lations between the portal venous and hepatic arterial blood flows did not differ from those of the dogs that had not received reserpine.

The portal venous and hepatic arterial systems are not related by the usual capillary venular connections. At least three forms of communication between these two systems have been described (*4*): (i) connections between portal and arterial sinusoids are noted in the periphery of the liver lobule; (ii) direct anastomoses exist between corresponding divisions of the portal vein and hepatic artery; and (iii) arterial branches frequently end in the terminal branches of the portal vein just before these branches empty into sinusoids. It has been estimated that at least 30 percent of the hepatic arterial blood is shunted into the portal venous system before the arterial blood reaches the sinusoids (*5*). Wakim observed that the portal blood flow is much faster distal to anastomatic communications with branches of the hepatic artery (*4*).

The hepatic artery–portal vein blood flow relations which we observed can be explained as simply the mechanical effect of the interposition of a slower-flowing stream in the path of a faster-flowing stream. The rate of flow in the slower system will be increased and the rate of flow in the faster system will be proportionately decreased. Decreasing the amount of slow flow is equivalent to removal of an impedance, and the rate of flow in the faster system should increase. Conversely, if the flow impetus of the faster system is decreased, the flow rate in the slower system should decrease. The relation between the systems is thus one of work mechanics, with a transfer of energy from the arterial to the venous system made possible by direct anastomoses of the two systems in the liver.

JESSIE L. TERNBERG
HARVEY R. BUTCHER, JR.
Department of Surgery, Washington University School of Medicine, St. Louis, Missouri

References and Notes

1. R. E. Condon, L. M. Nyhus, N. S. Chapman, H. N. Harkins, *Gastroenterology* **43**, 547 (1962); J. L. Bollman, M. Khattab, R. Thors, J. H. Grindlay, *Arch. Surg.* **66**, 562 (1953).
2. H. Schweigk, *Arch. Exp. Pathol. Pharmakol.* **168**, 693 (1932); S. Soskin *et al.*, *Am. J. Physiol.* **124**, 558 (1938).
3. Square-wave electromagnetic flow meter, model 202, from Carolina Medical Electronics, Inc.
4. K. G. Wakim, *Am. J. Med.* **16**, 256 (1954).
5. N. Rabinovici and J. Vardi, *Surg. Gynec. Obst.* **120**, 38 (1965).

2 August 1965

Physiological Predetermination: Imbibition, Respiration, and Growth of Lima Bean Seeds

Abstract. *Temperatures 15°C or lower during the first hour of imbibition immediately inhibited respiration in lima beans, with proportional inhibition of subsequent growth of seedlings. Correlations between initial respiration rates and growth rates of seedlings were found in different lots of seeds.*

Almost 50 years ago Kidd and West (*1*) reported that conditions during the early stages of germination could "pre-determine" subsequent growth of plants. More recent experiments (*2*) have shown that in lima beans (*Phaseolus lunatus*, L., var. Early Thorogreen) temperatures of 15°C or lower during the first hours of germination may severely inhibit later growth of seedlings. Susceptibility of lima beans to chilling injury is predetermined during seed development; seeds that bleach during maturation are more sensitive than normal seeds. In corn (*Zea mays*), measurements (*3*) of respiration rates 1 to 3 hours after the start of imbibition detected injury by heat or freezing and could be used to predict growth of seedlings. It is important both to find practical ways to predict plant behavior and to explain the mechanism of predetermination. We now report data showing that respiration is associated with chilling injury of lima beans, that the extent of the injury can be estimated from measurements of respiration, and that respiration rates are also related to subsequent growth of seedlings even in the absence of injurious treatments.

Chilling treatments that inhibited respiration also inhibited growth of seedlings (Table 1). Imbibitions at 15°C inhibited respiration and seedling growth, and the more severe treatment at 5°C inhibited respiration more markedly, eventually killing the seeds. It was shown (*2*) that bleached seeds are more susceptible to chilling injury than normal green seeds, and that susceptibility is limited to the first few hours of imbibition. After measurement of respiration, seeds were planted on moist paper towels and incubated in darkness at 25°C. Respiration rates 4 hours after the beginning of imbibition were correlated, at the 5-percent level of significance, with axis lengths of seedlings after 5 days of germination (Fig. 1). Comparison of initial respiration rates with fresh weights of seedlings of different lots of green and bleached seeds, at the 1-percent level of significance (Fig. 2, *A* and *B*, respectively), indicates a relation, between respiration rates at the start of germination and subsequent growth of seedlings, similar to that reported for corn (*3*). The correlation indicates that measurements of respiration can lead to a rapid estimate of the extent of injury caused by chilling treatment.

In one lot of seeds, respiration of bleached seeds was less than that of green seeds during the temperature-sensitive period, but not subsequently. These results, and observations that anaerobic conditions enhance the chilling injury (*2*), suggest that respiration rates during imbibition may be a factor in determining susceptibility to chilling injury. Relations between levels of respiratory energy, measured as concentrations of adenosinetriphos-

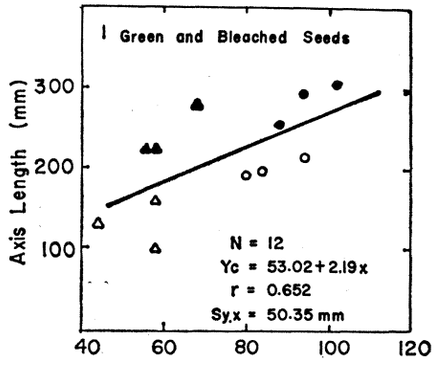

Fig. 1. Comparison of respiration of green and bleached seeds of lima beans 4 hours after initial imbibition with axis length 5 days later. ●, green seeds, imbibition at 25°C; ▲, green seeds, imbibition at 5°C; ○, bleached seeds, imbibition at 25°C; △, bleached seeds, imbibition at 5°C.

Table 1. Influence of imbibition temperatures on respiration and growth of seedlings in green and bleached seeds of lima bean.

Temp., first hour (°C)	Respiration, O_2, each (μl/hr)*		Growth (mm)	
	2-hr	6-hr	Roots	Shoots
		Green		
25†		95	169	115
15†		60	131	111
25‡	34	91	106	99
5‡	4	52	0	0
		Bleached		
25†		87	122	79
15†		54	71	59
25‡	25	80	60	48
5‡	5	46	0	0

* Respiration measured at 25°C. †, ‡ Seedling growth measured 5 (†) and 4 (‡) days after the start of imbibition.

19 NOVEMBER 1965

Table 2. Effects of 15°C chilling treatment on the respiration rates of cotyledons and embryonic axes excised from green seeds of lima bean. Treatment was administered during the first hour (cotyledons) or 30 minutes after initial imbibition (axes). Controls were maintained at 25°C. Cot, cotyledon.

Time after planting (hr)	Respiration, O_2 (μl/hr)			
	Per two Cot		Per axis	
	Control	Exp.	Control	Exp.
2	65	27	11	8
2½	70	45	12	9
3	70	50	12	9
3½	85	65	14	10
4	96	82	14	10
5	94	98	16	11
6	93	95	15	11
7	85	85	13	9

phate, and the physical characteristics of mitochondrial membranes have been reported (*4*). In lima beans the surface area of the seed increases about fourfold during imbibition; subsequent rates of increase in surface area are lower and limited to the elongating regions of the embryonic axis. Marked leaching of organic materials from the seed accompanies chilling injury (*2*), which fact indicates extensive membrane damage. The results support the assumption that relations involving respiratory energy may, like the relation with mitochondrial membranes (*4*), also apply with other kinds of cellular membranes.

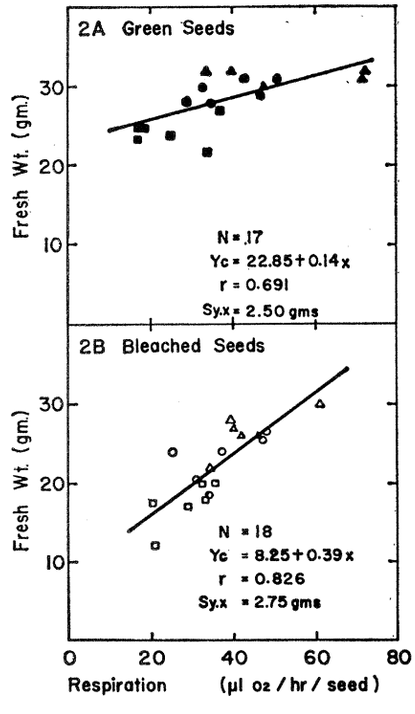

Fig. 2. (*A*) Comparison of respiration of green seeds of lima bean 4 hours after initial imbibition with fresh weights of seedlings 5 days later; ●, lot B; ▲, lot C; ■, lot D; (*B*) same comparison as in *A*, but with bleached seeds.

Chilling treatments inhibited respiration of excised cotyledons and embryonic axes (Table 2); respiration of the axes remained inhibited throughout the experiment, but that of the cotyledons equaled the values of controls within 5 hours of initial imbibition. Axes from bleached seeds respired less than those from green seeds, but there was no such difference in the respiration rates of cotyledons. Embryonic axes are among the first tissues to become hydrated during imbibition (*2*).

The data suggest the following explanation of chilling injury in lima beans: During low-temperature imbibition, the respiratory mechanism is too slow to supply energy sufficient for orderly stretching of the membranes. Extensive damage to membrane in critical sites, such as the embryonic axis, may lead to subsequent inhibition of growth of the seedling, attack by microorganisms, or even to death of the seedling.

While this report was in press, Shimon Klein and Yael Chen (*5*) wrote to us essentially as follows. Pollock and Toole recently showed that temperature during the early imbibitional stage is critical for normal growth and development of lima bean seeds and excised embryonic axes. Woodstock and Pollock [this report] have extended these experiments to include data on the influence of imbibition temperature on respiration rates; while using essentially similar methods to study similar experimental material for a different purpose, we have confirmed and extended their data.

Table 3 shows the rates of uptake of O_2 (per gram initial weight) by axes imbibed at 25°C and the rates of uptake by chilled axes as percentages of uptake of O_2 by the control. Early chilling for 30 minutes reduced uptake of O_2 by 20 to 40 percent during hours 1 to 3 of imbibition and by 30 to 70 percent during hours 6 to 9. An early morphological effect of the chilling is a diminished and abnormal elongation of the lima bean radicle (no divisions occur during the first 24 hours of imbibition), while elongation of the hypocotyl is not affected. No such localization of the effect of chilling occurs in connection with the respiratory apparatus. When isolated axes were thus chilled and uptake of O_2 was measured separately in the normally elongated hypocotyls, the same decrease in respiration rate was found as in the whole axes. Results were similar when the hypocotyls were isolated from the dry axes and were imbibed and chilled separately.

Table 3. Rates of uptake of O_2 at 26°C by isolated intact axes and by isolated hypocotyls (all chilled) of germinating lima bean seeds during the early hours of imbibition. Chilled tissues imbibed for the first 30 minutes at 5°C and thereafter at 25°C.

Hour of imbibition	O_2 uptake (μl min^{-1} g^{-1}*)	Uptake ratio chilled: unchilled (%)
	Intact axes	
0.5 – 1	14 – 20	
1 – 2	25 – 29	62 – 68
2 – 3	25 – 31	60 – 68
3 – 4	26 – 30	60 – 80
4 – 5		
5 – 6		
6 – 7	33 – 37	30 – 35
7 – 8	40 – 60	25 – 70
8 – 9	50	60
	Hypocotyls	
6 – 7	33 – 37	30 – 35
7 – 8	40 – 45	26 – 72
8 – 9	40	65

* Initial weight before imbibition.

Thus the respiratory apparatus, or parts thereof, in both radicle and hypocotyl is sensitive to chilling during the earliest phase in water uptake by the axes, and the respiration rate is reduced very early and permanently in both organs, although morphologically the effect of the cold treatment is manifest only in decreased elongation of the radicle. Imbibing the chilled and nonchilled axes in the presence of gibberellic acid in concentrations ranging between $3 \times 10^{-3}M$ and $3 \times 10^{-5}M$ had no effect on elongation or on uptake of O_2 by the isolated axes.

L. W. Woodstock
B. M. Pollock
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, Maryland

References

1. F. Kidd and C. West, *Ann. Appl. Biol.* **5**, 1 (1918); **6**, 1 (1919).
2. B. M. Pollock and V. K. Toole, *Plant Physiol.*, in press.
3. L. W. Woodstock and J. J. Feeley, *Proc. Assoc. Offic. Seed Analysts*, in press; L. W. Woodstock, *Bioscience*, in press.
4. J. M. Lyons, T. A. Wheaton, H. K. Pratt, *Plant Physiol.* **39**, 262 (1964); A. Gomez-Puyou, M. Tuena, C. Campillo-Serrano, J. Diaz-Maqueo, *Arch. Biochem. Biophys.* **109**, 221 (1965).
5. Department of Botany, Hebrew University, Jerusalem.
6. Work of Klein and Chen financed by USDA grant P.L. 480.

10 August 1965; 29 September 1965