Humoral Agent from Calf Lung

Producing Pulmonary Arterial Vasoconstriction

Abstract. *Saline washings obtained in vivo from the lung of young calves produce pulmonary hypertension upon intravascular (systemic or pulmonary) injection into either the dog or the calf. This pulmonary hypertension is produced by vasoconstriction of small, precapillary pulmonary vessels. The active agent, pulmonary arterial constrictor substance, differs chemically and physiologically from other substances which have been investigated with respect to vasomotor activity in the pulmonary circulation. Although the chemical nature of the active agent is not known it appears to have a relatively large molecular weight. Whether this agent plays a role in the physiological regulation of the pulmonary circulation is not known.*

The mechanisms involved in the regulation of the pulmonary vascular bed are obscure. In recent years, the development of pulmonary vasoconstriction in various normal and abnormal states has been documented (*1*, *2*). However, the precise genesis of pulmonary vasoconstriction is not known.

This report describes the demonstration of a substance in the lungs of young calves that produces pulmonary arterial vasoconstriction upon intravascular injection into either the calf or the dog. Although the role of this substance (if any) in the physiologic regulation of the pulmonary circulation remains to be established, its demonstration raises the possibility of humoral regulation of pulmonary hemodynamics.

Pulmonary arterial vasoconstrictor substance (PACS) was obtained from the lungs of 2- to 3-week-old calves (*3*). Calves were lightly anesthetized with thiopental and then given continuous intravenous infusion of succinyl choline. Ventilation was maintained by a Harvard pump. A Carlens catheter was inserted through a low tracheostomy and hooked on the carina. The right and left lungs were separated by inflation of the catheter balloons. In the calf the right upper lobe bronchus arises proximal to the carina. Therefore the proximal balloon was placed on the tracheal portion of the catheter and the left bronchus balloon was maintained in its usual position. Ventilation of the right lung was maintained with room air. Approximately 1 liter of physiological saline solution was equilibrated with approximately 100 percent N_2 for 30 to 60 minutes. This solution was then instilled into the left lung by a slow drip. After instillation the fluid was collected by gravity drainage. Usually 5 to 10 cycles of wash and collection were performed over a 60- to 120-minute period. During this procedure arterial oxygen tensions decreased and pulmonary artery mean pressures (PAP) increased over control values. Approximately 500 ml of saline solution were recovered after the successive washings. The final solution was foamy (presumably reflecting the presence of surface-active material) and opalescent.

A total of 18 calves were used for the preparation of separate batches of lung washings. Sixteen of the 18 washings showed pulmonary pressor activity. The other two batches were obtained from animals used in other experiments, which may explain the lack of pressor activity.

The effects of the saline washings on pulmonary and systemic hemodynamics were studied in 17 dogs and 5 calves, as follows. The animal to be studied was anesthetized as before. Catheters were placed in the pulmonary artery, the right atrium, and the femoral artery. The pulmonary artery catheter was advanced into the wedge position for measurement of "pulmonary capillary" pressure (*4*). Vascular pressures were measured by Statham strain gauges and recorded on an appropriate recorder. Cardiac outputs

Table 1. Summary of effects of PACS administration on hemodynamics. PAP is mean pulmonary artery pressure; FAP, mean femoral artery pressure; "PCP," "pulmonary capillary pressure"; PVR, total pulmonary vascular resistance (PAP ÷ cardiac output) P_aO_2 arterial oxygen tension. he "dose" was PACS, in the case of experimental animals (E), and saline solution, in the case of control animals (C).

Animal No.	Dose (ml)	PAP (mm-Hg)		FAP (mm-Hg)		Cardiac output (lit./min)		Heart rate (beats/min)		"PCP" (mm-Hg)		PVR units		P_aO_2 (mm-Hg)	
		C	E	C	E	C	E	C	E	C	E	C	E	C	E
							Dogs								
1	100	30	46	158	166	3.02	2.73	180	150			69	129		
2	50	20	31	143	140			180	180						
3	100	27	42	160	172			180	180						
4	30	31	37	125	135	3.90	2.40	198	186			60	117		
5	50	16	32												
6	50	15	23												
7	50	16	23	140	147	2.3	3.1				8	58	59		
8	50	19	28	173	175	2.8	2.4					66	92		
9	100	19	36	141	136	7.97	1.47	165	168	10		25	190	103	99
10	50	17	23	168	170	2.41	2.46	210	210	7	7	60	75	77	95
11	50	20	33	160	155	2.98	2.71	180	180		10	56	97		
12	50	27	36	152	130	4.87	1.51	180	135	9	9	57	175	99	98
13	50	20	23	163	149	3.08	1.12	180	180	9	9	57	157	99	107
14	50	16	35												
15	50	23	42	142	132	2.37	1.42	156	150	9	10	78	158	99	109
16	50	16	23	148		2.50	0.71	150	150	10	11	70	255	103	108
17	50	21	23	170		4.32	1.61	210		8		35	99	88	
							Calves								
1	300	35	49	132	135						15				
2	150	31	53	172	180	10.8	9.8			10	14	22	32	47	44
3	200	37	63	113	101	7.5	5.1			19	17	48	99	68	45
4	200	32	49	152	171	4.6	7.9	195	195	16	16	40	49	52	63
5	300	23	30	163	168	7.4	9.2	204	210	9	7	26	26	16	69

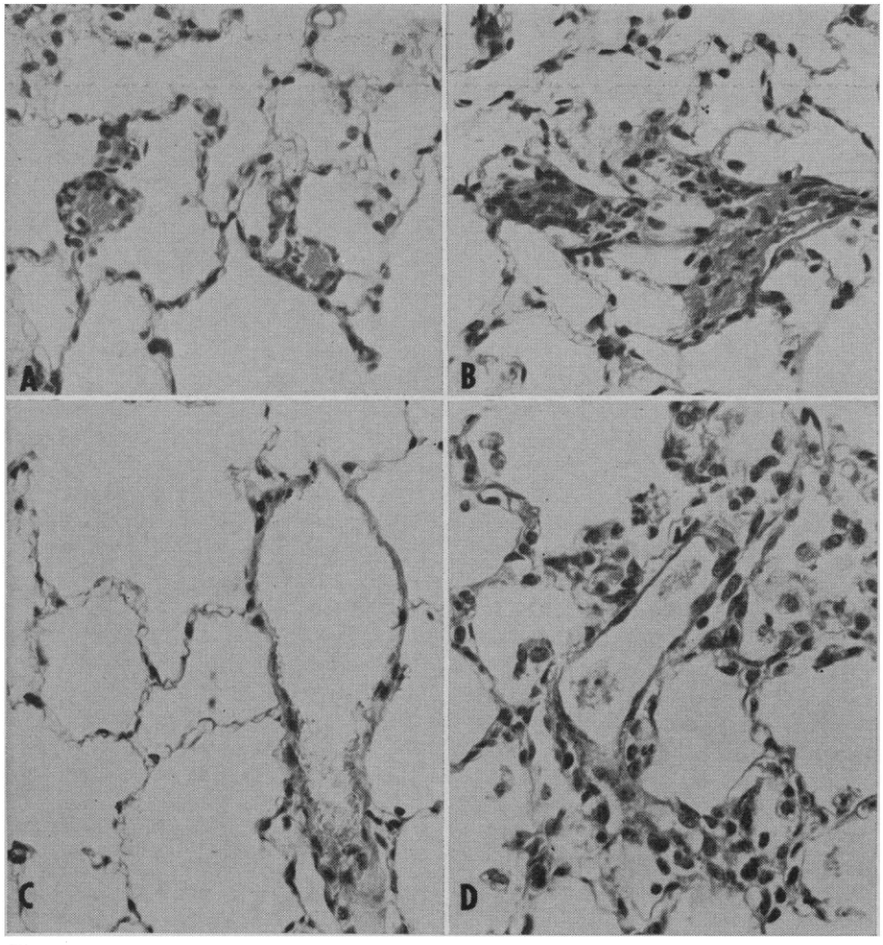

Fig. 1. (A) Fibrin clots in pulmonary capillaries. Nonheparinized dog. (B) Fibrin clots with enmeshed neutrophils in arterioles. Nonheparinized dog. (C) Vessels free of clot. Heparinized dog. (D) Vessels free of clot. Heparinized calf. (× 300)

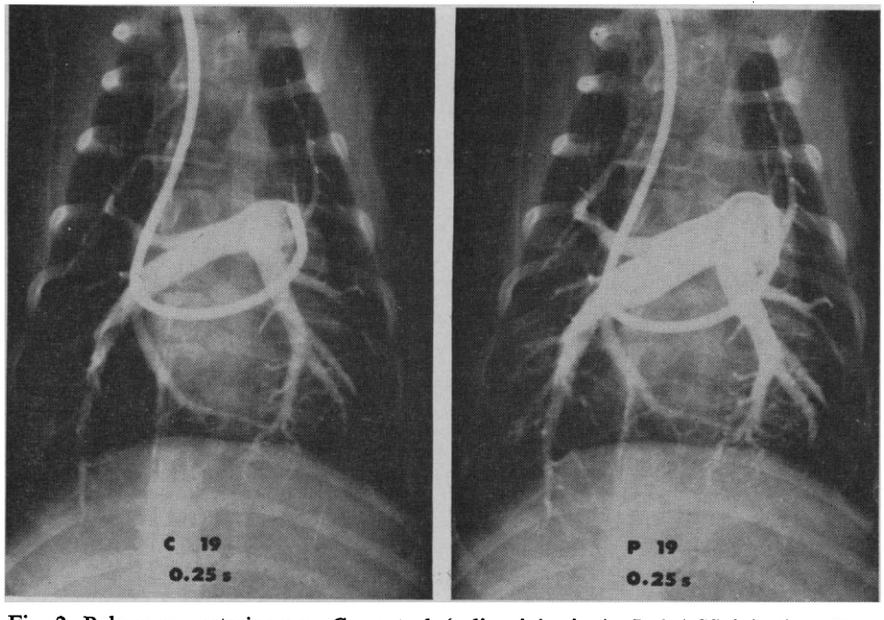

Fig. 2. Pulmonary arteriogram. *C*, control (saline injection); *P*, PACS injection. Each film obtained 0.25 second after injection of contrast material. Note marked increase in diameter of main stem, main stem branches, and lobar and lobular subdivisions of pulmonary artery.

were measured by means of the dye-dilution technique with an automatic sampling densitometer and Cardiogreen as the indicator material. The saline washing was injected into the right atrium. Injection of dye to measure cardiac output was also made into the right atrium. Arterial pO_2 was measured polarographically. Not every measurement was made in every animal. In all studies control observations were made after injections of physiological saline solution, the volume injected being the same as the volume of PACS used in the experimental period.

In three dogs pulmonary angiograms were obtained after the injection of saline and after the injection of PACS (*5*). In three dogs and one calf the effects of PACS on hemodynamics after femoral arterial injection were compared with the effects of right atrial injection. In an additional nine animals (seven dogs and two calves) the effects of femoral arterial injection of PACS on PAP were compared with the effects of injection of 0.9 percent NaCl solution.

Although there were individual variations in the levels of pulmonary artery pressure, cardiac output, and so forth, the pattern of response was essentially the same. These data are summarized in Table 1. The PAP values listed in the table, following the injection of PACS, are the peak pressures obtained during the given experiment. The pulmonary capillary pressures, femoral arterial pressures, and heart rates were obtained simultaneously with the PAP. The cardiac output measurements were obtained 2 minutes after the injection of PACS, and the pulmonary vascular resistance was calculated on the basis of the cardiac output and PAP found at that time (*6*).

In the dogs the injection of a single dose of 50 ml of saline washing led to an increase of approximately 50 percent in mean PAP over control values. This increase in mean PAP occurred as a result of increases in both systolic and diastolic pressures. Peak effect resulted from 50-ml injections (approximately 8 percent of the total volume of washing from a calf), and further doses generally resulted in no further increase. Approximately 1 to 2 minutes after injections of PACS, PAP began to increase; peak effect was noted 3 to 4 minutes after injection and remained elevated for 15 to 60 minutes after injection. The increase in

PAP occurred without substantial change in heart rate, "pulmonary capillary" pressure, femoral arterial pressure, or arterial oxygen tension. There was some variation in cardiac output. Generally, cardiac outputs following PACS injection were somewhat less than cardiac outputs following saline injection. However, pulmonary hypertension also developed when the post-injection cardiac output did not change or become elevated. Regardless of the level of cardiac output, calculated pulmonary vascular resistance was elevated by the injection of PACS.

Results with the calf as a test animal were qualitatively similar. Each animal showed a rise in PAP following injection of PACS. This increase averaged approximately 53 percent of control values. The increase could be demonstrated 2 minutes after injection, peak effect was found 2 to 3 minutes after injection, and hypertension persisted for 30 to 60 minutes after a single injection. As little as 50 ml of PACS produced a rise in PAP. Maximum effect was obtained with injections of 200 to 300 ml of washings. Larger doses produced no further increase of PAP. There were no substantial changes in heart rate, "pulmonary capillary" pressure, femoral arterial pressure, or arterial oxygen tension. Three of the five calves showed an increase in cardiac output at the time this parameter was measured. These outputs were measured before peak pressor activity, which may account for the relatively small changes in calculated pulmonary vascular resistance in these animals.

It was necessary to rule out mechanical occlusion of pulmonary blood vessels by emboli or thrombotic material as the basis of the pulmonary hypertension. This was accomplished in two ways. Injection of PACS into a femoral artery produces pulmonary hypertension qualitatively and quantitatively indistinguishable from that produced by right atrial injection (Table 2). It appears unlikely that embolic material could pass through systemic capillaries and produce embolic occlusion of pulmonary blood vessels.

Gross and histologic studies of the lungs of dogs and calves were performed after the injection of PACS (*7*). Careful evaluation of the pulmonary vascular head showed no gross embolic occlusion. Frozen sections stained with Oil Red 0 revealed no fat emboli. Microscopic examination did reveal fibrin thrombi involving occasional pulmonary capillaries and pulmonary arterioles in some animals. This type of lesion has been found in pulmonary hypertension of diverse etiology (*8*).

Table 2. Effects of PACS on pulmonary artery mean pressure. Control animals (C) were injected with saline solution; experimental animals (E) were injected with PACS.

Animal	PAP (mm-Hg) after an injection into:			
	Right atrium		Femoral artery	
	C	E	C	E
Dog 1	20	22	16	23
Dog 2	18	23	19	27
Dog 3	18	26	20	33
Calf 1	37	63	47	65
Dog 4			19	27
Dog 5			18	22
Dog 6			21	24
Dog 7			26	36
Dog 8			19	27
Dog 9			16	23
Dog 10			17	24
Calf 2			32	38
Calf 3			32	43

In order to investigate the possible role of these thrombi in the mechanism of PACS-induced pulmonary hypertension, the following study was performed. PACS was administered to eight dogs. Four animals were pretreated with heparin (10 mg/kg) to render the blood incoagulable. The other four dogs were given only PACS. Pulmonary hypertension developed in all eight animals. None of the heparin-treated dogs showed fibrin thrombi, whereas three of the four animals without heparin did develop these lesions. Pretreatment with heparin in two calves likewise prevented the development of thrombotic lesions despite the development of pulmonary hypertension. Representative sections of the lung in heparin-treated and untreated animals are shown in Fig. 1. It seems clear that thrombotic lesions are not the cause of the pulmonary hypertension and that the mechanism of pulmonary hypertension following PACS injection is pulmonary vasoconstriction rather than mechanical occlusion of pulmonary blood vessels.

The site of vasoconstriction is of interest; PACS produces an increase in PAP with essentially no change in "pulmonary capillary" pressure, which indicates that the site of vasoconstriction is precapillary in location.

Pulmonary angiography indicates that it is small pulmonary vessels which undergo vasoconstriction. Figures 2 and 3 show typical angiograms. It can be seen that the injection of PACS produces a marked increase in the diameter of the main pulmonary artery,

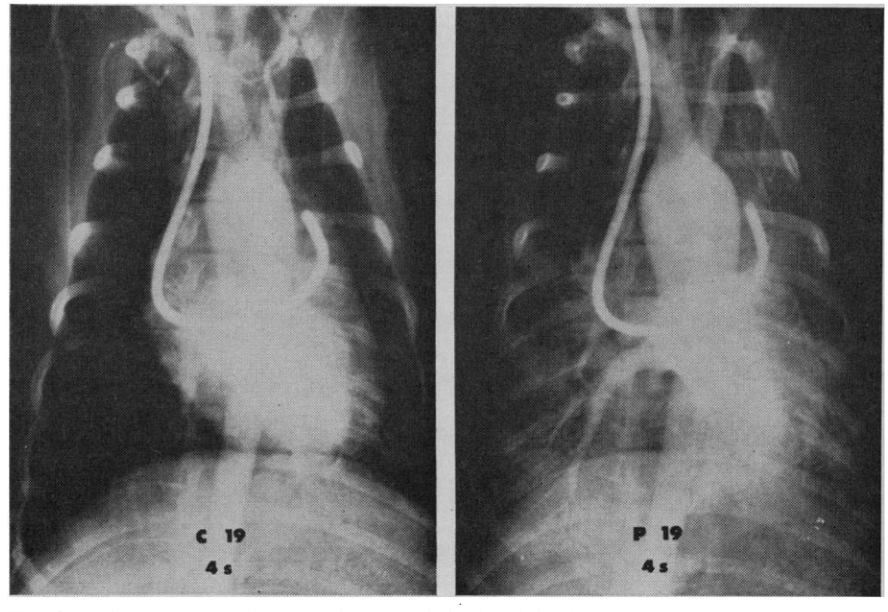

Fig. 3. Pulmonary arteriogram. *C*, control (saline injection); *P*, PACS injection. Each film obtained 4 seconds after injection of contrast material. Note changes in diameter of large branches of pulmonary arteries. Note also that pulmonary venous phase has been completed and opacification of left heart and aorta is taking place in the control film. In the PACS film, the pulmonary venous phase is still not completed.

main stem branches, and lobar and lobular subdivisions of the pulmonary artery, which indicates that the site of increased vascular resistance is distal to large pulmonary arteries. It thus seems clear that PACS produces vasoconstriction of small, precapillary (arteriolar) branches of the pulmonary artery.

The relationship of PACS to other substances capable of pulmonary pressor activity is of importance. Preliminary studies on the isolation of the pressor material indicate that full activity is retained after filtration through 5-μ Millipore filters and therefore the active principle of PACS must be smaller than 5 μ in diameter. Treatment of the washings in an ultrafiltration apparatus results in essentially all of the activity being retained on the inside of the filtration membrane. Apparently the diameter of active material is greater than 50 Å. From these studies it is clear that the substance involved is not any previously described species of relatively small molecular weight. Likewise, the mode of action of PACS differs from that of other agents previously investigated for pulmonary pressor activity (histamine, serotonin, bradykinin, adenosine triphosphate, epinephrine, and norepinephrine) in its relatively slow onset of action, its prolonged effect, and its ability to produce brisk pulmonary hypertension without profound effect on cardiac output, blood oxygen tensions, heart rate, or systolic blood pressure.

The ability of PACS to evoke pulmonary vasoconstriction in the calf as well as in the dog is likewise of importance. This observation indicates that the pressor activity does not result from an unusual allergic response produced by the injection of PACS. It likewise suggests that PACS may be of broad comparative physiologic importance, since it is active in species of two different orders of mammals.

It may be of interest to speculate on a possible relationship between hypoxia and the elaboration of PACS. It is generally accepted that hypoxia does produce pulmonary vasoconstriction (*1, 2*). Hypoxemic pulmonary vasoconstriction occurs in the isolated lung, and is evoked in the face of high oxygen tension, by agents such as CN^- and dinitrophenol which disrupt oxidative metabolism (*1, 9*). Both findings are consistent with a humoral mechanism for hypoxic pulmonary vasoconstriction. Although not universally accepted, the bulk of evidence favors a precapillary location as the likely site of hypoxic pulmonary vasoconstriction (*1*).

There are several lines of indirect evidence that the elaboration of PACS might be related to local pulmonary hypoxia. The demonstration of PACS in the young calf, an animal with a brisk vasoconstrictive response to hypoxia, is suggestive. The fact that the experimental conditions under which PACS is obtained were invariably associated with hypoxemia and pulmonary hypertension is likewise suggestive. The ability of PACS to produce specific precapillary pulmonary vasoconstriction resembles the pattern of pulmonary vasoconstriction produced by hypoxia. The saline solution used to obtain PACS was equilibrated with approximately 100 percent N_2. This was done as a possible approach to increasing the yield of PACS should it prove that the elaboration of PACS was directly related to hypoxemia. Since filling the lung with saline in vivo is associated with deficient O_2 exchange, it is not possible to state in absolute fashion that PACS was elaborated in response to hypoxemia. Nor was the question answered by studies in which the saline was equilibrated with 100 percent O_2 rather than 100 percent N_2. Even with the saline at a pO_2 of 760 mm-Hg, arterial hypoxia and pulmonary hypertension developed. The precise relationship between hypoxia and PACS may possibly be clarified by studies of the elaboration of PACS under hyperbaric conditions.

The precise role of PACS in the physiological regulation of the pulmonary circulation and its relationship to hypoxia will require chemical isolation of the substance involved.

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References and Notes

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3. The term "agent," as used in the title and abstract, is convenient and is used in a descriptive and not a chemical sense. Until the chemical species involved is isolated it is not possible to be certain that one is dealing with a single agent. For similar reasons, the term "PACS" is employed to describe what is essentially the physiological activity of a saline washing which undoubtedly contains many different chemical species. However, there is an acceptable scientific precedent for such terminology. The term "renin" was used prior to chemical characterization to describe a pressor substance obtained from kidney tissue.
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5. We are grateful to Dr. Klaus M. Bron, University of Pittsburgh Medical School, for performing the angiographic studies.
6. Pulmonary vascular resistance (PVR) is actually equal to [PAP (mean) minus left atrial pressure (mean)] ÷ cardiac output. In the present studies measurements of left atrial pressure were not obtained and pulmonary capillary ("PC") pressures were not measured in all animals. Therefore, PVR was approximated as the ratio of PAP (mean) to cardiac output. Since there was no significant change in "PC' pressure, this approximation appears to be acceptable.
7. We are grateful to Dr. Robert S. Totten, University of Pittsburgh Medical School, for performing the pathological studies.
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Geochemical Evidence of Present-Day Serpentinization

Abstract. *Ultrabasic (pH > 11) water issues from some fresh ultramafic bodies. The properties of the ultrabasic solutions are believed to be due to current reactions yielding serpentine from primary olivines and pyroxenes. The low concentrations of divalent iron, divalent magnesium, and dissolved silica from the serpentinization require an increase in rock volume.*

Structural relations and the absence of metamorphic aureoles indicate that many ultramafic bodies of the alpine type have reached their present positions through cold intrusion in tectonically mobile belts. The ubiquitous alteration of the original olivine and pyroxene to minerals of the serpentine group, however, is generally considered to have occurred at elevated temperatures, early in the history of an ultramafic body. We present evidence from studies of natural water that, in addition to the conventional interpretation, serpentinization may also be occurring locally at comparatively shallow depth and low temperatures at the present time. We do not know how much of the serpentine in the geologic record has been formed by the process we describe.

Waters of two chemically distinctive types are found in springs issuing from ultramafic rocks in California and Oregon. Most abundant is a moderately alkaline (*p*H range 8.3 to 8.6) magnesium bicarbonate water of meteoric origin. Less abundant, but of great potential significance, is a previously