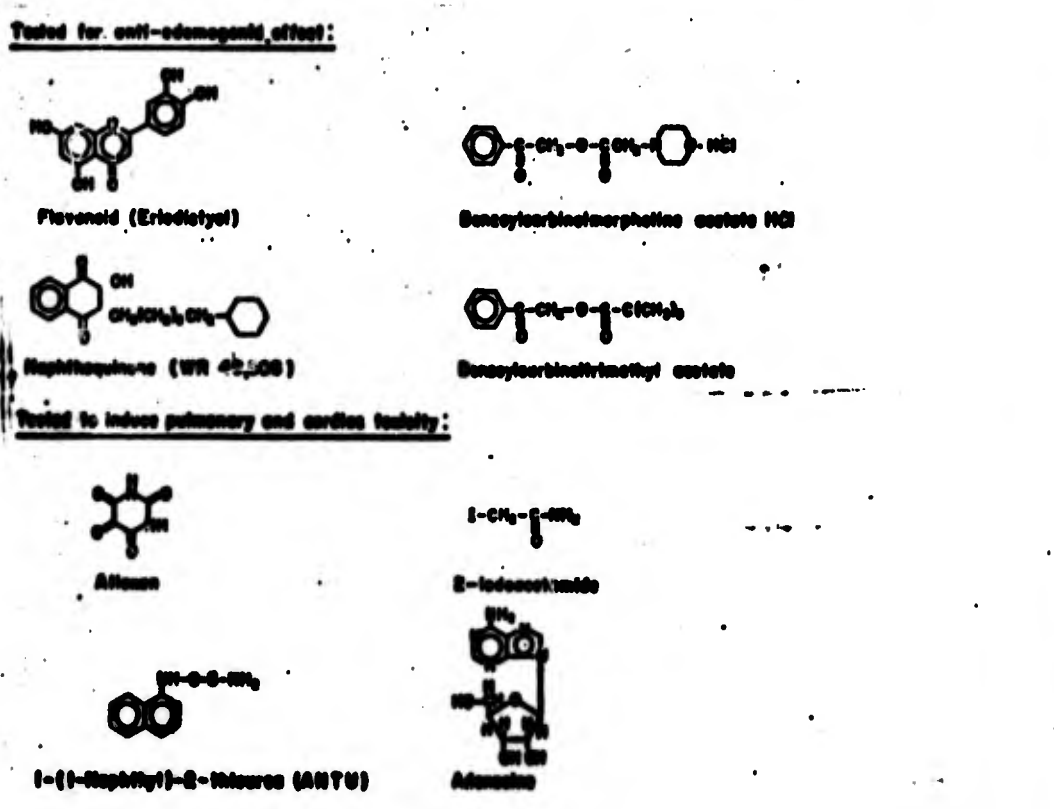
TABLE OF CONTENTS

ABSTRACT

Contract No. DADA-17-71-C-1060 was initiated for the purpose of developing new drugs for the treatment of acute pulmonary insufficiency. Two compounds have been selected for further investigation: Eriodictyol administered orally to rhesus monkeys prevented or reduced the pulmonary congestion and edema induced by iodoacetamide. Other compounds known to provoke pulmonary edema in the dog (alloxan and ANTU) did not induce similar effect in the monkey. The naphthoquinone compound previously demonstrated to protect rodents from pulmonary edema was less effective in monkeys. In the dog heart, eriodictyol is non-toxic and potentiates the coronary vasodilator action of adenosine, a substance known to be released by hemolysis.

Two water-soluble benzoylcarbinols were tested in mice. Both were effective in preventing carbon dioxide-induced pulmonary edema. However, benzoylcarbinol-trimetyl acetate produced pulmonary hemorrhages. Benzoylcarbinolmorpholine acetate was selected for future studies to determine if intravenous injection would be effective in treating or reversing acute pulmonary insufficiency.

The chemical structures of anti-edemagenic and other compounds are illustrated below.



DRUG THERAPY OF ACUTE PULMONARY INSUFFICIENCY

with special reference to anti-edemagenic compounds.

During the past year, there were three significant developments in our contract. First, is the confirmation that the compounds previously reported to exert pulmonary antiedemagenic action in rodents are also effective in the rhesus monkey. Second is the selection of eriodictyol as an orally administered agent for the prevention of pulmonary edema. Third is the initial search for an intravenously administered agent for the treatment of pulmonary edema. The experiments relating to these three items are discussed in this report which cover the second year of the contract.

Part I. Testing of antiedemagenic compounds in rhesus monkeys (Table 1 to 3).

In the dog experiments reported in the Progress Report No. 1, alloxan was used to provoke pulmonary insufficiency. It was tested in three rhesus monkeys anesthetized with sodium pentobarbital 30mg/kg intravenously. In three monkeys, the infusion of 5 or 10 mg/kg/min caused death after the following lethal dose of alloxan: 833, 900, and 1100 mg/kg. There was no pulmonary edema and respiratory insufficiency; death was caused by cardiac failure. Another chemical agent known to produce pulmonary edema in dogs was then tested in three monkeys. Alpha-naphthylthiourea (ANTU) was infused at the rate of 2 or 4 mg/kg/min. The lethal doses in three monkeys were: 140, 264 and 320 mg/kg. None of these monkeys showed acute pulmonary insufficiency. There was therefore a difference in sensitivity of the lungs to alloxan and ANTU between the dog previously reported (1) and the monkey reported presently.

Iodoacetamide proved to be an effective agent in producing acute respiratory insufficiency in rhesus monkey. This compound was infused at a rate of 10 mg/kg/min,

and the lethal doses for three monkeys were as follows: 410, 420, 440 mg/kg. Prior to death, there was in increase in pulmonary resistance, decrease in pulmonary compliance, decrease in respiratory minute volume, decrease in systemic arterial blood pressure and decrease in arterial blood oxygen tension. At autopsy, the lungs were hemorrhagic and the wet weight and moisture content were higher than those of control monkeys. The lungs were tested for static compliance by measuring pressure-volume curves. The critical pressure that caused inflation was -16 cm H_2O for iodoacetamide treated monkeys and -11cm H_2O for control monkeys (Figs. 1 and 2). There was also a reduction in total phospholipid content from 16.7 mg/g lung in control to 14.5 mg/g for iodoacetamide treated lung. These changes indicate acute pulmonary insufficiency based on signs of pulmonary edema, pulmonary congestion and reduced pulmonary compliance.

A naphthoquinone compound (WR 49,808) was administered orally, 500 mg/kg for two or three days prior to provoking acute pulmonary insufficiency with iodoacetamide. In three pretreated monkeys, the mean lethal dose for iodoacetamide was increased to 496 mg/kg which is higher but not statistically different from mean lethal dose of monkeys that were not pretreated. The severity of pulmonary edema judged by lung weight/body weight and percentage moisture of the lung of pretreated monkeys was significantly less indicating some protection. Three additional monkeys were pretreated with WR 49,808 by intraperitoneal injection of 500 mg/kg one hour prior to the injection of iodoacetamide. There was protection from the edema-inducing action of iodoacetamide based on the measurements in vivo and postmortem.

Eriodictyol was examined and its protective action was more conspicuous than that exerted by the naphthoquinone compound. Three monkeys were pretreated with eriodictyol 500 mg/kg orally for 2 or 3 days, and three additional monkeys with the

same dose administered intraperitoneally for 2 or 3 days. The conclusion that eriodictyol prevents acute pulmonary insufficiency provoked by iodoacetamide is based on the following observations:

1. The monkeys pretreated orally with eriodictyol had a mean lethal dose for iodoacetamide of 536.7 ±34.8 mg/kg which is statistically greater than the mean lethal dose for monkeys without pretreatment. The intraperitoneal injection of eriodictyol did not cause a significant increase in mean lethal dose of iodoacetamide.

2. The fall in arterial blood pH and oxygen tension was less in monkeys pretreated orally with eriodictyol than the monkeys receiving iodoacetamide without pretreatment.

3. There was no fall in pulmonary compliance in monkeys pretreated orally with eriodictyol.

4. The percentage moisture content of the lung of monkeys pretreated with eriodictyol was not different from the normal lung indicating that eriodictyol prevented pulmonary edema induced by iodoacetamide.

5. The critical inflating pressure of the lung from monkeys pretreated with eriodictyol of -12.07 cm H_2O approached the value for normal lung (-11.08 cm H_2O) and less than the value for the lung of iodoacetamide-treated monkeys without pretreatment (-16.00 cm H_2O).

6. The phospolipid content of lung of eriodictyol pretreated monkeys had a mean value of 15.0 mg/g lung tissue. This value is higher than that for iodoacetamide treated monkeys. The increase in phospholipid content reflects a correction of deficiency in surfactant activity.

(Tables 1 to 3 and Figures 1 and 2 are in the following pages).

Table 1. Summary of in vivo and postmortem measurements of monkeys with acute pulmonary insufficiency 40 min after starting infusion of iodoacetamide (mean±SE)

Procedure	No. of Exps.	Arterial pH Contr.	Arterial pH Resp.	Arterial pCO_2 mmHg Contr.	Arterial pCO_2 mmHg Resp.	Arterial pO_2 mmHg Contr.	Arterial pO_2 mmHg Resp.	Pulm. Compl. ml/cmH_2O Contr.	Pulm. Compl. ml/cmH_2O Resp.	g. W. L. W. kg. B. W.	g. W. L. W. g. D. L. W.	Phospho-lipids mg/g	Lethal dose mg/kg	Critical point -cmH_2O
Control	3	--	--	--	--	--	--	--	--	6.50 ±0.58	4.50 ±0.20	16.7 ±0.90	--	11.08 ±0.51
Iodoacetamide	3	7.36 ±0.01	7.13* ±0.05	33.3 ±1.3	34.0 ±13.5	88.7 ±0.3	65.0 ±12.6	9.67 ±0.33	7.50* ±0.29	10.7* ±0.17	5.20* ±0.12	14.5* ±0.55	423.3 ±8.8	16.00* ±1.58
WR-49808 (oral pretr.)	3	7.34 ±0.03	7.28 ±0.09	33.0 ±1.2	16.7 ±2.8	91.7 ±10.9	101.0 ±1.0	6.83 ±0.44	7.83 ±0.44	8.30** ±0.62	4.80** ±0.06	14.1 ±0.40	496.7 ±44.8	12.87 ±0.58
WR-49808 (i.p. pretr.)	3	7.41 ±0.02	7.26 ±0.02	36.7 ±0.3	17.0 ±1.5	96.3 ±4.7	87.7 ±14.1	9.83 ±0.17	9.50 ±1.32	8.80** ±0.65	4.50** ±0.23	14.4 ±0.20	460.0 ±50.3	--
Eriodictyol (oral pretr.)	3	7.41 ±0.02	7.27 ±0.09	35.7 ±3.3	25.3 ±6.6	99.0 ±7.4	81.3 ±18.7	6.00 ±0.58	6.77 ±0.50	8.50** ±0.59	5.00 ±0.15	15.0*** ±0.43	536.7** ±34.8	12.07** ±0.29
Eriodictyol (i.p. pretr.)	2	7.36	7.24	34.5	26.0	96.0	67.0	5.50	5.00	8.90	5.00	13.70	380.0	--

* Different from control, $p < 0.05$
** Different from iodoacetamide, $p < 0.05$
*** Not different from control, $p > 0.05$

Table 2. Summary of in vivo measurements in 20 monkeys 40 min following intravenous infusion of alloxan, ANTU or iodoacetamide.

Procedure	Exp. No.	Pulmonary Resistance cmH_2O/LPS Control	Response	%Δ	Pulmonary Compliance ml/cmH_2O Control	Response	%Δ	Resp. Min Volume ml/min Control	Response	%Δ	Heart Rate beats/min Control	Response	%Δ	Aortic Blood Pressure mm Hg Control	Response	%Δ
Alloxan	1	23.8	30.0	+26	10.0	9.0	-10	630	765	+21	170	160	-6	90	71	-21
5 or 10 mg/kg/min	2	25.0	22.0	-12	8.5	10.0	+17	729	880	+21	230	210	-9	140	104	-26
	3	26.7	25.5	-4.4	10.0	8.5	-15	432	510	+18	180	170	-5	100	100	0
	Mean	25.2	25.8	+3.2	9.5	9.2	-2.5	597	718	+20	193	180	-6.7	110	92	-15.7
	±SE	±0.8	±2.3	±11.6	±0.5	±0.4	±10.2	±87	±109	±1.0	±19	±15	±1.2	±15	±10	±8.0
ANTU	4	28.0	30.0	+7	8.0	7.0	-13	543	550	+1	180	185	+3	95	80	-16
2 or 4 mg/kg/min	5	23.5	27.7	+5	8.0	6.0	-25	675	144	-79	180	80	-56	95	30	-68
	6	27.3	30.0	+10	10.0	9.5	-5	540	550	+2	180	175	+3	125	90	-28
	Mean	26.3	28.2	+7.3	8.7	7.5	-14.2	586	413	-25	180	147	-17	105	67	-37
	±SE	±1.4	±1.8	±1.5	±0.7	±1.0	±5.8	±45	±137	±27	±0	±34	±20	±10	±19	±16
Iodoacetamide	7	27.0	27.0	0	9.0	7.5	-17	270	192	-29	175	60	-66	120	25	-79
10 mg/kg/min	8	28.0	32.0	+14	10.0	7.0	-30	360	288	-20	170	80	-53	120	30	-75
	9	21.1	30.7	+46	10.0	8.0	-20	300	120	-60	150	70	-53	127	25	-80
	Mean	25.4	29.9	+20	9.7	7.5	-22	310	200	-36	165	70	-57	122	26.7	-78
	±S. E.	± 2.2	± 1.5	±14	±0.3	±0.3	± 4	±26.5	±48.7	±12	±7.6	±5.8	±4	± 2.3	±1.7	±2
WR 49,808 orally	10	30	28	-7	7.5	8.0	+7	360	432	+20	180	230	+27	130	75	-42
500 mg/kg 2 or 3X	11	28	31	+11	7.0	7.0	0	528	540	+2	195	200	+3	145	50	-66
followed by iodo-	12	28	26	-7	6.0	8.5	+41	346	336	-3	175	160	-9	125	45	-64
acetamide 10 mg/																
kg/—in	Mean	28.7	28.3	-1	6.8	7.8	+16	411	436	+6	183	197	+7	133	57	-57
	±S. E.	± 0.6	± 1.5	±6	±0.4	±0.4	±12	± 58	±59	±7	±6	± 20	±10	±6	±9	±8

WR 49,808 intra-	13	22.0	28.0	0	10.0	12.0	+20	600	720	+20	230	170	-26	120	70	-42
peritoneally 500 mg/kg	14	21.0	21.0	0	9.5	7.5	-21	600	430	-28	210	100	-52	130	50	-62
1 or 2X followed by	15	20.0	20.0	0	10.0	9.0	-10	620	450	-27	180	100	-44	105	45	-57
Iodoacetamide 10 mg/kg/min	Mean	21.0	21.0	0	9.8	9.5	-4	607	533	-12	207	123	-41	118	55	-54
	±S.E.	± 0.6	±0.6	±0	±0.2	±1.3	±12	±7	±94	±16	±15	±23	±8	±7.3	±7.6	±6
Eriodictyol orally	16	18.0	18.0	0	7.0	7.0	0	405	767	+89	160	190	+19	140	65	-53
500 mg/kg 2 or	17	27.3	25.6	-6	5.0	7.5	+50	410	486	+19	190	186	-2	135	67	-50
3X followed by	18	26.3	24.0	-8	6.0	5.8	-3	294	280	-5	160	180	+12	145	85	-41
Iodoacetamide																
10 mg/kg/min	Mean	23.9	22.5	-5	6.0	6.8	+16	370	511	+34	170	185	+10	140	72	-48
	±S.E.	± 2.9	±2.3	±2	±0.6	±0.5	±17	±38	±141	±28	±10.0	±2.9	±6	±2.9	±6.4	± 4
Eriodictyol intra-	19	19.1	18.3	-4	5.0	5.0	0	612	300	-51	180	80	-56	110	25	-77
peritoneally 500	20	21.0	21.0	0	6.0	5.0	-17	324	200	-38	200	100	-50	110	40	-64
mg/kg 2 or 3X																
followed by iodo-	Mean	20.1	19.7	-2	5.5	5.0	-8.5	468	250	-45	190	90	-53	110	33	-71
acetamide 100 mg/																
kg/min																

Table 3. Details of in vivo measurements in monkeys receiving edema-provoking agents and anti-edemagenic drugs.

Procedure	Exp. No. (min)	Pulmonary Resistance cmH_2O/LPS	Pulmonary Compliance ml/cmH_2O	Resp. Min. Volume ml/min.	Heart Rate b/min.	Aortic B. P. mmHg	Art. pH	Art. pCO_2 mmHg	Art. pO_2 mmHg
Alloxan	1 (0)	23.8	10.0	630	170	90.0			
5 mg/kg/min	(10)	23.8	10.0	720	160	95.0			
	(30)	21.1	11.3	756	160	70.0			
	(60)	31.7	8.0	780	160	64.0			
	(90)	31.7	8.0	780	140	40.0			
	(120)	34.5	8.0	1092	120	35.0			
	(143)	34.5	8.0	120	60	30.0			
	Max. %Δ	+45	-20	-81	-65	-67			
	Animal died after 145 min.; lethal dose: 833 mg/kg								
Alloxan	2 (0)	25	8.5	729	230	140			
10 mg/kg/min	(10)	25	8.5	972	230	148			
	(30)	20	10.0	850	220	100			
	(60)	22	8.5	907	200	85			
	(80)	28.6	6.5	440	80	35			
	Max. %Δ	+14	-24	-40	-65	-75			
	Animal died after 90 min.; lethal dose: 900 mg/kg								
Alloxan	3 (0)	26.7	10.0	432	180	100			
10 mg/kg/min	(10)	28.6	9.0	540	180	115			
	(30)	28.6	9.0	540	180	85			
	(60)	24.2	8.0	540	160	100			
	(90)	25.0	8.0	486	140	96			
	(100)	30.8	5.0	553	120	65			
	(105)	30.8	5.0	324	80	35			
	Max. %Δ	-15	-50	-25	-56	-65			
	Animal died after 110 min.; lethal dose: 1100 mg/kg								
ANTU	4 (0)	28.0	8.0	544	180	95	7.35	30	90
2 mg/kg/min	(10)	30.0	8.0	580	190	88	7.33	30	89
	(30)	30.0	7.0	619	185	80	7.35	39	74
	(60)	30.0	6.5	518	180	60	7.24	30	70
	(90)	30.0	6.0	518	180	50	7.17	18	60
	(120)	30.0	6.0	454	175	40			
	(125)	30.0	6.0	232	100	30			
	Max. %Δ	+7	-25	-57	-44	-68	-2.4	-40	-33
	Animal died after 132 min.; lethal dose: 264 mg/kg								

Table 3 (continued)

Procedure	Exp. No. (min)	Pulmonary Resistance $cmH_2O/L/PS$	Pulmonary Compliance ml/cmH_2O	Resp. Min. Volume ml/min.	Heart Rate b/min.	Aortic B. P. mmHg	Art. pH	Art. pCO_2 mmHg	Art. pO_2 mmHg
ANTU	5 (0)	23.5	8.0	675	180	95	7.41	30	80
4 mg/kg/min	(10)	23.5	8.0	675	180	85	7.39	33	66
	(30)	24.2	6.0	504	180	55	7.23	42	42
	(32)	24.7	6.0	144	80	30	7.00	51	31
	Max. %Δ	+5	-25	-79	-56	-68	-5.5	+70	-61
	Animal died after 35 min. : lethal dose: 140 mg/kg								
	6 (0)	27.3	10.0	540	180	125	7.38	32	75
	(10)	27.3	10.0	570	200	110	7.38	28	80
	(30)	30.0	10.0	570	190	100	7.34	32	73
	(60)	27.3	9.0	540	160	54	7.28	32	74
	(75)	32.5	8.0	456	140	35	7.23	30	53
	Max. %Δ	+19	-20	-16	-22	-72	-2.0	-6	-29
	Animal died after 80 min. : lethal dose: 320 mg/kg								
Iodoacetamide	7 (0)	27.0	9.0	270	175	120	7.37	32	89
10 mg/kg/min	(10)	27.0	9.0	420	175	75	7.31	28	122
	(30)	27.0	8.0	260	140	45	7.19	30	100
	(40)	27.0	7.5	192	60	30	7.19	22	80
	Max. %Δ	0	-17	-29	-66	-75	-2.4	-31	-10
	Animal died after 42 min. : lethal dose: 420 mg/kg								
	8 (0)	28.0	10.0	360	170	120	7.34	32	89
	(10)	30.0	8.0	378	180	60	7.29	32	83
	(30)	32.0	8.0	462	180	57	7.34	14	82
	(40)	32.0	7.0	288	80	30	7.17	19	75
	Max. %Δ	+14	-30.0	-20	-53	-75	-2.3	-41	-16
	Animal died after 44 min. : lethal dose: 440 mg/kg								
	9 (0)	21.1	10.0	300	150	127	7.36	36	88
	(10)	30.7	8.0	240	160	70	7.25	42	78
	(30)	30.7	9.0	150	145	45	7.13	51	50
	(40)	30.7	8.0	120	70	25	7.04	61	40
	Max. %Δ	+46	-20	-60	-53	-80	-4.3	+69	-55
	Animal died after 41 min. : lethal dose: 410 mg/kg								
WR-49808,	10 (0)	30.0	7.5	360	180	130	7.38	35	70
per os,	(10)	30.0	7.5	396	195	100	7.45	23	111
2 X 500 mg/kg	(30)	28.0	7.5	495	200	70	7.40	21	120
followed by	(40)	28.0	8.0	432	230	75	7.42	11	103
Iodoacetamide,	Max. %Δ	-7.0	+7.0	+20	+27	-42	+0.5	-69	+47
10 mg/kg/min	Animal died after 52 min. : lethal dose: 520 mg/kg								

Table 3 (Continued)

Procedure	Exp.No. (min)	Pulmonary Resistance cmH_2O/LPS	Pulmonary Compliance ml/cmH_2O	Resp. Min. Volume ml/min.	Heart Rate b/min.	Aortic B. P. m.mHg	Art. pH	Art. pCO_2 mmHg	Art. pO_2 mmHg
WR-49808,	11 (0)	28	7.0	528	195	145	7.34	33	100
per os,	(10)	31	6.5	576	200	60	7.36	22	120
2 X 500 mg/kg	(30)	31	7.0	972	210	75	7.44	17	120
followed by	(38)	31	7.0	540	200	50	7.33	19	100
Iodoacetamide,	Max. %Δ	+11	0	+2	+3	-66	0	-42	0
10 mg/kg/min	Animal died after 41 min. : lethal dose: 410 mg/kg								
WR-49808	12 (0)	28	6.0	346	175	125	7.29	31	105
per os,	(10)	28	6.0	324	180	50	7.17	38	100
3 X 500 mg/kg	(30)	26	8.0	336	180	55	7.09	35	98
followed by	(40)	26	8.5	336	160	45	7.09	20	100
Iodoacetamide,	Max. %Δ	-7.0	+41	-3	-9	-64	-2.7	-35	-5
10 mg/kg/min	Animal died after 56 min. : lethal dose: 560 mg/kg								
WR-49808,i.p.	13 (0)	22.0	10.0	600	230	120	7.44	36	102
2 X 500 mg/kg,	(10)	22.0	10.0	612	190	85	7.44	36	103
followed by	(30)	22.0	12.0	840	180	80	7.45	19	109
Iodoacetamide,	(40)	22.0	12.0	720	170	70	7.38	21	108
10 mg/kg/min	(50)	20.0	12.0	760	140	35	7.30	15	115
	Max. %Δ	-9	+20	+27	-39	-71	-1.9	-58	+13
	Animal died after 56 min. : lethal dose: 560 mg/kg								
	14 (0)	21.0	9.5	600	210	130	7.38	37.0	87
	(10)	24.0	9.0	650	180	80	7.41	25.0	95
	(30)	22.0	9.0	660	210	75	7.43	25.0	100
	(40)	21.0	7.5	530	100	50	7.27	20.0	80
	Max. %Δ	0	-21	-12	-52	-62	-1.5	-46	-8
	Animal died after 42 min. : lethal dose 420 mg/kg								
WR-49808,i.p.	15 (0)	20.0	10.0	620	180	105	7.40	37	100
1 X 500 mg/kg,	(10)	20.0	10.0	500	193	70	7.37	29	88
followed by	(30)	20.0	9.0	475	220	50	7.33	18	72
Iodoacetamide,	(35)	20.0	9.0	450	100	35	7.22	16	68
10 mg/kg/min	Max. %Δ	0	-10	-27	-44	-67	-2.4	-57	-32
	Animal died after 40 min. : lethal dose 400 mg/kg								
Eriodictyol,	16 (0)	18.0	7.0	405	160	140	7.37	29	102
per os,	(10)	18.0	7.0	486	190	110	7.36	30	112
2 X 500 mg/kg	(30)	18.0	7.0	721	190	90	7.37	20	112
followed by	(40)	18.0	8.0	767	190	65	7.34	16	107
Iodoacetamide,	(50)	18.0	7.0	--	180	45	--	--	--
10 mg/kg/min	Max.%Δ	0	0	+89	+13	-68	-0.4	-45	+4.9
	Animal died after 60 min. : lethal dose 600 mg/kg								

Table 3 (Continued)

Procedure	Exp. No. (min)	Pulmonary Resistance cmH_2O/LPS	Pulmonary Compliance ml/cmH_2O	Resp. Min. Volume ml/min.	Heart Rate b/min.	Aortic B. P. mmHg	Art. pH	Art. pCO_2 mmHg	Art. pO_2 mmHg
Eriodictyol,	17 (0)	27.3	5.0	410	190	135	7.42	39	85
per os,	(10)	27.3	6.2	356	210	110	7.38	37	85
2 X 500 mg/kg,	(30)	25.6	5.8	585	205	97	7.40	19	95
followed by	(40)	25.6	7.5	486	186	67	7.34	22	97
Iodoacetamide,	(50)	25.6	7.5	162	80	32	7.10	38	45
10 mg/kg/min	Max. %Δ	-6	+50	-60	-58	-76	-4.3	-2.6	-47
	Animal died after 53 min. : lethal dose 530 mg/kg								
Eriodictyol,	18 (0)	26.3	6.0	294	160	145	7.43	39	110
per os,	(10)	24.0	7.0	288	200	107	7.39	38	108
3 X 500 mg/kg,	(30)	24.0	6.0	569	190	108	7.47	19	122
followed by	(40)	24.0	5.8	280	180	85	7.37	22	92
Iodoacetamide,	Max. %Δ	-9	-3	-5	+13	-41	-0.8	-44	-16
10 mg/kg/min	Animal died after 48 min. : lethal dose 480 mg/kg								
Eriodictyol,	19 (0)	19.1	5.0	612	180	110	7.31	38	87
i. p.	(10)	19.1	5.0	639	160	45	7.35	25	102
2 X 500 mg/kg	(30)	18.3	5.0	720	200	45	7.37	17	98
followed by	(35)	18.3	5.0	300	80	35	7.18	29	60
Iodoacetamide,	Max. %Δ	-4	0	-51	-56	-68	-1.8	-24	-31
10 mg/kg/min	Animal died after 39 min. : lethal dose 390 mg/kg								
Eriodictyol,	20 (0)	21.0	6.0	324	200	110	7.41	31	105
i. p.	(10)	21.0	6.0	390	200	70	7.38	30	112
3 X 500 mg/kg	(30)	21.0	5.7	420	220	55	7.42	20	114
followed by	(35)	21.0	5.0	200	100	40	7.29	23	74
Iodoacetamide,	Max. %Δ	0	-17	-38	-50	-64	-1.6	-26	-29
10 mg/kg/min	Animal died after 37 min. : lethal dose 370 mg/kg								

Figure 1. Pressure-volume curves of excised lungs from monkeys of normal control, iodoacetamide-treated and pretreated with WR 49,808 followed by iodoacetamide.

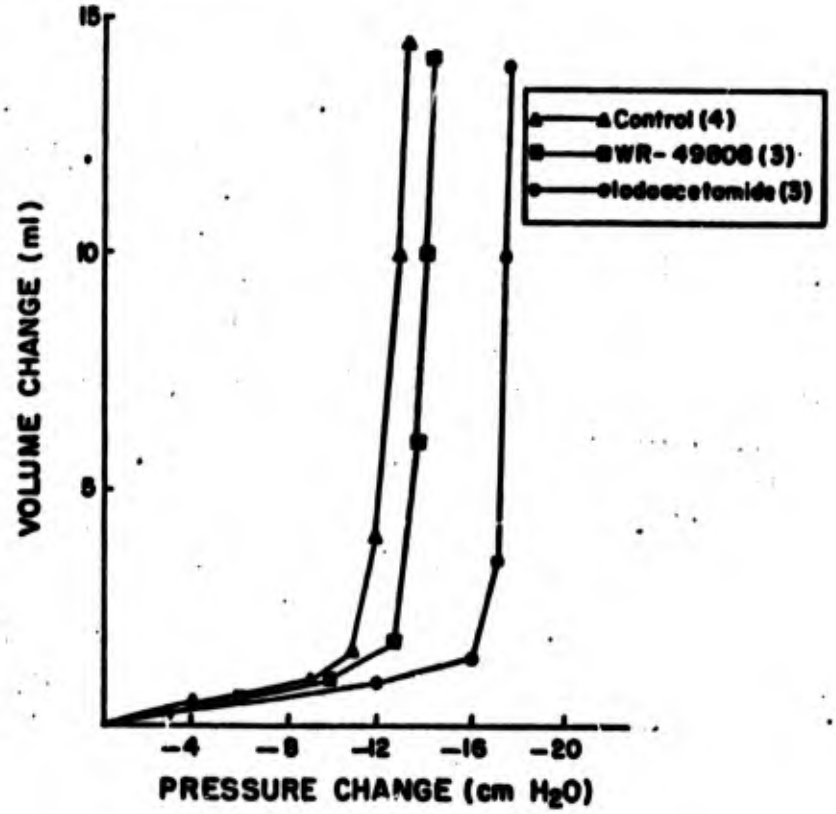
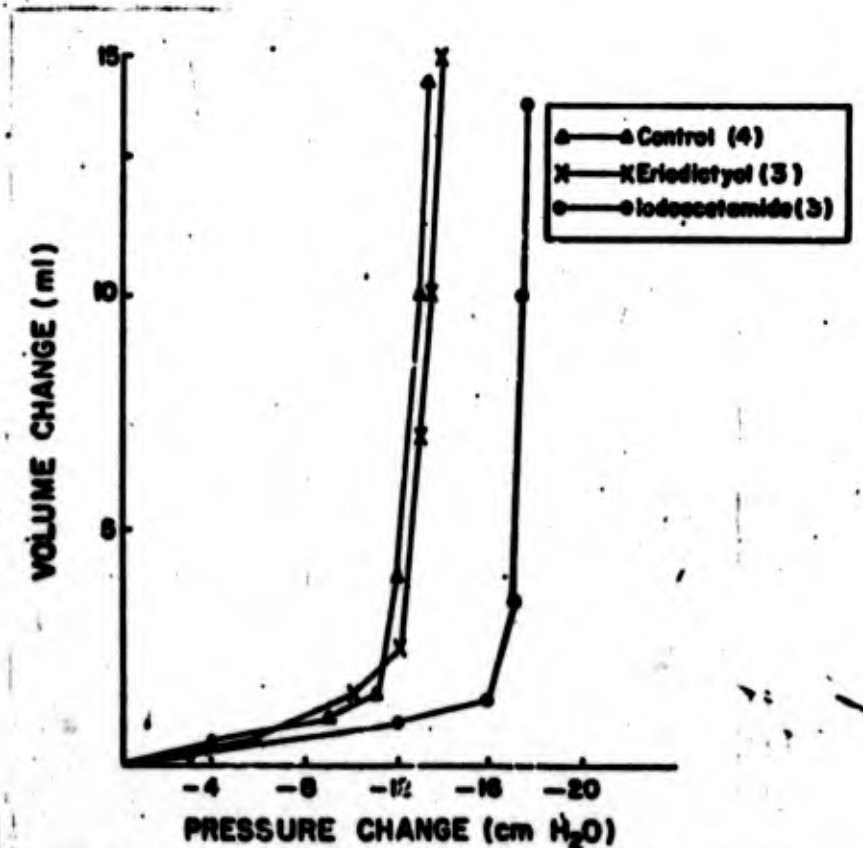


Figure 2. Pressure-volume curves of excised lungs from monkeys of normal control, iodoacetamide-treated and pretreated with eriodictyol followed by iodoacetamide.



Part II. Pharmacology of Eriodictyol.

Eriodictyol is a flavonoid originally isolated from the weed Eriodictyon californicum. It occurs in nature as a glycoside in lemon and when ingested, the enzymatic action in the intestine releases the aglycone, eriodictyol. Eriodictyol has been used by Williams and Hedgecock (1962) for the treatment of Menieres disease, presumably by reducing capillary permeability in the inner ear. The present observations that eriodictyol also reduces capillary permeability in the pulmonary capillaries present some theoretical considerations relating to a comparison of the formation of endolymph of the inner ear and the edema fluid in the pulmonary tissue. These are beyond the scope of the present investigation.

From the practical standpoint, information on pharmacology and toxicology of eriodictyol has been collected and a New Drug Application (NDA) has been prepared so that this drug can be tested in humans. Briefly, eriodictyol is non-lethal even in doses of 5 g/kg in animals. It has no adverse effect on the nervous, gastro-intestinal and respiratory systems. In the heart, another flavonoid, methyl hesperidin, was reported to produce potentiation of coronary vasodilation by adenosine (4). Adenosine is released from hemolyzed erythrocytes so that it became necessary to test eriodictyol for interaction with adenosine.

Six dogs, weighing from 15 to 20 kg, were used in this investigation. They were anesthetized with sodium pentobarbital (25 mg/kg intravenously). The chest was opened via a midsternal incision. The lungs were ventilated artificially, a strain gauge arch was sutured to the surface of the left ventricle supplied by the anterior descending branch, and the aortic blood pressure was measured through a catheter inserted into one femoral artery.

In the first group of three dogs the anterior descending branch of the left coronary artery was exposed and cannulated for perfusion. A Sigmamotor pump supplied this

branch continuously with femoral arterial blood. Eriodictyol, adenosine and angiotensin were administered into the perfused coronary artery.

The second group of three dogs received intravenous injections of adenosine, angiotensin and eriodictyol. The coronary artery remained intact and the coronary sinus was cannulated to measure the coronary blood flow by means of a Morawitz cannula and a Shipley-Wilson rotameter. Coronary vascular resistance was estimated by dividing the aortic blood pressure in mm Hg by the coronary sinus flow in ml/min (5).

Coronary arterial injections of adenosine. Adenosine was administered in three doses (0.1, 1.0 and 10 mg/kg) directly into the perfused coronary artery. The smallest dose of 0.1 mg/kg reduced the force of ventricular contraction, slowed the heart rate and reduced the aortic blood pressure, but had no significant effect on coronary perfusion pressure. The next dose of 1.0 mg/kg influenced all four parameters, including coronary perfusion pressure, which was reduced, indicating vasodilation (Table 4).

Coronary arterial injections of eriodictyol and adenosine. Eriodictyol was also administered in three doses (0.1, 1.0 and 10 mg/kg). A temporary reduction occurred in coronary perfusion pressure, indicating coronary vasodilation. There was a transient decrease in ventricular force, a decrease in heart rate and a fall in aortic blood pressure, lasting for one to two minutes. After complete recovery, the injections of adenosine were repeated. No important alterations in coronary vascular and ventricular contractility responses to adenosine appeared. However, there was a reduction in the intensity of the bradycardia response to adenosine following the intracoronary administration of 0.1 mg/kg eriodictyol (Table 4).

Coronary arterial injection of angiotensin and eriodictyol. The same three dogs reported on the preceding paragraphs also received an injection of angiotensin, which

caused an elevation of coronary perfusion pressure, indicating vasoconstriction (Table 5). Following the injections of eriodictyol, there was a reduction in intensity of coronary vasoconstrictor response (Fig. 2).

Intravenous injections of eriodictyol. The second group of three dogs received intravenous injections and the coronary circulation was interrupted on the venous side. The administration of 1 and 10 mg/kg eriodictyol caused an increase in coronary blood flow, accompanied by a reduction in aortic blood pressure. This combination of elevated flow and reduced pressure indicated a decrease in coronary vascular resistance (Table 6). The accompanying changes in heart rate and ventricular force were not significant.

Intravenous injections of adenosine and angiotensin. The repeated injections of angiotensin after 10 mg/kg eriodictyol initiated a reduction in coronary vasoconstrictor response.
With 1 mg/kg eriodictyol there was a detectable exaggeration of the increase in coronary blood flow elicited by adenosine (Fig. 4). Therefore, eriodictyol administered intravenously produced a
sensitivity of the coronary blood vessels to adenosine and angiotensin.

Conclusions. Eriodictyol reduced the vasoconstrictor effect of angiotensin on the canine coronary vessels. The reduction in effect was apparent whether eriodictyol was administered intracoronarily or intravenously. However, with regard to adenosine the potentiation of its coronary vasodilator effect was not detectable when it was administered into the coronary artery but was seen after intravenous injection. The phenomenon of sensitization to adenosine probably depends on a metabolic conversion of either adenosine or eriodictyol outside of the coronaries.

(Tables 4 to 6 and Figures 3 and 4 are in the following pages).

Table 4. Influence of eriodictyol on responses to adenosine in the heart with perfused coronary artery.

Eriodictyol (Intracoronary Dose)	Adenosine (Intracoronary Dose	Mean ± SE for three anesthetized dogs							
		Cor. Perfusion		Ventricular Force		Heart Rate		Aortic BP	
		mm Hg	%Δ	g	%Δ	/min	%Δ	mm Hg	%Δ
Control		99 ± 4		110 ± 5		166 ± 13		122 ± 3	
	Adenosine (0.1 mg/kg)	96 ± 3	- 3 ± 3	93 ± 3	-15* ± 2.7	146 ± 3	-12* ± 1.8	105 ± 5	-14* ± 4.1
	Adenosine (1.0 mg/kg)	84 ± 6	-15 ± 6	76 ± 19	-31 ±17.2	130 ± 9	-22* ± 5.4	92 ± 13	-25 ±11
	Adenosine (10 mg/kg)	76 ± 8	-23* ± 8	71 ± 16	-35 ±14.5	121 ± 8	-27* ± 4.8	74 ± 10	-39* ± 8.2
Eriodictyol (0.1 mg/kg)		92 ± 8	- 7 ± 8	86 ± 13	-22 ±11	144 ± 4	-13* ± 2.4	145 ± 4	+19* ± 3.0
	Adenosine (0.1 mg/kg)	88 ± 8	- 4 ± 8.7	73 ± 6	-15 ± 6.9	139 ± 5	- 3** ± 3.5	89 ± 10	-39** ± 6.9
	Adenosine (1.0 mg/kg)	84 ± 7	- 9 ± 7.6	63 ± 6	-27 ± 6.9	135 ± 4	- 6** ± 2.8	82 ± 11	-44** ± 7.6
	Adenosine (10 mg/kg)	82 ± 5	-11 ± 5.4	48 ± 15	-44 ±17.9	130 ± 7	-10** ± 4.8	66 ± 12	-55 ± 8.3
Eriodictyol (1.0 mg/kg)		87 ± 5	-12 ± 5	78 ± 17	-29 ±15.6	138 ± 3	-17** ± 1.9	87 ± 10	-29* ± 8.2
	Adenosine (0.1 mg/kg)	84 ± 4	- 3 ± 4.6	66 ± 9	-15 ±11.5	128 ± 4	- 7 ± 2.9	80 ± 9	- 8 ±10.3
	Adenosine (1.0 mg/kg)	81 ± 2	- 7 ± 2.3	72 ± 17	- 8 ±21.7	120 ± 10	-13 ± 7.2	60 ± 14	-31 ±16.0
	Adenosine (10 mg/kg)	78 ± 5	-10 ± 5.7	61 ± 21	-22 ±26	112 ± 9	-19 ± 6.5	53 ± 5	-39 ± 5.7
Eriodictyol (10 mg/kg)		86 ± 6	-13 ± 6.2	78 ± 15	-20 ±14	132 ± 7	-20* ±4.2	87 ± 7	-29* ± 5.7
	Adenosine (0.1 mg/kg)	80 ± 6	- 7 ± 6.9	76 ± 16	- 3 ±20.5	122 ± 8	- 8 ± 6.3	72 ± 12	-17 ±13.7
	Adenosine (1.0 mg/kg	80 ± 4	- 7 ± 4.7	71 ± 18	- 9 ±23	115 ± 8	-13 ± 6.3	68 ± 13	-22 ±14.9
	Adenosine (10 mg/kg)	77 ± 6	-10 ± 6.7	47 ± 19	-40 ±24	103 ± 6	-22 ± 4.7	54 ± 9	-18 ±10.3

* $p < 0.05$ compared to control values.

** $p < 0.05$ compared to control responses to adenosine.

Table 5. Influence of eriodictyol on responses to angiotensin in the heart with perfused coronary artery.

Eriodictyol (Intracoronary Dose)	Angiotensin (Intracoronary Dose)	Mean ± SE for three anesthetized dogs							
		Cor. Perfusion		Ventricular Force		Heart Rate		Aortic BP	
		mm Hg	%Δ	g	%Δ	/min	%Δ	mm Hg	%Δ
Control		99 ± 4		110 ± 5		116 ± 13		122 ± 3	
	Angiotensin (0.1 μg/kg)	104 ± 4	+ 5 ± 3.9	100 ± 17	- 9 ±15.5	163 ± 12	+ 2 ± 7.2	127 ± 6	+ 4 ± 4.9
	Angiotensin (1.0 μg/kg)	10° ± 5	+10 ± 5	80 ± 30	-27 ±27	162 ± 9	- 2 ± 5.4	144 ± 15	+18 +12.3
	Angiotensin (10 μg/kg)	128 ± 12	+29 ±12	102 ± 16	- 7 ±15	152 ± 12	- 8 ± 7.3	142 ± 4	+16* ± 3.2
Eriodictyol (0.1 mg/kg)		92 ± 8	- 7 ± 8	86 ± 13	-22 ±11	144 ± 4	-13* ± 2.4	145 ± 4	+19* ± 3.0
	Angiotensin (0.1 μg/kg)	92 ± 8	0 ± 8.6	91 ± 5	+ 6 ± 5.8	145 ± 3	+ 1 ± 2.1	124 ± 13	-14 ± 8.9
	Angiotensin (1.0 μg/kg)	87 ± 6	- 5 ± 6.5	73 ± 22	-15 ±26	137 ± 9	- 5 ± 6.3	122 ± 11	-16 ± 7.7
	Angiotensin (10 μg/kg)	97 ± 3	+ 5 ± 3.3	74 ± 24	-14 ±28	141 ± 7	- 2 ± 4.8	120 ± 13	-17 ± 8.0
Eriodictyol (1.0 mg/kg)		87 ± 5	-12 ± 5	78 ± 17	-29 ±15.6	138 ± 3	-17* ± 1.9	87 ± 10	-29* ± 8.2
	Angiotensin (0.1 μg/kg)	91 ± 6	+ 4 ± 6.9	77 ± 19	- 1 ±24.3	138 ± 3	0 ± 2.2	102 ± 10	+17 ±11.4
	Angiotensin (1.0 μg/kg)	99 ± 7	+14 ± 8	74 ± 22	- 5 ±28	138 ± 0.5	0 ± 0.4	117 ± 15	+34 ±17
	Angiotensin (10 μg/kg)	87 ± 4	0 ± 4.5	97 ± 14	+24 ±18	128 ± 4	- 7 ± 2.9	130 ± 20	+49 ±22.9
Eriodictyol (10 mg/kg)		86 ± 6	-13 ± 6.2	78 ± 15	-20 ±14	132 ± 7	-20** ± 4.2	87 ± 7	-29** ± 5.7
	Angiotensin (0.1 μg/kg)	86 ± 6	0 ± 7	81 ± 12	+ 4 ±15.0	132 ± 4	0 ± 3.1	103 ± 14	+18 ±16.1
	Angiotensin (1.0 μg/kg)	91 ± 6	+ 6 ± 6.8	86 ± 17	+10 ±22	133 ± 2	+ 1 ± 1.6	110 ± 18	+26 ±20.6
	Angiotensin (10 μg/kg)	94 ± 9	+ 9 ±10.5	82 ± 22	+ 5 ±28	127 ± 2	- 4 ± 1.5	116 ± 8	+35** ± 9.1

* $p < 0.05$ compared to control values.

** $p < 0.05$ compared to control responses to angiotensin.

Table 6. Influence of eriodictyol on responses to adenosine and angiotensin in the heart with intact coronary artery.

Eriodictyol (Intravenous Dose)	Angiotensin or Adenosine (Intravenous Dose)	Mean ± SE for three anesthetized dogs															
		Coronary Sinus Flow			Coronary VR			Ventricular Force			Heart Rate			Aortic BP		
		Immediate ml/min	%Δ	Delayed ml/min	Immediate mm/ml	%Δ	Delayed mm/ml	Immediate g	%Δ	Delayed g	Immediate /min	%Δ	Delayed /min	Immediate mm Hg	%Δ	Delayed mm Hg
Control		76 ± 22			1.45 ±0.34			118 ± 13			114 ± 0.3			97 ± 12		
	Angiotensin (1 μg/kg)	84 ± 37	+ 2 ±15.6	108 ± 48	1.62 ± 0.40	+11* ± 2	1.67 ± 0.40	94 ± 16	-18 ±18	159 ± 20	120 ± 6	+ 3 ± 4	123 ± 8	108 ± 17	+13 ±14	144 ± 20
	Adenosine (1 mg/kg)	109 ± 37	+38* ±10	111 ± 35	0.47 ±0.09	-38* ± 5.2	0.63 ± 0.15	73 ± 10	-38* ± 5.2	130 ± 10	68 ± 7	-42* ± 5.6	104 ± 2	45 ± 8	-50* ±13.5	60 ± 3
	Angiotensin (1 μg/kg)	60 ± 8	-13 ±14.7	86 ± 27	1.81 ±0.20	+34 ±18	1.50 ± 0.30	97 ± 18	-19 ± 15.1	148 ± 9	110 ± 2	- 6 ± 3.1	120 ± 3	106 ± 3	+14 ±18	113 ± 8
Eriodictyol (0.1 mg/kg)		72 ± 4	-15 ±21.2	... ...	0.93 ±0.01	-26 ±19.7	... ...	119 ± 2	-13 ± 7.2	... ...	114 ± 0.3	- 0.3 ± 0.8	... ...	67 ± 3	-30* ± 5.5	... ...
	Adenosine (1 mg/kg)	113 ± 36	+64 ±37	110 ± 37	0.42 ±0.04	-55** ±10	0.66 ± 0.17	72 ± 12	-40 ± 9	127 ± 15	65 ± 7	-43 ± 6	105 ± 2	46 ± 7	-29 ± 13	67 ± 4
	Angiotensin (1 μg/kg)	76 ± 32	+33 ±40.3	116 ± 52	1.56 ±0.43	+61 ±79	1.79 ± 0.54	92 ± 17	-33 ± 3.6	137 ± 32	113 ± 4	- 1.2 ± 3.2	121 ± 3	108 ± 1	+62** ± 9.0	149 ± 5
Eriodictyol (1 mg/kg)		88 ± 15	+29 ±27.8	... ...	0.86 ±0.15	-38 ± 6.9	... ...	121 ± 2	+ 5 ±14.4	... ...	115 ± 0.6	+ 0.26 ± 0.26	... ...	72 ± 2	-23 ±11.6	... ...
	Adenosine (1 mg/kg)	154 ± 26	+86 ±52	95 ± 18	0.30 ±0.07	-20 ±13.7	0.51 ± 0.22	100 ± 5	-17 ± 4.3	135 ± 6	82 ± 20	-29 ±17.7	108 ± 0	42 ± 1.5	-42 ± 1.5	69 ± 3
	Angiotensin (1 μg/kg)	105 ± 28	+38** ± 3.0	134 ± 26	1.33 ±0.32	+55 ±34	1.06 ± 0.31	111 ± 11	- 5 ±10.9	193 ± 20	117 ± 2	+ 1.1** ± 2.1	116 ± 3	120 ± 0	+60** ± 1.2	127 ± 7
Eriodictyol (10 mg/kg)		89 ± 16	+48 ±50	... ...	0.74 ±0.19	-34 ±34.4	... ...	122 ± 1	+ 6 ±13.8	... ...	113 ± 0.5	- 1.2 ± 0.29	... ...	60 ± 2	-24 ±20	... ...
	Adenosine (1 mg/kg)	177 ± 29	+113 ± 77	43 ± 16	0.25 ±0.16	-59 ±18	0.67 ±0.16	110 ± 9	- 9** ± 6.5	151 ± 17	90 ± 18	-19** ±15.2	112 ± 4	40 ± 5	-42 ± 2.6	57 ± 2
	Angiotensin (1 μg/kg)	161 ± 45	+106** ± 82	161 ± 45	0.66 ± 0.20	+ 2 ±45.8	0.61 ±0.18	124 ± 0	+ 1 ± 0.7	180 ± 30	113 ± 2	+10 ± 5.8	113 ± 2	84 ± 4	-13 ±26	82 ± 4

* $p < 0.05$ compared to control.

** $p < 0.05$ compared to control responses.

Figure 3. Influence of eriodictyol on increase in coronary blood flow in response to adenosine in the dog. Note potentiation of response to adenosine.

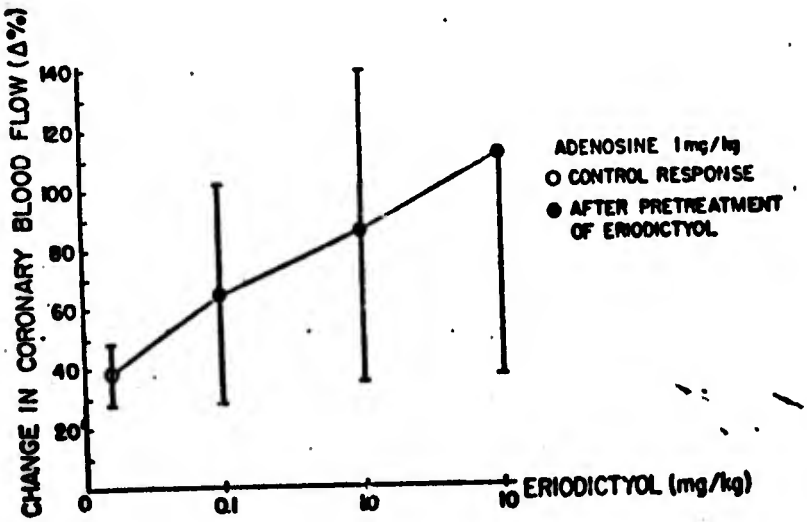
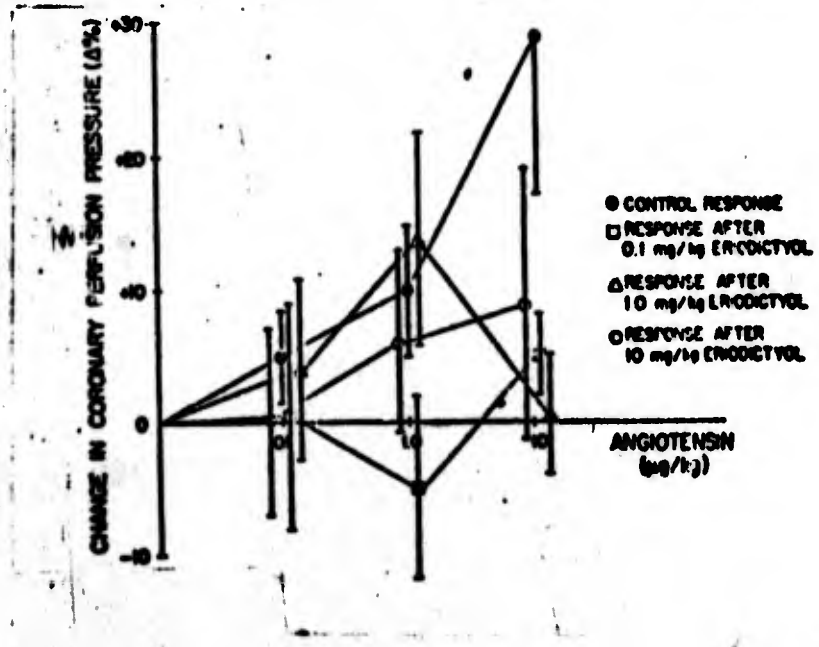


Figure 4. Influence of eriodictyol on coronary vasoconstrictor response to angiotensin in the dog. Note reduction in response following eriodictyol.



Part III. Search for Intravenous Preparation for Treatment of Acute Pulmonary Insufficiency.

The 15 compounds reported in Progress Report No. 1 were water-insoluble and could not be injected intravenously. An examination of the literature indicated that there are several water-soluble compounds which reduce the capillary permeability of the skin. Two compounds reported in 1963 by Tommasini, Parenti, Longoni and Berti (7) were obtained and are as follows: benzoylcarbinolmorpholine acetate and benzoylcarbinoltrimethyl acetate. Both are water soluble and are suitable for intravenous injection.

The initial testing for prevention of pulmonary edema was performed in male Swiss mice inhaling 25% carbon dioxide (6). In addition to the control and the carbon dioxide groups of mice, several mice were pretreated with one of the following: benzoylcarbinolmorpholine acetate hydrochloride, benzoylcarbinoltrimethyl acetate, eriodictyol and WR 49, 808. Although the last two compounds have been previously tested, they were used again for comparison of effective dosages.

The results indicate that both benzoylcarbinols are effective in preventing pulmonary edema induced by carbon dioxide inhalation. An intraperitoneal dose of 2. 5 mg/kg of either benzoylcarbinol afforded a protection similar to 25 mg of eriodictyol or WR 49, 808. The examination of the lungs of mice pretreated with benzoylcarbinoltrimethyl acetate revealed petechial hemorrhages which were absent in mice pretreated with benzoylcarbinolmorpholine acetate hydrochloride. The former has been excluded from further consideration and future work will be on the latter.

(Table 7 appears in the following page).

Table 7. Summary of antiedemagenic effect of benzoylcarbinols, eriodictyol and naphthoquinone (WR 49,808): Mean ± SE

Procedure	Dose mg/kg	No. of Mice	Body Weight g	Lung Weight % B W	P versus Control	P versus CO_2	Lung moisture %	P versus Control	P versus CO_2
Control		5	30.8 ±1.16	0.62 ±0.02			79.4 ±0.68		
Carbon dioxide	25%	5	20.8 ±1.28	0.92 ±0.04	< 0.001		83.2 ±0.49	< 0.001	
Eriodictyol	25	5	20.2 ±1.28	0.92 ±0.073		NS	79.4 ±0.40		< 0.001
Eriodictyol	50	5	21.2 ±2.06	0.92 ±0.073		NS	79.4 ±0.81		< 0.001
Eriodictyol	100	5	22.4 ±0.51	0.84 ±0.024		NS	78.6 ±0.927		< 0.001
Carbon dioxide	25%	5	23.8 ±3.70	0.94 ±0.040	< 0.001		84.4 ±1.86	< 0.05	
WR 49,808	25	5	20.6 ±0.75	0.90 ±0.063		NS	78.2 ±0.37		< 0.001
WR 49,808	50	5	23.6	0.84		NS	77.2		< 0.001
WR 49,808	100	5	26.0 ±0.89	0.74 ±0.060		< 0.01	79.6 ±1.07		< 0.05
Carbon Dioxide	25%	5	19.2 ±1.02	1.0 ±0.07	< 0.001		84.2 ±1.11	< 0.001	
Benzoylcarbinol-trimethyl acetate	2.5	5	22.2 ±0.80	0.90 ±0.084		NS	78.0 ±0.45		< 0.001
	5.0	5	20.0 ±2.66	1.0 ±0.09		NS	80.6 ±1.08		< 0.05
	10	5	23.2 ±1.06	1.0 ±0.10		NS	79.0 ±0.55		< 0.001
	25	5	19.4 ±1.63	0.88 ±0.019		NS	79.0 ±0.95		< 0.001
Carbon dioxide	25%	5	21.4 ±2.16	0.94 ±0.116	< 0.01		83.8 ±1.77	< 0.05	
Benzoylcarbinol-morpholine acetate	2.5	5	21.6 ±0.81	0.94 ±0.024		NS	81.2 ±1.15		NS
	5.0	5	21.6 ±0.81	0.86 ±0.039		NS	79.0 ±0.71		< 0.01
	10	5	29.2 ±0.37	0.64 ±0.024		< 0.01	79.6 ±0.63		< 0.05

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