tube elements in the major role of connector between regions of supply and those of utilization ("sinks"). Tissue autoradiography showing a concentration of activity in companion cells (*1*) has cast some doubt on the validity of this conclusion. Mechanical and thermal damage to the vascular system results in reduced phloem-limited translocation; application of metabolic inhibitors indicates the process to be metabolically dependent in the phloem itself. These methods, however, do not differentiate between enucleated sieve tube elements and the nucleated cells of the phloem.

In view of the report (*2*) that nucleoplasm is more radiosensitive than cytoplasm, we felt that application of x- or β-radiation (*3*) to the phloem tissue might differentially affect the enucleated sieve tube elements and the nucleated cells of the phloem. For this study, bean plants, *Phaseolus vulgaris* var. Black Valentine, were cultivated hydroponically in a controlled-environment room. Translocation was estimated by direct counting of dried stem sections with a Geiger-Müller tube and conventional scaler after application of phosphorus-32 or of a dried ethanol extract after application of carbon-14.

Phosphorus-32 was applied as phosphate HCl (about *p*H 3) as a drop to the upper side of the leaf; C^{14} was supplied as CO_2 to the under side of the leaf by a leaf cup (*4*). The supply leaf petioles of five groups of two bean plants each were x-irradiated in doses of from 1000 to 50,000 r. Four days after irradiation, 3 μc of P^{32} were applied to the treated leaf, and after a 6.5-hour migration period, the amount and distribution of the P^{32} was determined. All differences between these plants and two control plants could be accounted for by random variation, indicating that treatment at these dosages had no effect. One bean petiole was subjected to irradiation of 683,000 reb (roentgen-equivalent-beta) over a 5.75-hour period with no apparent reduction in translocation of P^{32}. Estimates indicated that phloem tissue absorbs approximately 2 percent of the x-ray energy and 5 percent of the β-ray energy.

The radioresistance of the translocatory pathway seems to be further circumstantial evidence pointing to the enucleate sieve tube as this pathway.

Skok (*5*) has suggested that translocation of carbohydrates into "sink" regions such as meristems is directly dependent upon the cellular activity of the region. Thus a metabolically active site would reduce the substrate concentration, establish a concentration gradient, and promote translocation into the region. Support was given to this suggestion when excision of the terminal buds of sunflower resulted in an approximate 50-percent reduction in upward translocation of C^{14} from the supply leaf without significantly affecting the amount moving downward. Subsequently, Crafts and Yamaguchi (*6*) reported that phloem translocation of herbicides was correlated with both sink activity (root growth) and source activity (amount of green in variegated leaves). If we suppose Skok's hypothesis to be valid, factors directly affecting the metabolism of the meristem would indirectly control translocation into the region.

Table 1. Effect of naphthaleneacetic acid (NAA) on translocation of C^{14} to apical regions. The results are averages for three plants.

Treatment	Count/min mg (dry weight)	
	Small leaf	Meristem
Control	350.1	272.6
9900 reb	62.0	72.7
9900 reb with NAA added to meristem	91.6	212.6

Exposure of meristems to relatively small doses of ionizing radiation results in temporary reduction in growth, presumably through a reduction in auxin and deoxyribonucleic acid concentrations (*7*). To determine whether the reported growth reduction was accompanied by concomitant reduction in translocation, the apical regions of eight bean plants were irradiated with a strontium-90 source at surface incident doses (two plants at each dose) of 0.1, 1, 10, and 100 kr, respectively. Phosphorus-32 was applied 48 hours after irradiation. After a 7-hour translocation period, the amount of activity in the terminal region of the plants treated with 10 and 100 kr was less than that in the control plants by 97 and 98 percent, respectively. Findings for the other treatment groups, were not significantly different from those for the control group, indicating a threshold within the 1- to 10-kr range.

Topical application of auxin can maintain normal growth after irradiation. No natural recovery has been reported after x-ray doses above about 2 kr (*7*). If the translocation to the apical meristem region is a function of growth, topical application of auxin to the irradiated bud should also maintain translocation.

Table 1 shows the results of an experiment on three groups of three plants each. The apical region for the two irradiated groups consisted of the meristem and a small trifoliate leaf. Immediately after irradiation and again 19 hours later, one of these groups received 10 μl of a 5-parts-per-million naphthaleneacetic acid solution applied to the meristem only. Carbon-14 dioxide was released to a fully expanded primary leaf approximately 21 hours after irradiation, and the experiment was terminated 3 hours later. Irradiation at this level significantly reduced carbon translocation to the irradiated portions; however, application of auxin did result in the translocation of significantly more activity to the meristem than to the irradiated leaf.

While there is no real way of differentiating causes and effects in this case, the known relationship between auxin and growth and the correlation between auxin and translocation demonstrated in this study clearly support the hypothesis that translocation is correlated with and probably dependent upon cellular or metabolic activity at the site of utilization (*8*).

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Transplantation and Malignancy in a Companion-Host System on the Chorioallantoic Membrane

Abstract. Pairs of tissue fragments in contact with one another were transplanted onto the chorioallantois. Ten days after transplantation, embryonic lung and liver were found to be accepted, without obvious infiltration of one another. When Walker tumor was substituted for either of the normal tissues, the pair of transplants was also accepted by the chorioallantois, and in addition there was extensive invasive replacement of the normal tissue by the cancer transplant.

In the search for the essence of cancer in a microsystem, reference is often made to bioassay methods for evidence that the elemental factor under study is related to some quality of

malignancy. Growth of a suspected tissue, transplanted in a conditioned host, has been used most extensively as an assay. The degree to which successful transplantation in a treated heterologous host can be equated with malignancy is uncertain, since many normal embryonic tissues and benign tumors will "take" in heterologous hosts while many highly malignant clinical cancers will not (*1*). Furthermore, the conditioned host has been considered an improper test object, even though the "take" exhibits all the microscopic features considered to be diagnostic of cancer by the pathologist (*2*).

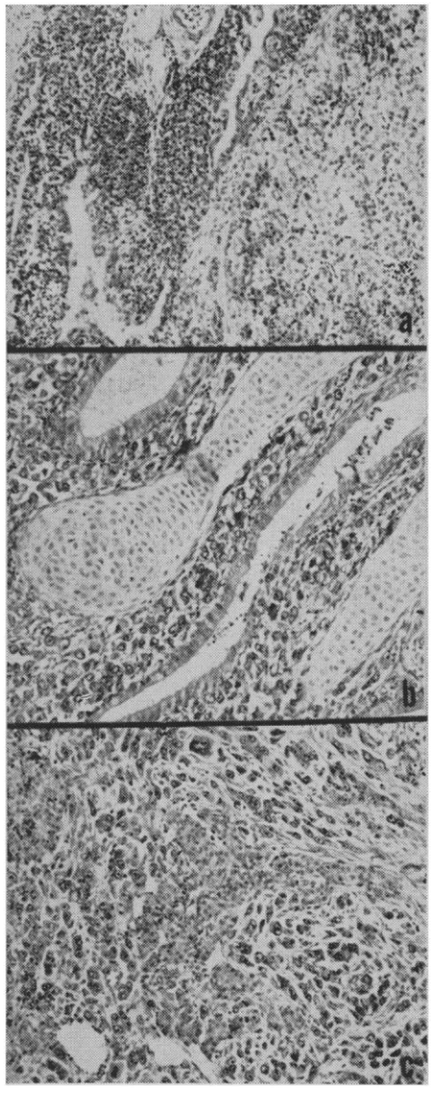

Fig. 1. Comparison of the interactions between three different pairs of transplants after 10 days on the chorioallantois (hematoxylin and eosin stain, about × 75). *a*, Lung (left) and liver (right). The integrity of each tissue is preserved. There is no invasive replacement of one tissue by the other. *b*, Lung and tumor. Except for bronchial epithelium and cartilage, the lung parenchyma has been completely replaced by tumor cells. *c*, Liver and tumor. The liver tissue (identified in the center of the field as acinar groups of cells with small nuclei) is invaded and extensively replaced by tumor cells.

In the studies described here we used a *companion host system* (*3*). These studies were undertaken to distinguish between phenomena that may be purely evidences of successful transplantation and others that clearly represent malignancy, that is, invasive replacement of normal parenchymal tissues (*4*). The tissues involved were three that are readily transplanted to the chorioallantoic membrane of the chick: chick embryonic liver and lung and the Walker carcinosarcoma 256 of the rat (*5*). Paired explants of these tissues were placed side by side on the chorioallantoic membrane as follows: liver plus lung, Walker tumor plus lung, and Walker tumor plus liver.

Transplants consisted of pieces 1 or 2 mm in diameter of Walker tumor tissue from subcutaneous passage in the rat, and of liver and lung from 15- or 16-day-old chick embryos. Pairs of explants were placed touching one another on the freshly dropped chorioallantoic membrane of 8- or 9-day-old recipients. In some series (see Table 1) the fragments were cemented together with a chick plasma clot. All the eggs were opened on the 18th day of incubation, 9 or 10 days after inoculation. The site of transplantation was identified, and semiserial sections were prepared of each.

On gross examination, the pairs of inocula that included a tumor fragment produced the largest nodules, some up to 1 cm in diameter. The pairs of normal tissues produced nodules of less than 5 mm in diameter.

On microscopic examination of combinations that included tumor, the tumor was seen to occupy two-thirds or more of the total mass of the transplant. Masses of tumor cells infiltrated the normal tissues extensively, and in many instances only focal residua of the normal tissue could be identified (Fig. 1). In three instances invasion was not observed although both transplants were identified. In these cases, either the inocula were separated or else massive necrosis and inflammation made positive identification of interaction impossible.

In the combinations of normal liver and lung, invasion or destruction of one tissue by the other was not observed. In the three instances listed with a plus-minus in the "invasion" column of Table 1, occasional glandular units of the liver and epithelial structures of the lung shared a common stromal bed, but replacement of one by the other was not suggested.

Under the conditions of our experiments, two qualities can be distinguished, transplantability and invasion. The latter is generally considered to be an essential feature of malignancy. The two transplanted embryonic tissues when presented as a pair do not suggest neoplasia. They retain their orderly architecture. Furthermore no replacement, destruction, or invasion of one tissue by the other is seen. When the Walker tumor is substituted for either normal partner, it not only "takes" in an aggressive fashion in the chorioallantoic membrane, but it invades and replaces the normal tissue with which it is in contact.

Some might argue that the observations described here are properly interpreted as simply the effect of the greater growth potential of the Walker tumor on the chorioallantoic membrane. We think that this kind of explanation contributes very little to an understanding of the mechanisms involved. Growth need not be infiltrative, but can be expansive with compression of normal parenchyma. The capacity for rapid growth of the Walker tumor is probably a necessary factor if invasion is to occur, but rapid growth does not in itself account for the invasive capacity of the tumor. It does not explain, for example, why cartilage and bronchial epithelium are spared when all other structures are replaced.

Many normal tissue "soils" can be exposed to the same tumor in companion host systems. We may be able to refine our assay of the functional capacity of a tumor in a highly pertinent context—invasive replacement of specific host tissues.

Furthermore, we hope that the two concurrent biologic events, transplantation and malignancy, occurring at the same site may be individually modified

Table 1. Data comparing interaction of tissues on the chorioallantoic membrane. In all series but 6 and 9 the explants were cemented together with chick plasma prior to completion of the inoculation.

Series No.	Membranes (No.)		
	No. examined	Both transplanted tissues identified	Invasion seen
	Liver and lung		
1*	12	7	(1 ±)
2†	11	8	(1 ±)
3*	7	6	0
4†	20	19	(2 ±)
	Walker tumor and lung		
5*	12	9	8
6†	9	8	8
7*	13	10	10
	Walker tumor and liver		
8*	12	9	8
9†	9	9	8
10*	11	8	8

* Eight-day-old chorioallantoic membrane inoculated with 15-day-old organ fragment.
† Nine-day-old chorioallantoic membrane inoculated with 16-day-old organ fragment.

with pharmacologic agents. Perhaps lung or liver can be changed from being a good soil for invasive tumor spread to a poor soil without important impairment of other normal attributes.

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Relationship between Reaction Time and Electroencephalographic Alpha Phase

Abstract. Demonstrations of a relationship between human 8 to 13 per second (alpha) electroencephalographic activity and simple visual reaction time can be made at reliable levels of confidence by (i) sampling reaction times to stimuli given at phases of the alpha cycle 10 msec apart, (ii) selecting the phase with the slowest reaction times, and (iii) collecting enough reaction times to stimuli at this and some other control phase for statistical comparison.

Several investigators (*1*) have speculated on the possibility of a parallel between phasic changes in behavior and the 8 to 13 per second activity of the electroencephalogram, known as the alpha rhythm. Lansing (*2*) compared reaction times to stimuli which coincided with six different portions of the alpha cycle. He found no reliable overall differences, and to achieve statistical significance he was forced to select retrospectively the slowest and fastest groups of reaction times for comparison. He also screened 100 subjects in order to find eight with enough alpha activity for his procedure.

Our paper describes a technique that (i) allows identification of that portion of the alpha cycle most likely to be associated with slow reaction times in advance of statistical evaluation, and (ii) is applicable to about a third of an unselected adult population by virtue of requiring only moderately dominant alpha activity.

The individual being tested is seated with eyes closed in a darkened room. Alpha activity is lead off from the left occipital area and either the left ear lobe or the mid-frontal scalp. This is amplified and fed into a circuit designed to generate an electrical signal only when the phase and amplitude of the alpha rhythm correspond with a predetermined setting. This initial signal is followed by a second signal after a delay that can be varied manually from 10 to 90 msec.

The second signal flashes a strong light in the subject's face and simultaneously starts a timer. The subject responds to this light by pressing a key; his reaction stops the timer, automatically prints the reaction time, and furnishes a voltage analog of reaction time to the Y axis of an automatic X-Y plotter. By supplying the X axis of this plotter with a voltage corresponding to the delay between the two signals, one can plot each reaction time as a function of approximate alpha phase at stimulation.

If an artifact never triggered the first signal of this device, and if alpha waves were perfect sine waves of fixed frequency, then the delay between the first and second signals would specify exactly the alpha phase at the instant of visual stimulation. However, neither of these two ideals is realized. Therefore, a sweep on a monitor oscilloscope is started by the first signal and this displays the electroencephalographic (EEG) activity for 150 msec. In this way the subject's EEG activity can be visualized at the instant of each stimulation. Stimuli that fail to coincide with the desired portion of an alpha cycle are discarded from further consideration. In spite of this check, however, there is possible error of as much as 15 msec for individual stimuli.

Using this device, one can present blocks of 10 to 20 stimuli at steps of 10 msec each along the alpha cycle, and in this way accumulate a scattergraph of reaction time as a function of approximate alpha phase at stimulation. One can then select the alpha phase at which stimuli elicit the slowest responses and compare this to one other phase—either a phase associated with fast responses or one about 180 degrees away. The results of selecting phases in this way are illustrated in Table 1. Data in this figure were collected after the initial screening runs by presenting 10 to 15 stimuli at a time at one phase. Rest periods were introduced after each 20 to 40 stimuli, and the order of phase presentation was alternated to minimize the effects of learning and fatigue on differences between reaction times to stimulation at the two selected phases.

These data represent only reaction times measured after a prediction; therefore, one-tailed tests of significance could be justified. In this case, six out of the eight subjects would be found to yield significant results at the 5 percent level or better. However, to test the over-all group, two-tailed probabilities are given in the Table. Combining these by the chi-square formula, we find that differences in reaction times to stimuli presented at different alpha phases in eight independent studies such as this would have occurred by chance about one time in 500.

At the onset, we elected to disregard reaction times over 300 msec as probably representing lapses of attention. The number of reaction times disregarded in this way are also given in Table 1. This procedure biased the data against the obtained results and significantly more reaction times were excluded from the slow samples than from the fast samples.

Because of individual variability, phase at stimulation is given in milliseconds from maximum occipital positivity. Average alpha cycle duration is also indicated for each subject. In this series stimuli presented nearest to maximum occipital positivity elicited slowest reaction times. This corresponds to the

Table 1. Visual reaction times to phasic stimuli. All times in milliseconds. Stimulus phase is given in milliseconds from maximum occipital positivity. $X^2 = -2\Sigma\log_e p = 38.9$, (16 df).

Subject	Predicted slow				Approx. av. alpha period	Predicted fast				Statistics	
	Stimulus phase	Reaction time				Stimulus phase	Reaction time			t	$p <$
		Under 300		Over 300 (No.)			Under 300		Over 300 (No.)		
		Mean	No.				Mean	No.			
L. H.	0	243	47	6	100	50	231	49	3	2.22	.05
D. M.	−20	240	40	3	100	−30	226	40	3	2.21	.05
I. V.	0	249	50	10	100	20	240	50	9	2.48	.02
A. W.	0	247	107	16	110	40	241	105	15	1.68	.10
I. S.	−10	262	46	20	100	40	250	51	12	2.50	.02
J. M.	−10	239	77	5	90	20	236	79	3	0.87	.40
P. P.	0	262	49	17	100	−40	252	40	9	1.80	.10
R. D.	0	215	73	9	100	40	216	80	2	0.15	.90