of these hearts provided normal values for comparison.

The catecholamine content of reimplanted hearts averaged 0.01 μg/g. The catecholamine content of the normal dog hearts averaged 0.75 μg/g.

The histamine content of reimplanted hearts averaged 4.80 μg/g, and the histamine content of the normal dog hearts averaged 4.58 μg/g. Individual values for histamine and catecholamine content are presented in Table 1.

These data indicate that the autotransplanted canine heart is depleted of catecholamine but not of histamine. Our results, therefore, support the hypothesis of von Euler that the histamine content of an organ, unlike the norepinephrine content, is not dependent upon the presence of sympathetic nerves (*6*).

The autotransplant provides a preparation which is important in homotransplantation studies. One may analyze changes in chemical composition and in pharmacological responsiveness of the organ graft without the changes superimposed by immunologic rejection. Moreover, an autotransplant provides assurance of total extrinsic denervation restricted to the heart. Such a denervated autotransplant maintains the animal's life, whereas the heterotopic cardiac homograft shows electrical and mechanical activity but is incapable of supporting life.

Our studies support the hypothesis of Wegmann (*7*) that a puppy heart transplanted to another dog's neck is depleted of catecholamines by virtue of denervation rather than by an immunologic phenomenon (*8*).

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Uptake of Strontium-85 by Alfalfa

Abstract. Experiments with alfalfa were carried out to study the possibility of changes with time in the availability of radiostrontium in soil. After the soil was treated once with Sr^{85}, the first crop was harvested after 60 days of growth. Four subsequent crops, cut at successive 4-week intervals, were examined. The difference in uptake between the second and fifth crops was statistically significant, suggesting that some fixation of Sr^{85} may occur in the soil. The effects of various applications of ammonium dihydrogen phosphate, monocalcium phosphate, calcium chloride, and potassium chloride on the uptake of Sr^{85} by alfalfa were also investigated. Of the experiments carried out, only the treatment with 1.0 meq of potassium per 100 g of soil resulted in a statistically significant reduction in strontium uptake.

In a study on changes with time in the availability of strontium-90 in soil, Squire (*1*) pointed out that such investigations are of importance in assessing the long-term consequences of the deposition of Sr^{90} on agricultural land. It is of interest to ascertain whether Sr^{90} could become progressively fixed in the soil and, therefore, less available to plants. The work of Morgan (*2*) showed that fallout Sr^{90} was not less available to ryegrass than freshly added Sr^{85}, thus indicating that the availability of Sr^{90} did not decrease appreciably with time. On the other hand, in studies with ryegrass planted on drums of soil whose surface had been treated with carrier-free Sr^{90}, Squire (*1*) noted that in four seasons there was a 20 to 30 percent decrease in the Sr/Ca ratio in the grass. It was suggested that this reduction may be attributable to either or both of two causes, namely, slow chemical changes that have reduced the availability of the Sr^{90} in the soil and the increasing penetration with time of the Sr^{90} down the soil profile. The effects of deeper penetration may be of considerable importance; Milbourn (*3*), after ploughing the soil to a depth of 11 inches instead of leaving its surface contaminated, has reported that the absorption of radiostrontium by the shallow-rooted ryegrass could be reduced by a factor of 4.

As an attempt to study further the possibility of changes with time in the availability of radiostrontium in soil, experiments with alfalfa were carried out because successive crops of the above-ground portion of the plant can be harvested at different time intervals. In addition, the effects of a number of chemical nutrients on the uptake of radiostrontium were investigated. Plants of the Grimm variety were grown, one per pot, in the greenhouse. Each pot contained 400 g of Saskatchewan Oxbow loam soil which had a *p*H of 7.2, and 19.2, 6.5, 1.9, and 0.1 meq of exchangeable Ca, Mg, K, and Na, respectively, per 100 g of soil. Before seeding, each pot of soil (except in one series) was first mixed thoroughly with 50 ml of solution containing approximately 0.1 mc of carrier-free Sr^{85}. In the single exception, the Sr^{85} solution was added to the surface of the soil without mixing. In the studies with chemical nutrients, each pot of soil was also mixed with 50 ml of solution containing various levels of ammonium dihydrogen phosphate, monocalcium phosphate, calcium chloride, or potassium chloride, or with 50 ml of distilled water as a control. During the entire period of the experiments, daily watering maintained soil moisture at its field capacity. After 60 days of growth, the first crop was harvested. The entire above-ground portion of each plant was analyzed for Sr^{85} uptake. Four more successive crops were cut at 4-week intervals, and each cutting was assayed for radioactivity (*4*). The results are summarized in Tables 1 and 2 (*5*).

From Table 1, it may be noted that

Table 1. Uptake of Sr^{85} by successive crops of alfalfa. Each value is the average of analyses of 36 plants.

Crop	Harvest date (days after germination)	Uptake (%) per gram of dried plant
1	60	1.65
2	88	1.35
3	116	1.29
4	144	1.28
5	172	1.20
Least significant difference		0.13*

* Significant at the 1-percent level.

Table 2. Uptake of Sr^{85} by alfalfa grown in soil with or without fertilizer treatment. Each value is the average of analyses of 20 plants.

Nutrient	Dosage (meq /100 g of soil)	Uptake (%) per gram of dried plant
None (surface application)		0.63*
None (control)		1.44
$NH_4H_2PO_4$	0.08 PO_4†	1.50
	0.8 PO_4	1.51
$Ca(H_2PO_4)_2$	0.08 PO_4, 0.03 Ca†	1.29
	0.8 PO_4, 0.3 Ca	1.33
$CaCl_2$	0.03 Ca	1.28
	0.3 Ca	1.37
KCl	1.0 K	1.13*
Least significant difference		0.17

* Significantly different from the control at the 1-percent level. † This dosage is equal to a treatment of about 60 lb of nutrient per acre of soil, assuming the weight of 1 acre of soil to be 2×10^6 lb.

successive crops continued to take up radiostrontium even though the soil was treated only once with Sr^{85}. The uptakes of crops 2, 3, 4, and 5 were comparable to one another, but they all show a statistically significant difference from crop 1. This is as would be expected since crop 1 resulted from a 60-day growth following germination, while each of the other crops was grown for only 28 days. The fact that there were no large differences in Sr^{85} uptake among the successive crops indicates that during the period covered by these experiments there was no extensive reduction in the availability of radiostrontium to the alfalfa. However, the difference in uptake for crops 2 and 5 was statistically significant, thus suggesting that there might have been a slight amount of fixation of the radiostrontium in the soil, just as Squire reported (*1*).

In the studies on possible influences of chemical nutrients on plant uptake of radiostrontium from soil, previous work in this laboratory has shown that with greenhouse experiments similar to those described here, monocalcium phosphate applied at a level of about 600 lb/acre of soil effected a statistically significant reduction of Sr^{85} uptake in the grain, chaff, stem, and leaf of Thatcher wheat (*4*). My results with alfalfa (Table 2) show that ammonium dihydrogen phosphate, a commonly used fertilizer, caused no reduction in strontium uptake. This behavior is the same as that found in Thatcher wheat. Of the other nutrient treatments studied (Table 2), only 1.0 meq of potassium per 100 g of soil resulted in a statistically significant reduction in Sr^{85} uptake.

It may also be noted from Table 2 that the addition of radiostrontium to the surface of the soil, without mixing, resulted in much less uptake than the control in which the Sr^{85} was mixed through the soil. This is not surprising since under the conditions of these greenhouse experiments, the roots of the alfalfa plant covered almost the entire bottom of the pot. Although ploughing is known to decrease the absorption of radiostrontium by shallow-rooted plants such as ryegrass (*3*), mixing surface-contaminated soil by ploughing probably will not be very effective in reducing strontium uptake by deep-rooted plants.

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Serial Propagation of Influenza B (Lee) Virus in a Transmissible Line of Canine Kidney Cells

Abstract. The serial propagation of influenza B (Lee) virus has been demonstrated in a transmissible line of canine kidney cells, as evidenced by the presence of cytopathic effects and hemagglutinins in each of six consecutive tissue-culture passages, and by egg infectivity titers of $10^{-4.3}$ and $10^{-4.1}$ per 0.1 ml in 3rd and 5th tissue culture fluids, respectively.

The propagation of influenza A and B viruses has been demonstrated in a variety of primary tissue-culture systems including minced chick embryo, human embryo lung and kidney, monkey kidney and bovine embryo kidney (*1, 2*). The propagation of influenza A, B, or C viruses could not be demonstrated in transmissible cell cultures such as HeLa, human conjunctiva, KB or human fibroblast (*2*). More recently Solov'ev and Alekseeva (*3*) have shown that a strain of Asian influenza virus was capable of being serially propagated in primary monkey kidney cells but incapable of maintaining infectivity beyond two passages in SOTs (cynomolgus monkey heart) cells (*4*), a transmissible strain derived from cynomologus monkey heart.

I believe this is the first report of the serial propagation of influenza virus in a transmissible strain of mammalian cells, derived from dog kidney and designated MDCK (*5*). The canine kidney cells had been through more than 35 serial passages prior to their use for the propagation of Lee influenza virus. These cells were grown initially on a medium consisting of 5 percent calf serum and 0.5 percent lactalbumin hydrolyzate (LAH) in Earle's balanced salt solution (EBSS) with 0.002 percent phenol red, 200 units of penicillin per milliliter, and 200 μg of streptomycin per milliliter. After the formation of a continuous cell sheet, in 2 to 3 days, and before virus inoculation, the fluids were changed to a 3 percent horse serum, 0.5 percent LAH, 0.1 percent yeast extract, and 0.1 percent bovine albumin in Earle's medium containing phenol red and antibiotics as above. The *p*H of all mediums was adjusted to approximately 7.2 with 7.5-percent $NaHCO_3$ solution. Cultures of MDCK were inoculated initially with 0.1 ml of a 10^{-2} dilution of egg-adapted influenza B virus (Lee) having an EID_{50} titer of $10^{-7.8}$ per 0.1 ml. Two serial passages of the virus in MDCK cells were made using undiluted harvest fluids, while the remaining serial passages were made with a 10^{-1} dilution of the harvested fluids.

The medium was changed once, 3 days after inoculation, and this fluid or the 6- or 7-day-old fluids, or both, were tested for hemagglutination (HA) at 4°C using 0.5-percent fowl erythrocytes. Egg infectivity (EID_{50}) titrations were conducted on tissue culture fluids from the 3rd and 5th passages, using 10- to 11-day-old embryonated eggs, by amniotic inoculation with 0.1 ml of fluid. Fluid from the 4th serial passage of virus in MDCK cells was shown to be neutralized in MDCK cultures by

Table 1. Growth of type B (Lee) influenza virus in a transmissible strain of canine kidney (MDCK) cells.

Tissue culture passage	Time of harvest (days)	Egg infectivity titer* (EID^{50})	Hemagglutination titer† (days)		Cytopathic effect (CPE)	
					Response‡	Time, post-inoculation (days)
0		5.8§	3	6 or 7		
1	3	N.D.‖	80	N.D.	4+	3
2	7	N.D.	<5	5	1+	7
3	6	4.3	<5	160	3+	6
4	3	N.D.	80	N.D.	2+	3
5	6	4.1	<5	1280	2–3+	6
6	3	N.D.	160	20	3+	3

* Expressed as the logarithm (base 10) of the number of 50 percent infectious doses in 0.1 ml of inoculum. † Expressed as the reciprocal of the initial dilution; 0.5-percent fowl erythrocytes used. ‡ Cells affected: 4+, 100 percent; 3+, 75 percent; 2+, 50 percent; 1+, 25 percent. § Titer of the original, diluted amniotic fluid inoculum. ‖ N.D., not done.