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

mitochondrial fraction was washed an additional time and that the "microsomes" were separated from the "soluble" fraction by centrifugation at 100,000*g* for 1 hour. Sucrose gradient centrifugation was performed (*4*) on the crude-mitochondrial fraction. The "myelin" fraction is the material at the interface between 0.32 and 0.8*M* sucrose; the "nerve ending" fraction is the material at the interface between 0.8 and 1.2*M* sucrose; and the "mitochondrial" fraction is the pellet at the bottom of the tube, the surface of which was rinsed twice with 0.32*M* sucrose before collection. Lysis of the nerve-ending fraction with water was effected since it has been shown (*8*) that "soluble" and "particulate" constituents can be isolated after such treatment. This was performed by dilution of the nerve-ending fraction from the sucrose gradient by dropwise addition of an equal volume of water, recovery of a pellet by centrifugation at 100,000*g* for 1 hour, and lysis of this pellet by suspension in 5 ml of water. The lysed nerve-ending fraction was separated into particulate matter (pellet of endings) and a supernatant (soluble portion of endings) by centrifugation at 100,000*g* for 1 hour. "Soluble" material was obtained from the myelin, mitochondrial, and crude-mitochondrial fractions as indicated in Table 2.

Table 1 shows the specific activities of the proteins isolated from the various subcellular fractions. The only major fraction which showed a continued rise in specific activity of protein over the period studied was the nerve-ending fraction. The substantial amount of labeling of this fraction at 2 hours need not be taken to indicate that there is biosynthesis of protein in the nerve endings proper, since this fraction is known (*4*) to be contaminated by membranous material which may derive from the cell body. This membranous material may well be what is rapidly labeled in the nerve-ending fraction. When the nerve-ending fraction from animals killed 2 hours after injection was lysed the "soluble" protein thereby released had a specific activity which was only 9 percent of that of the soluble protein of the whole homogenate. The specific activity of the soluble protein of the endings increased from 49 to 219 in the interval between 2 and 49 hours and to 254 at 130 hours.

In contrast to this, the specific activity of the whole homogenate and of the soluble fraction of the whole homogenate fell substantially during this period. Treatment of other "particulate" fractions with water also released some protein which remained in the supernatant after centrifugation at 100,000*g* for 1 hour. In none of these other fractions was a prominent progressive increase in specific activity of "soluble" protein found (Table 2). The fact that these specific activities do not fall as quickly as that of the soluble protein from the whole homogenate may be due, in part, to contamination with some nerve endings as is obviously the case with the crude-mitochondrial fraction.

The magnitude of the alteration in specific activity of the soluble protein from the endings is emphasized in Table 3 which shows that its specific activity rises from 9 percent to 141 percent that of the specific activity of the soluble protein from the whole homogenate in the period between 2 and 130 hours. The data are expressed in ratio form in an attempt to cancel out the influence of the turnover of brain protein during the course of the experiment which would tend to obscure the progressive increment in radioactive protein in the nerve endings. The finding that the specific activity of the soluble protein of the endings is greater than that of the whole homogenate at 130 hours is probably due to the fact that the latter is derived from both neurons and glia whereas the former is presumably derived only from neurons which may have a higher rate of incorporation of amino acids into protein.

Since it is theoretically possible that the progressive appearance of labeled protein at the nerve endings is due to delayed incorporation of some precursor into protein at this site, the amount of residual precursor was estimated by adding trichloroacetic acid (to a concentration of 10 percent) to a portion of the whole homogenate and

Table 1. Specific activity (count min^{-1} mg^{-1}) of protein derived from subcellular fractions. The fractions were diluted to 5 ml and trichloroacetic acid was added to a concentration of 10 percent. The mixture was heated at 90° to 95°C for 15 minutes, chilled on ice for 15 minutes, centrifuged, and the precipitate was washed twice with 10 percent trichloroacetic acid, twice with ethanol-ether (2:1) and then twice with ether. The residue was dissolved in 5 ml of 0.1*N* NaOH and portions were removed for protein determination (*10*), 30 to 60 μg of protein being used in 4.4 ml of reaction mixture. Protein content was read from a standard curve (prepared with crystalline bovine serum albumin) which was linear in the concentration range used for the unknowns. Ten micromoles of leucine were added to portions containing 1 mg protein (albumin was added to make 1 mg if necessary), HCl was added to neutrality, and trichloroacetic acid was added to give 5 ml of a 10 percent solution. The samples were kept on ice for 2 hours, then poured onto Millipore filters (pore size 0.45 μ) which were then washed three times with 10 percent trichloroacetic acid. The filters were mounted on planchettes, dried, and counted on a low background gas flow counter.

Fraction	Hours after injection					
	2	4	13	27	49	117
Homogenate	333	352	328	346	268	177
Nuclei	398			330		163
Microsomes	492	572	550	520	414	202
Soluble	534	568	530	589	406	219
Myelin	203	244	254	252	214	164
Mitchondria	247	222	260	271	253	165
Nerve endings	112	121	145	161	166	171
	After lysis with water					
Particulate of endings	132	147	154	198	170	168
Soluble portion of endings	49	58	102	145	219	218

Table 2. Specific activity (count min^{-1} mg^{-1}) of "soluble" protein obtained from various fractions. The crude mitochondrial and purified mitochondrial fractions were suspended in 0.32*M* sucrose and centrifuged at 100,000*g* for 1 hour. The myelin and nerve-ending fractions were removed from the sucrose gradient, diluted with 2/3 or 1 volume of water, respectively, and centrifuged at 100,000*g* for 1 hour. Each of the pellets were then carefully suspended in 5 ml of water and centrifuged at 100,000*g* for 1 hour. The resultant supernatants were taken to be the "soluble" portion of each fraction.

Soluble portion of	Hours after injection		
	3	40	130
Homogenate	600	401	182
Crude mitochondria	180	221	189
Myelin	182	240	214
Mitochondria	377	309	214
Nerve endings	68	175	254

determining the radioactivity in the material which remained soluble after this treatment. Two hours after injection each whole brain contained only about 150 counts per minute of radioactive material which was soluble in trichloroacetic acid; 4 hours after injection there was virtually no detectable radioactivity in the trichloroacetic acid-soluble fraction. Therefore the large amount of precursor which was injected either escaped from the brain or was incorporated into protein within a relatively short period of time so that precursor, sufficient to account for the delayed incorporation observed, was not, apparently, present. It thus seems more reasonable to interpret the results as indicating that protein that is synthesized in the nerve cell body in the period shortly after injection is transported, by axoplasmic flow, to the nerve ending. What is probably being measured here is the time required for protein to flow from the various cell bodies along axons of various lengths to nerve endings. The average rate of axoplasmic flow cannot be calculated since distances traveled are not known. The data suggest that some cell bodies are close enough to their nerve endings to transmit new protein to the endings within hours after its synthesis in the cell body; and that within days a substantial number of nerve cell bodies have transmitted newly synthesized protein to their endings. These observations are also of interest since it should be possible to use this method to measure relative rates of appearance of new protein at nerve endings under various experimental conditions whose influence on axoplasmic flow could thus be estimated. It should also be noted that although the progressive increment in radioactive protein in the nerve endings is more prominently shown in the "soluble" component of this fraction, there is also an increase in the radioactivity of "particulate" protein of the endings between 2 and 27 hours, which is more marked than the increments seen in the other fractions. Whether or not this is due to axoplasmic flow of "particulate" components of the nerve endings, as suggested by other studies (*9*), cannot be stated with confidence since the changes are not as prominent as those found with the "soluble" protein and could be due to incomplete lysis of the endings.

Table 3. Ratio of specific activity of "soluble" protein from nerve endings to specific activity of "soluble" protein from whole homogenate.

Hours after injection	Ratio
2	0.09
3	.11
4	.10
13	.19
27	.25
40	.44
49	.54
117	1.00
130	1.41

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Staphylolytic Enzyme from Chalaropsis: Mechanism of Action

Abstract. *The staphylolytic enzyme recently isolated from cultures of a* Chalaropsis *species by Hash is shown to be an acetylmuramidase that cleaves all the glycosidic linkages of* N-*acetylmuramic acid and* N,O-*diacetylmuramic acid in the cell wall of* Staphylococcus aureus *strain Copenhagen. It is similar in specificity to the "32 enzyme" from* Streptomyces albus *but it differs from egg-white lysozyme whose activity is inhibited by the presence of O-acetyl groups.*

Hash has reported (*1*) that an unidentified species of the fungus *Chalaropsis* produces several extracellular enzymes which lyse *Staphylococcus aureus* and several other gram-positive bacteria. One of these enzymes, the B enzyme, obtained as a relatively pure protein, catalyzed hydrolysis of the cell wall of *S. aureus*, with liberation of reducing groups and of material reactive as acetylhexosamine in the Morgan-Elson reaction. We now report that this enzyme is an acetylmuramidase, similar to the "32 enzyme" isolated from *Streptomyces albus* G by Ghuysen *et al.* (*2*); it catalyzes hydrolysis of glycosidic linkages of both *N*-acetylmuramic acid and *N,O*-diacetylmuramic acid in the cell wall of *Staphylococcus aureus* (*3, 4*).

The methods used were the same as those used to study the "32 enzyme" (*3, 4*). Purified cell walls of *S. aureus* strain Copenhagen (300 mg) were incubated for 11 hours at 37°C in 11 ml of 0.025*M* acetate, *p*H 4.7, with 0.5 mg of B enzyme from *Chalaropsis* (*5*). Lysis of cell walls (measured by decrease of turbidity) and maximum hydrolysis of glycosidic linkages (measured by reducing power) were reached after 6 hours of incubation (Fig. 1). The cell walls contained equal amounts of acetylmuramic and glutamic acids (0.47 μmole/mg), and 0.46 μmole of reducing group was liberated, indicating that one glycosidic linkage had been cleaved for each repeating unit.

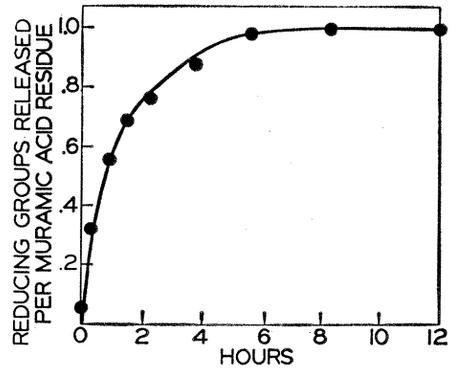

Fig. 1. Release of reducing power during the lysis of cell walls of *S. aureus* strain Copenhagen (300 mg) by B enzyme from *Chalaropsis* (0.5 mg) in 11 ml of 0.025*M* acetate, *p*H 4.7, at 37°C.