survival rate of 52 ± 8 percent (*6*).

The fact that the survival rates of second grafts are varied, depending on the length of time that the first graft survived, suggests that the influence of the first graft can be of several kinds and that these are related to the cutoff periods of between 1 and 3 months. First grafts fail after more than 3 months almost solely as a result of a slow, relentless rejection process. These patients are generally a homogeneous group who if grafted again perhaps benefit from some enhancement or tolerance effect produced by the first graft. First grafts which fail between 1 and 3 months are often acutely rejected. This mode of rejection apparently leads to heightened sensitivity with lower second graft survival rates.

Patients who lose grafts in the first month constitute the most heterogeneous group. Some of the transplants are hyperacutely rejected. Second grafts into such patients have a low survival rate. Some grafts are removed because of surgical failure or of preservation failure. Technical failure apparently does not sensitize the host, confirming earlier data of Straffon *et al.* (*7*).

The basic problem which remains to be answered is whether the first kidney transplant actively influences the fate of second grafts by immunization or enhancement or both or whether it only serves to select out patients with different degrees of immunologic responsiveness. At first sight, since the overall second transplant survival rate is the same as the first transplant survival rate, it might be assumed that the first graft does not condition the host in any way. Closer examination of the time at which the first graft was rejected appears to show that rejection at 1 month is associated with no influence of the first graft, whereas hyperacute rejection and rejection between 1 and 3 months is associated with lower survival rates of the second grafts. Patients who hyperacutely reject grafts tend to acutely reject their second grafts. Of greatest interest is that patients who slowly reject their first graft tend to have a high second graft survival rate. Certain patients may be inherently "slow rejectors," and such patients with poor immunologic responsiveness (*8*) may also slowly reject second grafts. Immunologic responsiveness, on the other hand, cannot totally explain the clinical kidney transplant results, for patients who reject a first graft are not uniformly immunologic responders to second grafts, but often retain their second grafts longer than their first. Also, second grafts in patients who slowly rejected their first grafts survive *longer* than overall first grafts. We conclude, therefore, that the first graft may under certain conditions induce enhancement or tolerance.

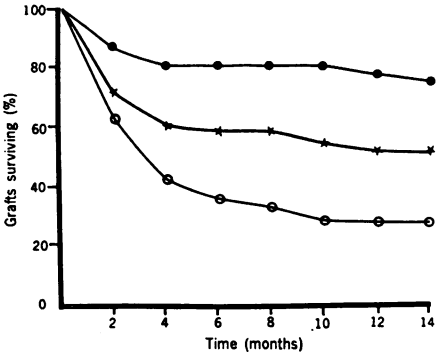

Fig. 2. Second graft survival rates in three subsets of cytotoxicity-negative recipients. Thirty-nine patients lost their first graft within 1 month (★), 35 patients in 1 to 3 months (○), and 36 patients after more than 3 months (●). It can be noted that transplant recipients who lost their first graft after more than 3 months have an unusually high second graft survival for cadaver kidney transplants.

GERHARD OPELZ
MAX R. MICKEY, PAUL I. TERASAKI
Departments of Surgery and
Biomathematics, School of Medicine, University of California, Los Angeles

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6. Five of these 39 patients had rejected their first transplant hyperacutely. When retransplanted, one of them had a hyperacute rejection again and two rejected their grafts in the second month; one had a functioning graft at 6 months and one at 1-year posttransplantation.
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21 August 1972

Silicon: An Essential Element for the Chick

Abstract. *Silicon is required for normal growth and development in the chick when a low silicon diet is fed in a trace element controlled environment. Day-old deutectomized cockerels fed a purified amino acid diet showed significantly retarded growth and development within 2 to 3 weeks. Chicks fed the same diet plus a silicon supplement showed 50 percent higher growth and normal development. Silicon meets the criteria for an essential trace element.*

Silicon is, next to oxygen, the most abundant element in the earth's crust, and at least trace amounts appear in most animal tissues (*1–3*). Although great importance has been attached to the study of the toxicity of the oxide, silica, and of certain fibrous silicates, mainly the involvement of silica in silicosis, there has been relatively little work concerned with the effect of silicon in normal metabolism, and until now there has been no proof that silicon plays any definite role in vital processes in animals or man. Silicon has generally been considered to be nonessential except in certain primitive organisms, notably diatoms, Radiolaria, and some sponges, which utilize silica as a component of body structure. I have now found that silicon is required for normal growth and development in the chick when a low silicon diet is fed in a trace element controlled environment, thus establishing silicon as an essential element (*4*).

Previous studies in this laboratory had suggested a possible role for silicon in bone formation. In vitro studies based upon electron microprobe analysis had shown the unique localization of silicon in active calcification sites in young bone (*5*). In the earliest stages

Table 1. Growth response of chicks to silicon supplementation.

Study No.	Chicks (No.)	Average daily weight gain in 23 days (g) (mean ± S.E.M.)		Difference (%)	P
		Unsupplemented group	Supplemented group		
1	36	2.37 ± 0.11	3.10 ± 0.10	30.0	< .01
2	30	3.25 ± 0.09	4.20 ± 0.09	30.0	< .02
3	48	2.57 ± 0.09	3.85 ± 0.11	49.8	< .01

of calcification in these sites, when the calcium content of the preosseous tissue is very low, there is a direct relationship between silicon and calcium. As a result of these findings it has been suggested that silicon is associated with calcium in an early stage of bone formation (*6*). Further in vivo experiments with rats have also shown a relationship between silicon and calcium in bone formation (*7*). It was demonstrated that dietary silicon increases the rate of mineralization; this was particularly apparent on a low calcium diet. A relationship has been established between silicon, magnesium, and fluorine in the formation of bone in the chick (*8*).

Some studies by other workers have also suggested the possibility that silicon might have a physiological function. For example, the level of silicon in the blood and certain tissues of rats has been reported to be affected by age, sex, adrenalectomy, and thyroidectomy (*9*). In addition to calcium, phosphorus, magnesium, iron, and certain vitamins, silicon, along with tin, vanadium, and fluorine, has been shown to have an effect on incisor pigmentation (*10*). However, none of the above studies have shown the essentiality of silicon.

Because of its abundance in the environment and in the laboratory, silicon offers an unusual challenge in the problem of avoiding contamination. In the work described here silicon contamination has been kept to a minimum by the use of silicon-low plastics and by using a specially constructed environmental chamber in which the trace element content of the air is greatly reduced.

A major prerequisite was the formulation of a low silicon diet. The basal diet (*11*), based on an optimal mixture of L-amino acids for the chick, is similar in amino acid composition to those diets described as producing optimum or near optimum growth by other workers. The salt mix (*12*), patterned after those described as optimal, contains the known required mineral elements in sufficient and balanced amounts except for calcium. Changes in the calcium level were made by the addition of calcium carbonate. Diets containing 0.90, 1.0, and 1.2 percent calcium have been used. The vitamin mix is more than adequate when supplied at the 1 percent level (*13*). All dietary components were analyzed repeatedly for silicon by emission spectrography, since appreciable variations in silicon content are found in many ingredients, even from the same supplier.

Although an effect of silicon had been demonstrated in the rat in earlier studies (*7*), the chick was chosen as the experimental animal replacing the rat for two reasons: first, because of its more rapid skeletal growth, and second, because of the likelihood of obtaining an earlier depletion of silicon, since the experimental diet can be fed at an earlier age. In the rat and other mammals, milk is a significant source of silicon, as would be expected if this element is required for growth and skeletal development. To reduce the parental contribution of silicon in the chick, the day-old cockerels were deutectomized, that is, the yolk which is inside the chick at the time of hatching was removed by making a small incision in the area of the umbilicus. Thus any silicon supplied by the yolk reserves was removed.

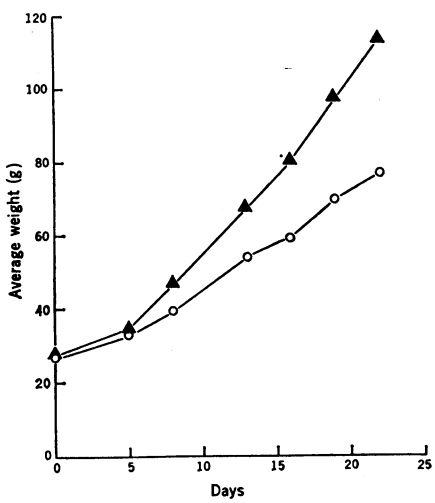

Fig. 1. Growth curves of chicks with and without silicon supplement. Upper curve (▲), basal plus silicon supplement. Lower curve (○), unsupplemented controls. Each group contains 24 chicks.

Each experiment consisted of six groups of eight White Leghorn cockerels. Three groups of eight chicks were fed the basal diet, and three groups the basal diet plus silicon supplement. Several silicon compounds have been used as supplements and the effectiveness has been found to vary, in some cases directly with solubility. In the three studies reported in detail here, silicon was supplied as sodium metasilicate, $Na_2SiO_3 \cdot 9H_2O$, at a level of 100 parts of silicon per million in the diet. Growth rates and the appearance of the animals were evaluated at 2- to 3-day intervals. The animals were killed at the end of a 25- to 35-day period. Blood, certain skeletal structures, and soft tissues were taken for analyses. Histopathological studies were also performed.

Differences between the chicks on the basal and silicon-supplemented diets were noted after approximately 1 to 2 weeks. As shown in Fig. 1, silicon was found to exert a significant effect on growth, the unsupplemented silicon group showing a lesser growth rate. At the end of 23 days the average weight for the low silicon group was 76 g, compared to a weight of 116 g for the supplemented group. In Table 1 the average daily weight gains are given for study 3, illustrated in Fig. 1, and for two earlier studies. In study 3 the average daily weight gains for the chicks on the basal diet is 2.57 g, compared to 3.85 g for the supplemented group. The percentage difference in the average daily weight gain over the 23-day period is 49.8 percent. In the earlier studies 1 and 2 the percentage difference in the average daily weight gain is 30 percent.

The difference between the chicks on the basal and silicon-supplemented diets is readily observable. The chicks on the basal diet are much smaller but in proportion. They appear stunted. On subsequent examination all organs appeared relatively atrophied. The legs and comb of the deficient chick are particularly pale. Macropathologic ex-

amination showed that the skin and mucous membranes are somewhat anemic. The subcutaneous tissue seems muddy to yellowish compared with a white-pinkish color of the supplemented bird. The deficient chick has no wattles and the comb is severely attenuated.

The leg bones of the deficient bird are shorter, of smaller circumference and thinner cortex. The metatarsal bones are relatively flexible and the femur and tibia fracture more easily under pressure than those of the supplemented group. The beak is also flexible in the deficient bird. The cranial bones appear somewhat flatter. The effect of silicon on skeletal development strengthens my earlier postulate that silicon is involved in an early stage of bone formation.

Several other observations support the conclusion that silicon is essential. The level of silicon found to be effective for normal growth and development in my studies is on the order of that present in plant and animal foodstuffs (*1–3*). Silicon appears to be invariably present in animal matter. All tissues and fluids examined in this laboratory and by other workers contain at least traces. The eggs of birds (*13*, *14*), milk (*13–16*), and the fetuses of mammals (*2*) have small but appreciable quantities. The blood of man and other species averages about 5 mg of silicon per liter, and this level is not increased by the inhalation of silica dust (*2*). The substantial amount of silicon in cow's milk seems to be little influenced by dietary silica intake (*15*, *16*). The constant low concentration of silica in organs other than lungs does not appear to vary appreciably during life, in contrast to the lungs, which may accumulate large amounts of silica from lifelong inhalation of dust.

The discovery has many implications, first from an evolutionary point of view, since silica is known to perform a skeletal role in some primitive organisms, and second because, although great importance has been attached to the study of the toxicity of silica and the fibrogenic and potential carcinogenic effects of fibrous silicates, this is the first time that it has been shown that silicon itself can be considered as a participant in normal metabolism.

EDITH M. CARLISLE
Environmental and Nutritional Sciences, School of Public Health, University of California, Los Angeles 90024

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12. The salt mix when fed at the 5.2 percent level contributed the following minerals, in grams per kilogram of diet: $CaHPO_4$, 28.4; Na_2HPO_4, 7.0; $MgSO_4 \cdot 7H_2O$, 6.14; $FeSO_4 \cdot 7H_2O$, 0.32; $MnSO_4 \cdot H_2O$, 0.5; $ZnSO_4 \cdot 7H_2O$, 0.32; $CuSO_4 \cdot 5H_2O$, 0.016; NaCl, 4.0; KCl, 7.0; KIO_3, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $CoSO_4 \cdot 7H_2O$, 0.001; Na_2SeO_3, 0.0004; and $NaHCO_3$, 1.0. $NaHCO_3$ was used to maintain a constant sodium level in all diets.
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Bacterial Ribonucleic Acid in the Frog Brain after a Bacterial Peritoneal Infection

Abstract. *Frogs were injected intraperitoneally with bacteria, and the RNA of the brains (which have protective barriers against the bacteria used) was extracted. Part of the RNA was bacterial RNA apparently resulting from the transcription of DNA transferred from bacteria to the brain cells.*

Plants or animal organs dipped in a suspension of bacteria synthesize bacterial DNA and bacterial RNA (*1–4*). This phenomenon, transcession (*4*), is due to the spontaneous release of DNA from bacteria (*5*) into cells of higher organisms. Up to now the results on animals (*3*) have been obtained with frog auricles. We now report bacterial RNA in the brain, which is naturally protected by barriers against bacteria (*6*).

Adult frogs were injected intraperitoneally with 1 ml of a suspension (10^8 bacteria per milliliter of Ringer solution, unless specified) of *Bacillus subtilis* (strain Caron), *Escherichia coli* (strain B), or *Agrobacterium tumefaciens* (strain B6). The controls were injected only with 1 ml of sterile Ringer solution. Addition of antibiotics did not change the results. Therefore a high concentration of antibiotics (for *B. subtilis*, 2.000 μg of ampicillin-cloxacillin, and for *E. coli* and *A. tumefaciens* 2.000 μg of colimycin) was injected into each animal 2 hours before labeling to obtain sterile organs. [^{3}H]Uridine (1 mc per animal) was injected intraperitoneally 12, 24, and 72 hours after infection. The animals were anesthetized with chloroform 3 hours later. The thoracic cavity was exposed, and 40 ml of sodium citrate (3.8 percent) was perfused at constant pressure (27 mm-Hg) into the ventricle. The aorta was clamped distally to the origin of the carotid artery; the jugular vein was sectioned, and after a moment the escaping liquid was clear, an indication of a well-washed veinous and arterial system. The brains were then removed, and the meninges were dissected away.

One longitudinal half was used for sterility tests and autoradiography, the other for RNA extraction. The first part was homogenized (*4*) and used for sterility tests by plating on petri dishes. We report only results with sterile brains. (In the absence of antibiotics, we found from 5×10^2 to 2×10^3 bacteria in five brains that probably had not been completely washed out by perfusion. These brains were not used.) For autoradiography (*7*, *8*) we took one sample from the hemispheres and another from the hypothalamus. The latter was not used for RNA extraction since its basis is not protected by a barrier (*9*). We used either classic (*8*) or water-soluble (*7*) autoradiographic technique. (Cryostat sections were made to check whether some bacteria had been washed away during dehydration of the paraffin sections.) The number of bacteria