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Embryo Transplanted Calves: The Pulmonary Hypertensive Trait Is Genetically Transmitted (40837)

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Cattle which develop pulmonary hypertension and right heart failure (brisket disease) at high altitude have offspring which develop excessive pulmonary hypertension with acute or chronic exposure to high altitude (1–3). Such cattle are "susceptible" to brisket disease. Cattle which are resistant to brisket disease at high altitude have offspring which develop relatively little pulmonary hypertension with acute or chronic exposure to high altitude. Such cattle are "resistant" to brisket disease. Although the ability to breed cattle to be either "susceptible" or "resistant" suggested that the trait was inherited, it could be modified or even acquired *in utero*. During pregnancy at 1500-m altitude "susceptible" cows have more pulmonary hypertension, lower values of cardiac output, and tendency toward decreased mixed venous oxygenation compared to "resistant" cows (4). A different intrauterine environment in susceptible cows could induce pulmonary vascular hypertrophy in the fetus that persisted after birth.

To test the hypothesis that lung vessels' hyperreactivity is genetically determined, we planned to transplant embryos from susceptible parents into cows likely not to have pulmonary arterial hyperreactivity; i.e., resistant cows. If the calves retained the pulmonary vascular hyperreactivity, the hypothesis would be supported. Further, the use of embryo transplantation could be of value in studying variability in calves with identical parents.

Materials and methods. With the technique of superovulation and embryo transplantation (5) six ova obtained from one Hereford cow known to be "susceptible" to excessive hypertension at high altitude were fertilized with semen from one bull known also to be susceptible to high-altitude pulmonary hypertension. The embryos were transferred to the uterus of ordinary recipient cows. Four viable calves were obtained. They were delivered on October 12, 14, 15, and 16, 1978, respectively. The calves were exposed to hypobaric hypoxia (P_B = 430 torr) for 2 hr at 163 days of age. Three of the recipient mothers were also exposed to the same environment. One of the recipient mothers could not be studied because she escaped en route to the laboratory. In fact the recipient mothers being range cows and rather wild, received 30 mg of xylazine (2(2,6-dimethylphenylamine)-4 *H*-5,6-dihydro-1,3-thiazine, Rompun[2]) to tranquilize them for movement to the laboratory and for catheterization. In these cows measurements were deferred for 2 hr after catheterization had been completed to allow dissipation of the drug effect. Both calves and cows underwent right heart catheterization in a hypobaric chamber with its door open to the ambient altitude of 1524 m (P_B = 630 torr). The animals were held in a standing position and restrained in standard cattle chutes. Pulmonary arterial and aortic catheters were introduced percutaneously as previously described (1). Pressures were measured using appropriate strain gauge transducers (Statham). Arterial and mixed venous blood were drawn for pH, P_{O_2}, and P_{CO_2} measurements using the corresponding electrodes and potentiometer from Radiometer. Differences between measurements were

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[2] Haver–Lockhart, Cutter Laboratories, Shawnee, Kan. Rompun is reputed to have brief (less than 1 hr) tranquilizing effect.

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TABLE I. INDIVIDUAL MEASUREMENTS OF ARTERIAL BLOOD GASES, CALCULATED ARTERIAL-MIXED VENOUS OXYGEN CONTENT DIFFERENCES ($C_aO_2 - \bar{C}_vO_2$), AND MEAN PULMONARY ARTERIAL PRESSURE ($\overline{PAP}$) IN THREE COWS AND FOUR CALVES (EACH CALF IS CITED ON THE SAME LINE WITH ITS FOSTER MOTHER)

	P_aO_2 torr		P_aCO_2 torr		pH_a units		$C_aO_2 - \bar{C}_vO_2$ ml%		$\overline{PAP}$ torr	
	Cow	Calf	Cow	Calf	Cow	Calf	Cow	Calf	Cow	Calf
					Measurements at 1524 m. P_B = 630 torr					
	83	77	30	43	7.49	7.38	4.3	4.0	41	42
	82	75	40	39	7.42	7.41	5.4	3.7	36	47
	—	76	—	36	—	7.41	—	4.8	—	41
	70	76	37	40	7.46	7.47	3.9	3.2	44	42
Mean	78	76	35	39	7.45	7.42	4.6	3.9	40	43
± SE	4.2	0.4	3.0	1.3	0.02	0.02	0.43	0.34	2.4	1.4
					Measurements at 4500 m. P_B = 430 torr					
	46	31	38	38	7.47	7.44	3.1	2.8	49	92
	40	29	34	36	7.47	7.43	4.1	2.2	42	82
	—	29	—	35	—	7.41	—	2.8	—	86
	40	30	38	37	7.46	7.42	3.1	2.5	59	71
Mean	42[a]	29[a,b]	36	36[a]	7.46	7.43	3.4[a]	2.6[a,b]	50[a]	83[a,b]
± SE	2.0	0.4	1.4	0.6	0.01	0.01	0.34	0.13	5.0	4.5

[a] Calves' and cows' values at high altitude differ ($P < 0.05$) from controls at low altitude.
[b] Calves' values differ ($P < 0.05$) from cows only at high altitude.

analyzed statistically using a paired *t* test for altitude responses and an unpaired *t* test for differences between calves and cows. A probability value of 0.05 or less was accepted as significant.

Results. Table I and Fig. 1. At the laboratory altitude of 1524 m the values of arterial blood gases and arterio-venous oxygen differences in both cows and calves were similar to values previously reported. Pulmonary arterial pressures in the cows were similar to those previously reported in postpartum cows (4). Pressures in the calves were similar to the values in the cows but higher than previously reported calves studied under similar conditions (6).

At the simulated high altitude of 4500 m the cows had decreased arterial oxygen tensions and pulmonary arterio-venous oxygen content differences. Pulmonary arterial pressures increased. The increase in pulmonary arterial pressure as related to the degree of hypoxia (as measured by P_aO_2) was similar to that previously observed during acute hypoxia in postpartum cows resistant to high-altitude pulmonary hypertension (4). At high altitude, the calves developed more severe hypoxemia and smaller arterio-venous oxygen differences than did the cows, but their pulmonary arterial pressures were higher. The degree of pulmonary hypertension as related to the degree of hypoxemia was more severe than has been previously observed in calves of similar ages susceptible to pul-

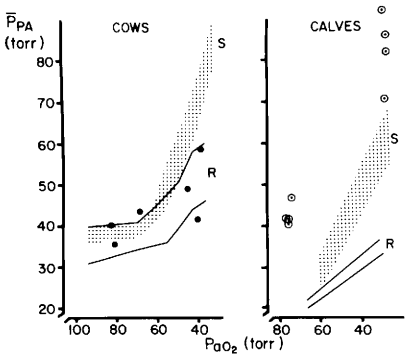

FIG. 1. Mean pulmonary arterial pressure ($\bar{P}_{pa}$) vs arterial oxygen tension (P_aO_2) for three cows (●) receiving the embryo transplants and four calves (⊙) resulting from embryo transplantation. The stippled area indicates the range of expected values for "susceptible" cows (4) and calves (1). The unbroken lines indicate the range of expected values of resistant cows (4) and calves (1). The cows had pressures within the range of "resistant" values and the calves had pressures even higher than expected from previous "susceptible" values.

monary hypertension at high altitude (6). The increase in pulmonary arterial pressure at high altitude in the calves (40 ± 5 torr) was much greater than that (10 ± 3 torr) in the cows.

Discussion. The purpose of the present study was to show whether the intrauterine environment or genetic factors determine the pulmonary vascular reactivity of cattle. The experimental design required, first, that embryos be obtained from cattle susceptible to pulmonary hypertension at high altitude. The present study of calves resulting from such embryos showed that at age 5 months the calves had remarkable pulmonary hypertension with acute exposure to simulated high altitude. In fact, the pressures were higher at both high and low altitude than susceptible calves age 5 months reported previously (6). Cardiac output was not measured in the present study but arterio-venous oxygen differences were similar to those reported for young calves during normoxia and acute hypoxia (1). Further, the present calves had had prior ventilatory measurements made within the chamber in another experiment, were accustomed to being handled, and were quiet during the present study. Thus, excitement or high cardiac output did not seem likely explanations for their high pressures. The genetic parents of the present four calves were selected for their propensity to pulmonary hypertension and, thus, their offspring could represent the upper range of pulmonary arterial pressure levels even for susceptible cattle. Further, the change in altitude from 1524 to 4500 m resulted in a large increase in pulmonary arterial pressure seen heretofore only in susceptible cattle. Thus, it appeared that the four calves had the excessive pulmonary vascular reactivity which characterizes susceptible cattle.

The second requirement of the study was that the fetal host, i.e., the females receiving the embryo transplants, be cattle resistant to pulmonary hypertension at high altitude. In the postpartum period the three cows we were able to study did have, with acute exposure to high altitude, mean increments in pulmonary arterial pressure of only one-fourth that of the four calves. The pressures in the cows both at low and high altitude were similar to those of "resistant" postpartum cows under similar circumstances. They were range cows of mixed breed which proved to be sufficiently wild to require tranquilization prior to study. Because of the rather brief duration of action of the tranquilizer the intentional delay in starting our experiments to allow for dissipation of the drug and the normal values of blood gases and pulmonary arterial pressure during the control period, it seemed unlikely that the drug accounted for the results in the cows. Rather, in view of the small pulmonary pressor response at high altitude in these cows compared to those of their calves, the similarity of the pressor responses of these cows to those of postpartum cows known to be "resistant," and the high-altitude origin of the cows, we considered it likely that, as planned, the cows were resistant to high-altitude pulmonary hypertension. If so, then cows of the resistant type, when transplanted with embryos from susceptible parents, delivered susceptible calves. Thus, the results appear to support the hypothesis that genetic factors rather than maternal environment affect the pulmonary vascular reactivity in the offspring.

Because one susceptible bull donated the sperm and one susceptible cow the ova, the four offspring of this union were full siblings and were available for study when they were of identical age. We have not previously had the opportunity to study four siblings in a single experiment. While all four showed large pressor responses to acute high-altitude exposure, the increases in mean pulmonary arterial pressure ranged from 28 to 50 torr. Thus, even here there was remarkable variability within the group suggesting either that the genetic influences are complex or there are environmental influences which were not controlled.

An additional observation was the greater hypoxemia in the calves than in the cows at high altitude in spite of similar effective alveolar ventilation (as judged by the similar values of P_aCO_2). The findings suggest impaired gas exchange in the calves stemming from shunting, a ventilation–perfusion mismatch or poorer dif-

fusion, or some combination of these. Our recent experience in calves suggests that intrapulmonary shunting increases with pulmonary hypertension (7), a mechanism which may have contributed to the relatively greater hypoxemia in the calves compared to the cows.

Summary. The superovulation and embryo transplantation techniques were used to obtain four sibling calves from four different mother cows. The semen and ova were obtained from one bull and one cow, respectively, known to have susceptible pulmonary hypertension when exposed to low-pressure environments. The recipient mothers were healthy ordinary cows purchased at high terrestrial elevations, thus, supposed to be resistant to high-altitude-induced pulmonary hypertension. Both cows and calves, 5 month of age, were tested for pulmonary hypertension in a hypobaric chamber at 4500 m simulated altitude (P_B = 430 torr) for 2 hr. The increase in pulmonary arterial pressure at high altitude in the calves (40 ± 5 torr) was much greater than that in the cows (10 ± 3 torr), demonstrating that they were susceptible and resistant cattle, respectively, to high-altitude-induced pulmonary hypertension. Therefore, susceptible calves were obtained from resistant cows. This study supports the hypothesis that pulmonary vascular hyperreactivity to hypoxia is genetically transmitted.

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