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.

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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

scored by autoradiography and the colony assays differs by less than 20 percent in control experiments where brains were dipped in a suspension of labeled bacteria. [^{3}H]RNA was extracted from the other half of the brain (*10*) and characterized by RNA-DNA hybridization in vitro according to the method of Gillespie and Spiegelman (*11*). Filters containing either no DNA or salmon sperm DNA served as controls. The results give the relation (expressed in percent) between the amount of denatured DNA trapped on the filter and the amount of [^{3}H]RNA hybridized. The estimate of the [^{3}H]RNA hybridized is based on the specific activity of the bulk RNA. This may lead to error, since it is likely that the RNA, being a mixture of bacterial and animal RNA, might not be uniformly labeled. We present our results as percentage of hybridization rather than as a percentage of the input counts hybridized. Indeed, in this case the problem of nonuniform labeling is not solved. Moreover, from one experiment to the other the bacterial RNA synthesized in animal cells could consist either of the repetition of few genes or of one copy of many genes, possibilities which would give completely different percentages. It should however be stressed that our results are not quantitative in an absolute sense.

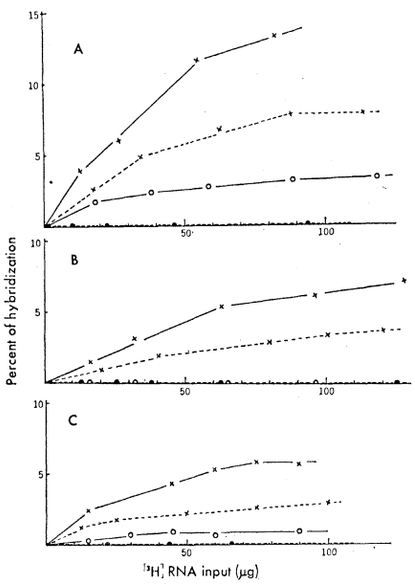

Fig. 1. Saturation curves of [^{3}H]RNA extracted from brains of frogs infected with bacteria; (X——X) for 12 hours; (X- - - -X) for 24 hours; (○——○) for 72 hours, or (○- - - -○) from brains of control frogs and labeled for 3 hours with [^{3}H]uridine. (A) The frogs were injected with a suspension of *E. coli* (strain B); (B) a suspension of *A. tumefaciens* (strain B6); (C) a suspension of *B. subtilis*. In (A) 21 μg of *E. coli* DNA, in (B) 18 μg of denatured *A. tumefaciens* DNA, and in (C) 16 μg of denatured *B. subtilis* DNA were trapped on each filter. The results give the relation, expressed in percentage, between the number of micrograms of denatured DNA trapped on the filter and the amount of [^{3}H]RNA (counts per minute per milligram) hybridized.

To check whether the labeled RNA in the brain had been synthesized in the brain cells, we excised the brains of some animals before labeling and placed them in an aerated Eagle solution (*12*) with [^{3}H]uridine (1 mc per 10 ml). Sterility was maintained by an addition of 200 μg of ampicillin-cloxacillin and 200 μg of penicillin. To ascertain whether DNA can circulate in the blood and penetrate the brain while keeping its primary and secondary structure, 300 μg of purified *A. tumefaciens* [^{3}H]DNA (density, 1.718 g/cm^3) was injected intraperitoneally. This foreign DNA was differentiated from the native DNA of the brain (density, 1.700 g/cm^3) by CsCl gradient centrifugation (*13*). The localization of the radioactive molecules was checked by autoradiography.

We also injected [^{3}H]RNA (300 μg per frog) extracted from bacteria labeled with [^{3}H]uridine to ascertain whether this RNA could circulate in the blood and penetrate into brain cells. [^{3}H]RNA recovered from these brains was characterized by DNA-RNA hybridization (*11*). The effect of purified bacterial DNA or RNA on the syntheses of RNA in brain cells was studied by injecting intraperitoneally unlabeled bacterial DNA or RNA (300 μg per frog) followed by 1 mc of [^{3}H]uridine. The bacterial DNA (*14*) and RNA (*15*) were prepared from labeled or nonlabeled bacteria. The frog DNA (*14*) was prepared from the intestine of nonlabeled animals. The infected frogs are highly resistant to septicemia and were in good condition.

Figure 1 shows the presence of bacterial RNA in the brains of infected frogs. The percentage of hybridization of DNA-RNA is high soon after the beginning of the infection and then decreases. Competition experiments with nonlabeled RNA extracted from the same strain of bacteria displaces about 70 percent of the [^{3}H]RNA hybridized. These competitions are specific.

Injection of bacteria into frogs does not seem to reduce the transcription of the brain since [^{3}H]RNA extracted from brains of infected frogs shows the same percentage of hybridization with frog DNA as [^{3}H]RNA extracted from control frogs. When 10^8 to 10^{10} bacteria are injected into the frog there is no significant difference in the amount of bacterial RNA found in the brains; with 10^7 bacteria there is a decrease.

If the brains were taken out after the injection of bacteria and labeled in vitro, bacterial [^{3}H]RNA could still be extracted from the brains (Fig. 2). The amount is much smaller than when the labeling is done in vivo. If the labeling takes place 5 hours after the extraction of the brain, no bacterial [^{3}H]RNA can be traced. However when brains of noninfected frogs were extracted and labeled in vitro, we observed a decrease in the synthesis of their own RNA.

When nonlabeled purified bacterial DNA (300 μg per frog) or RNA (300 μg per frog) was injected intraperitoneally to frogs which were then labeled in vivo (1 mc of [^{3}H]uridine per frog), no bacterial RNA could be detected in their brains. However the bacterial

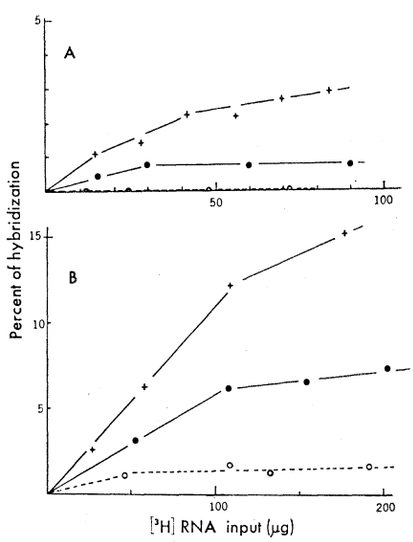

Fig. 2. Saturation curves on [^{3}H]RNA extracted from brains of frogs. (+——+) The labeling with [^{3}H]uridine in vivo; (●——●) labeling in vitro with [^{3}H]uridine just after the extraction; (○- - - -○) labeling in vitro 5 hours after the extraction. (A) The frogs were infected for 12 hours with a suspension of *A. tumefaciens* (strain B6); (B) infection of 1 ml of Ringer solution. In (A) 18 μg of denatured *A. tumefaciens* DNA and in (B) 16 μg of frog denatured DNA are trapped on each filter. The results give the relation, expressed in percentage, between the number of micrograms of denatured DNA trapped on the filter and the amount of [^{3}H]RNA (counts per minute per microgram) hybridized.

DNA can cross the brain barrier and reach the brain cells without losing its primary and secondary structures. Indeed after *A. tumefaciens* [^{3}H]DNA has been injected intraperitoneally, an important part of the radioactive molecules of the DNA extracted from the brains sediment at the level of the bacterial DNA after CsCl gradient centrifugation. Autoradiographs show that the radioactivity is located mainly in the nuclei. The foreign [^{3}H]RNA injected is probably completely broken down and reutilized for synthesis since after extraction of the [^{3}H]RNA in the brain no bacterial RNA can be traced.

Our results show that the bacterial RNA extracted comes from the brain cells and not from contaminating bacteria. The following observations support this argument. The brains extracted from the infected frogs are sterile as shown by plating. This is confirmed by autoradiography, which shows no labeled bacteria even though it is difficult by this technique to score bacteria when there are less than 3×10^3 bacteria per brain. If we take into consideration only the autoradiographs and, for the sake of the argument, admit that we have missed 2×10^3 bacteria per brain, this amount could not account for the bacterial RNA found in the animals. Indeed, if, before RNA extraction, 10^7 bacteria labeled in vitro with [^{3}H]uridine (for 3 hours in the presence of brain tissues in Ringer solution) are added to five brains of control frogs labeled in vivo with [^{3}H]uridine, no bacterial RNA can be detected by our hybridization method (the total amount of RNA contained in 10^7 bacteria is negligible compared to the bulk of the animal RNA). Apparently the bacterial RNA in the brain cells depends on the capacity of these cells to synthesize their own RNA. Indeed when the brains of the infected frogs are labeled in vitro, the amount of bacterial RNA recovered from the brain cells decreases while the synthesis of frog RNA is lowered in the same cells. This is also an indication that the bacterial RNA extracted does not come from bacteria.

The bacterial RNA is synthesized directly in the brain cells. Indeed brains of infected frogs labeled in vitro still synthesize bacterial RNA. Under these conditions the labeled bacterial RNA cannot be transferred from labeled bacteria in the circulatory blood system into the brain cells. Besides, the bacterial RNA does not seem to be able to reach the cells before being broken down. Moreover, bacterial RNA is synthesized in frog auricles that have been in direct contact with the bacteria-free supernatant of *B. subtilis* (*16*). It is difficult to conceive of the same experiment with brains which survive in vitro with difficulty.

Purified bacterial DNA is not transcribed once injected, even though it readily penetrates the brain cells without losing its primary and secondary structures. Bacterial DNA can be transcribed in plant or animal cells only if it is accompanied by its own DNA-dependent RNA polymerase (*2, 16*) as seems to be the case when it is spontaneously released by living bacteria.

These results suggest that, after a bacterial infection, even an organ which has a protective barrier against bacteria can be reached by their informative molecules. The pathological or neurophysiological implications of these results are still difficult to evaluate.

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Hemoglobin Lepore Trait: Globin Synthesis in Bone Marrow and Peripheral Blood

Abstract. *There was decreased synthesis of the β-globin chain in the peripheral blood, and equal synthesis of α- and non–α-chains in the bone marrow of three patients with hemoglobin Lepore trait, similar to the findings in patients with heterozygous β-thalassemia. There is a relative instability of the synthetic mechanism for normal β-chain in these patients.*

Hemoglobin Lepore (Hb Lepore) is a structurally abnormal hemoglobin composed of two normal α-chains, and two chains containing an NH_2-terminal portion with the amino acid sequence of δ-chains, and a COOH-terminal portion with the sequence of β-chains (*1, 2*). The Lepore globin chains presumably arose from nonhomologous pairing and subsequent crossing-over between the genes for δ- and β-chains. Three different types of Lepore globin have been described which differ in the region of crossing-over: Hb Lepore Hollandia (*2, 3*) Hb Lepore Baltimore (*4*), and Hb Lepore Boston (*1, 5*). With Hb Lepore trait there is mild anemia, hypochromia, and microcytosis, similar to that seen in heterozygous β-thalassemia, while with homozygous Hb Lepore there is a severe anemic disorder similar to that with homozygous β-thalassemia. These clinical observations indicate that the synthesis of the Lepore chain is markedly decreased, as compared to that of α-chain.

There is a reduced synthesis of structurally normal β-chain in patients with β-thalassemia. In the peripheral blood of patients with heterozygous β-thalassemia, the amount of radioactive amino acid incorporated into the β-chain is approximately one-half that incorporated into the α-chain; in homozygotes, incorporation into β-chains is less than one-fourth that incorporated into α-chains (*6, 7*). A decrease in β-chain synthesis is also found in the bone marrow cells of patients with homozygous β-thalassemia (*8*). In con-