ence of this second enzyme may explain why complete β-galactosidase deficient mutants of *Neurospora* have not been reported.

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4. Ground, lyophilized mycelia were extracted into 0.0125*M* tris-HCl buffer, *p*H 8.0.
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Polyoma Virus: Production in Bacillus subtilis

Abstract. *Particles of complete polyoma virus are produced in competent* Bacillus subtilis *incubated with DNA isolated from purified, conventionally grown polyoma virus. The virus grown in* B. subtilis *is biologically identical to polyoma virus produced by animal cells. Quantitative parameters of the system have been established, and fluctuation tests indicate that viral replication occurs within the infected bacteria.*

Abel and Trautner (*1*) reported that vaccinia virus is formed by *Bacillus subtilis* when competent bacteria are incubated with DNA extracted from this animal virus. The virus obtained was biologically identical with conventionally grown virus used for DNA extraction. Biochemical evidence was not presented to show whether the virus replicates within the bacterium or whether the vaccinia DNA entering the bacterium merely directs the synthesis of new coat protein. Nevertheless, these important findings add further support to the concept of the universality of the genetic code. In addition, this system could be of importance for experimental virology if other animal viruses could be propagated in these bacteria, and if the synthesis of viral nucleic acids could be demonstrated to occur in this system. We now report the production of polyoma virus in *B. subtilis* and present indirect evidence for virus replication within the infected bacteria.

Competent cultures of *B. subtilis* 168-23 (a diauxotroph requiring histidine and indole) were prepared, and their competence was measured by methods previously described (*2*). Competence of the bacteria was determined by their ability to produce bacteriophage from purified phage DNA ("transfection," *3*) and to be transformed by homologous wild-type bacterial DNA. The degree of competence of the bacteria used for the polyoma experiments was such that, when they were incubated with bacteriophage SP8 DNA (1.2 μg/ml), 7.5×10^{-5} of them were infected and produced phages. The fraction of these bacteria transformed to histidine independence by exposure to DNA (1 μg/ml) from prototrophic bacteria was 2.3×10^{-3}. The total number of viable bacteria in the culture was 3.5×10^{7} per milliliter.

The "transfecting" capacity of polyoma DNA for this *B. subtilis* culture was tested essentially by the method described by Abel and Trautner (*1*). Two samples of polyoma DNA which contained 1.38×10^{12} and 1.26×10^{12} molecules per milliliter were tested. Polyoma DNA has a molecular weight of 3.5×10^{6}, is double-stranded and has a ring form (*4*). It was extracted from purified polyoma virus (*5*) by phenol (*4*), and samples were prepared by tenfold dilutions to contain from 10^{11} to 10^{2} molecules of DNA per milliliter. One-tenth milliliter of each dilution of polyoma DNA was added to 0.9 ml of the competent *B. subtilis* 168-23 culture described above and incubated at 37°C for 28 hours. The bacterial growth was removed and suspended in Eagle's medium, and the bacteria were disrupted by grinding with alumina or by grinding in a Nossal cell disintegrator. The debris was removed by centrifugation at 3000 rev/min and the supernatant was dialyzed against standard buffer at 4°C (*5*). The supernatant was tested for its plaque-forming capacity on monolayers of mouse-embryo cells (*6*) and for its focus-forming capacity on agar-suspension and monolayer cultures made with secondary fibroblast cells of rat embryo of the inbred Lewis strain susceptible to polyoma virus (*7*).

The data for the plaque-forming capacity are given in Table 1. The number of plaque-forming units (PFU) detected as a function of DNA concentration remains constant over a dilution range of five orders of magnitude; it decreases with further dilution of the polyoma virus DNA. No polyoma virus was produced with any concentration of polyoma DNA that had been incubated with deoxyribonuclease (20 μg/ml) before adding it to the competent bacteria. Both samples of polyoma DNA diluted to contain approximately 10^{6} molecules per milliliter resulted in the production of about 10^{6} PFU/ml, corresponding to about 1 polyoma PFU recovered for each molecule of DNA added to the competent bacteria. To explain these data the following possibilities were considered: (i) Most of the polyoma DNA molecules were taken up by the bacteria and simply enclosed in a protein coat, or (ii) only a certain fraction of the DNA molecules were incorporated, but those that were incorporated initiated the synthesis of more than one polyoma virus per infected bacterium.

To test these alternatives, a modified fluctuation test was performed. Competent *B. subtilis* were exposed, as in previous experiments, to polyoma DNA (10^{6} molecules/ml), evenly spread onto agar plates and incubated at 37°C for 28 hours. Twenty samples, each ap-

Table 1. The number of plaque-forming units (PFU) of polyoma virus formed by *Bacillus subtilis* as a function of polyoma DNA concentration.

Sample 1*		Sample 2*	
DNA concentration (molecules/ml)	PFU/ml	DNA concentration (molecules/ml)	PFU/ml
1.38×10^{11}	7.1×10^{5}	1.26×10^{11}	6.3×10^{5}
1.38×10^{10}	8.2×10^{5}	1.26×10^{10}	6.8×10^{5}
1.38×10^{9}	6.3×10^{5}	1.26×10^{9}	5.3×10^{5}
1.38×10^{8}	7.6×10^{5}	1.26×10^{8}	7.1×10^{5}
1.38×10^{7}	7.5×10^{5}	1.26×10^{7}	5.8×10^{5}
1.38×10^{6}	7.1×10^{5}	1.26×10^{6}	6.7×10^{5}
1.38×10^{5}	3.1×10^{4}	1.26×10^{5}	1.3×10^{4}
1.38×10^{4}	1.7×10^{2}	1.26×10^{4}	6.0×10^{2}
1.38×10^{3}	3.0×10^{1}	1.26×10^{3}	6.0×10^{1}
1.38×10^{2}	0	1.26×10^{2}	0

* All deoxyribonuclease-treated samples of polyoma DNA were inactive in this assay.

proximately 1/1000 of the total area of the agar surface, were punched out with Pasteur pipettes, and the bacteria were dispersed in 1 ml of Eagle's medium by treatment with high frequency sound. By microscopic count each sample contained 1×10^8 bacteria per milliliter with a maximum deviation of 10 percent. The bacterial suspensions were processed as previously described and assayed for their plaque-forming capacity. The number of PFU found in the entire contents of each of these 20 samples were: 8, 34, 228, 2546, 0, 108, 8, 1250, 82, 46, 0, 153, 1864, 504, 63, 0, 124, 564, 423, and 64. Since the numbers of PFU from individual samples are not normally distributed, the results are consistent with the hypothesis that a fraction of the bacteria synthesizes more than one polyoma particle per molecule of DNA taken up. The ratio of physical particles to plaque-forming units of polyoma virus produced by *B. subtilis* has not yet been established. It is likely, however, that this ratio is similar to that of conventionally grown virus, which is about 100:1. If this assumption is valid, the hypothesis of *de novo* synthesis of virus would be further strengthened.

Attempts were made to determine the time required for synthesis of polyoma virus by competent bacteria. Parallel samples of *B. subtilis* exposed to polyoma DNA were spread onto agar plates and incubated at 37°C. At intervals thereafter the bacterial growth was harvested from a set of plates, processed as previously described, and the supernatant was assayed for plaque-forming capacity. No plaques were found in samples incubated less than 12 hours, and the maximum number was produced by samples incubated 24 hours or longer. Because of technical inconsistencies these data are not completely decisive, but they indicate that under comparable conditions intact polyoma virus is synthesized much more slowly than are the bacterial viruses that infect *B. subtilis*.

The capacity of polyoma virus grown on *B. subtilis* to transform normal secondary fibroblasts from susceptible rat embryo of the inbred Lewis strain into neoplastic cells was tested in agar-suspension and monolayer cultures (*7*). Normal cells in contact with a solid substrate and immersed in a liquid nutrient medium grow rapidly until the solid substrate is covered by a continuous layer of cells, often the thickness of a single cell (monolayer). Then growth stops. The cessation of growth is caused by regulatory mechanisms which are sensitive to the establishment of reciprocal contacts between cells. When normal cells are infected by cancer-producing virus, some of them undergo, in one or more steps, a characteristic change called transformation. The decreased response to regulatory influences, which is characteristic of transformed cells, has made it possible to develop methods for assaying the transforming titer of virus preparations. In a monolayer culture the transformed cells that arise after virus infection grow to form easily recognizable colonies, called foci.

Statistical considerations show that a focus is produced by a single virus particle, thus the number of foci is a measure of the transforming titer of the virus. The transforming capacity of polyoma virus grown in *B. subtilis* was the same as that of conventionally grown virus. The ratio of PFU to FFU (focus forming units) was 10^3:1 in both cases. The cellular efficiency was about 1 percent.

The plaque-forming and focus-forming capacity of polyoma virus grown in *B. subtilis* was destroyed by antibody at the same rate as that for purified polyoma virus used to prepare antibody. Neither of these capacities was affected by treatment with deoxyribonuclease.

Thus, polyoma virus, a small DNA-containing tumor virus, has been grown in *B. subtilis* and the data indicate that polyoma DNA is probably synthesized in the infected bacteria.

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Delayed Appearance of Labeled Protein in Isolated Nerve Endings and Axoplasmic Flow

Abstract. *Subcellular fractions of mouse brain were prepared by differential and sucrose density-gradient centrifugation at intervals up to 130 hours after intracerebral injection of C^{14}-labeled leucine. The specific activity of the nerve ending fraction continued to rise during this period. Between 2 and 130 hours after injection there was a fivefold rise in the specific activity of the soluble protein prepared by lysing the nerve ending particles with water while the specific activity of the soluble protein from the whole homogenate concomitantly fell almost by a factor of three. The data are interpreted as being consistent with the appearance of protein in nerve endings by axoplasmic flow.*

The hypothesis that neuronal protein flows from its site of synthesis in the body of the nerve cell through the axon to the nerve ending has been supported by nerve constriction experiments (*1*) and by autoradiographic studies (*2*). Much of the evidence for this phenomenon has been reviewed by Weiss (*3*).

Recently, methods have been developed (*4, 5*) for the isolation of nerve endings from brain homogenates by centrifugation of a "crude mitochondrial" fraction through a discontinuous sucrose gradient. In the experiments reported here it was found that this technique could be used to measure the appearance of radioactive protein in nerve endings after animals had been injected with labeled precursor.

Female Swiss albino mice (*6*) 12 to 13 weeks old, each weighing 28 to 32 g, were anesthetized with ether and bilaterally injected with a total of 20 μl of leucine-1-C^{14} (*7*) containing 4.8×10^5 count/min. The injections, made with a microsyringe and a 25-gauge needle, were placed 1 to 2 mm lateral to the midline of the skull on a line drawn between the anterior insertion of the ears at a depth of 3.5 mm from the surface of the scalp. The animals (four in each group) were killed by decapitation and the cerebral hemispheres were removed by transection at the level of the midbrain. The remainder of the brain was discarded. The cerebral hemispheres were homogenized and subcellular fractions were prepared by the method of Gray and Whittaker (*4*), except that the crude