numerous mononuclears and granulocytes, many of which contained brightly fluorescent coccoid bodies (Fig. 1E). Gram stains of tissues revealed that such cells contained Gram-positive cocci. Specimens of skin taken from areas remote from the inflammatory lesions showed only very few macrophages, none of which contained any fluorescence. Specimens of skin taken from lesions of delayed hypersensitivity in three of five animals challenged intravenously with RITC-labeled streptococci had large numbers of streptococcal chains in the tissue debris in the dermis as well as within phagocytic cells at the site of inflammation. Two animals that developed delayed hypersensitivity to BGG and were challenged with TiO_2 particles showed accumulation of the particles in the inflammatory sites. Both animals that developed delayed hypersensitivity to BGG and that were challenged intravenously and intraperitoneally with FITC-labeled goat antiserum to BGG showed the regular pattern of macrophage accumulation in the skin at the site of delayed hypersensitivity lesions, but no trace of fluorescence was found in any of the cells. On the other hand, examination of the peritoneal exudates of these animals showed that many of the granulocytes and macrophages had brightly fluorescent amorphous intracellular masses, which indicates that the phagocytes had taken up the labeled protein.

Two rabbits that were injected with TF and challenged with FITC- or RITC-labeled streptococci developed endocardial lesions that consisted of large accumulations of granulocytes and fibrin-like amorphous material. The majority of granulocytes were filled with large numbers of brightly fluorescent cocci. Also two guinea pigs that developed delayed hypersensitivity to BGG and were challenged intraperitoneally with FITC-labeled streptococci had focal lesions in the peritoneal linings in which large numbers of granulocytes containing fluorescent cocci were found.

Results of this preliminary study indicate that streptococci or inert particles circulating in the blood or in the peritoneal cavity later on become localized within phagocytic cells in sites of tissue injury previously produced by a streptococcal "toxin" or by immunological reactions of delayed hypersensitivity in rabbits or guinea pigs, respectively. Chase (*10*) noted a similar phenomenon in which guinea pigs injected with complete Freund's adjuvant developed spontaneous disseminated granulomata. Once dissemination had occurred, various stimuli such as the injection of tuberculin or the application of skin irritants produced new granulomatous lesions in the skin at the challenge sites.

While the uptake and localization of both cocci and titanium particles seem similar, and therefore nonspecific, the importance of the former lies in the demonstrated capacity of some streptococcal components to persist for long periods of time and to produce and perpetuate chronic lesions (*11, 12, 7*). Furthermore, not only the toxic factor used in this study but also other streptococcal products [streptolysin O (*13*), streptolysin S (*2, 14*), proteinase (*15*), and others] can cause cellular damage and produce tissue trauma. It is thus tempting to speculate that the combination of naturally occurring group A streptococcal infection (producing circulating "toxic factors"), when followed by a bacteremia or by circulatory transport of cocci-laden phagocytes, might lead to chronic nonsuppurative sequelae. Clearly, however, the outcome of the streptococcal localization in distant, toxin-traumatized tissues must depend on the length of persistence of some of their products in tissues. Long persistence of streptococcal products in rabbits was found to cause chronic inflammatory lesions in rabbit skin (*11*) and, as demonstrated elsewhere (*7*) and in this study, in other rabbit organs as well.

Also, Mallory and Keefer (*16*) observed lesions of multiple organs in fatal cases of infections caused by hemolytic streptococci. These were late lesions with monocytic elements in tissues of patients with bacteremia who survived more than 10 days. It is intriguing to consider the possibility that the combination of effects seen in these patients could have been caused in a fashion similar to what has been observed in the rabbit model. Finally, it is possible that chronic diseases of obscure etiology may be caused by similar mechanisms involving bacteria other than hemolytic streptococci.

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Herpes Simplex Virus: Dry Mass

Abstract. *Dry mass of herpes simplex virus particles was measured by quantitative electron microscopy after isolation by surface spreading and critical-point drying of infected cells. The core weighed about 2×10^{-16} gram, the empty naked capsid 5×10^{-16} gram, the full naked capsid 7×10^{-16} gram, and the enveloped nucleocapsid 13×10^{-16} gram.*

Herpes simplex virus strain 11140 (*1*) was grown in monolayer cultures of a line of kidney cells derived from LEW-BN rats. Virus particles were isolated by spreading the cells, 20 to 40 hours after infection, on a trough filled with distilled water at *p*H 6.4. For electron microscopy, they were

picked up by touching the surface with grids coated with carbon Formvar and then dried by the critical-point method (*2*). Grids prepared in this way usually contained several disrupted interphase nuclei, with only remnants of cytoplasmic structures such as mitochondria. Virus particles could be found within the nucleus or lying separately around the nucleus. These unstained virus particles could be identified in various stages of maturation (Fig. 1). Isolated virus particles were photographed at 100 kv in a Siemens Elmiskop I at a magnification of 34,000 to 38,000. In electron microscopy, the object's mass (relative dry weight) is proportional to the transmission for visible light of the object's image in the electron micrograph (the negative), provided that certain operating conditions of the microscope and of the photographic processing are fulfilled (*3*). Quantitative photometric evaluation of the electron micrographs was carried out with an integrating photometer (IPM-2, Carl Zeiss, Oberkochen, West Germany), an instrument specifically designed for this purpose. Absolute dry weights of the virus particles were determined in proportion to small polystyrene spheres of known masses (serving as standards) which were photographed under the same conditions (*3*).

Table 1. Diameter and dry mass of herpes simplex virus particles dried by the critical-point met od. Results are given as means plus or minus the standard deviation.

Diameter (Å)	Dry mass (10^{-16} g)
Core	
676 ± 76	2.07 ± 0.95
Empty capsid (maximum)	
1062 ± 89	5.22 ± 1.10
Full capsid (minimum)	
973 ± 78	7.55 ± 1.11
Enveloped nucleocapsid	
1335 ± 241	13.33 ± 2.56

The results of our mass determinations on particles of herpes simplex virus (19 enveloped, 31 full, 17 empty capsids, 13 cores) are summarized in Table 1. Enveloped nucleocapsids had the highest degree of variation in their dry mass, with a range from 10.7 to 19.4 × 10^{-16} g. Naked, empty, and full capsids were often photographed together on the same plate so that comparison of their mass became very accurate (*3*). The diameters (maximum 1062 Å, minimum 973 Å) of the hexagonal shape of these capsids dried by the critical-point method agree well with the average diameter of 1050 Å of herpes capsids measured in negatively stained preparations (*4*). The difference of 2.33 × 10^{-16} g found between full and empty capsids is considered to reflect the mass of the core. A similar weight of 2.07 × 10^{-16} g was found for isolated round to oval structures resembling cores. Our value for the complete core is higher than the weight of 1.66 × 10^{-16} g for the total amount of DNA of herpes simplex virus, calculated from the average molecular weight of 100 × 10^6 daltons for herpes simplex DNA (*5*). If we calculate the specific densities (mass per volume) of the measured cores, considering them as spheres, the densities of 1.28 g/cm³ (core weight, 2.07 × 10^{-16} g) or 1.44 g/cm³ (core weight, 2.33 × 10^{-16} g) are considerably lower than the buoyant density 1.727 for pure herpes simplex DNA (*6*). The standard deviations of the measurements reported here do not allow a definitive interpretation of this difference in specific densities; but one cannot exclude the possibility that the lower density of the core may be due to some association of protein with the DNA. We also measured ten isolated, smaller, round structures which had a diameter range of 304 to 473 Å and a weight range of 0.3 to 0.6 × 10^{-16} g. These structures may be that part of the core which is composed of DNA.

We think that this first attempt to measure directly the mass of individual virus particles by electron microscopy is a valuable quantitative procedure for characterizing viruses. The preparative method, furthermore, allows one to isolate viruses from small amounts of cellular material without further purification to localize the virus particles within the cell and to study the morphology of viruses without any staining or contrasting procedure.

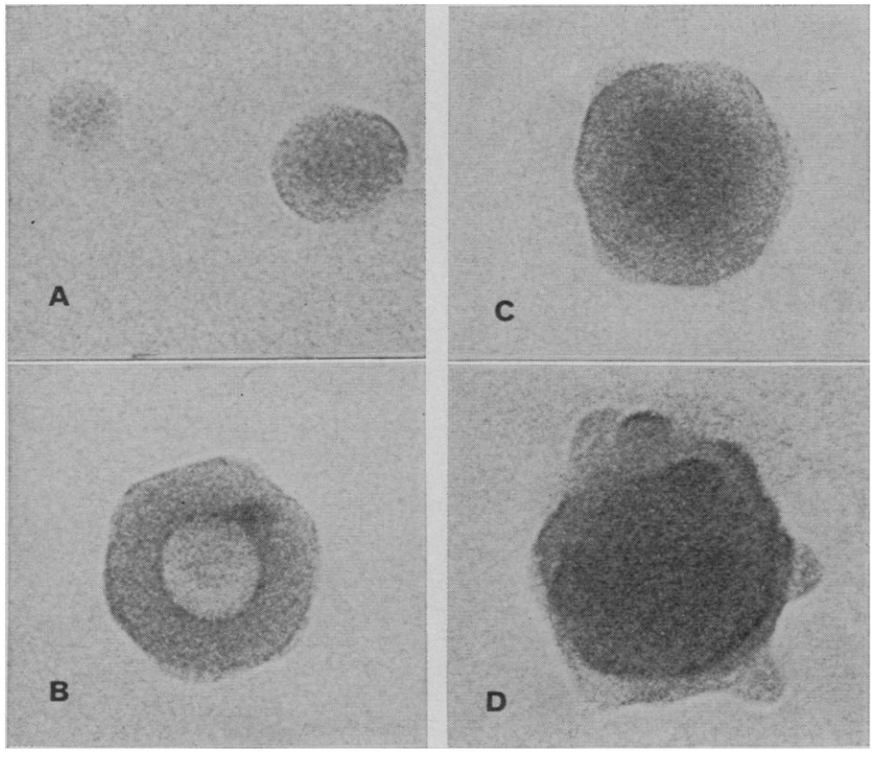

Fig. 1. Steps of virus maturation as seen in unstained herpes simplex particles dried by the critical-point method. (A) Core; (B) empty capsid; (C) full capsid; (D) enveloped nucleocapsid (× 280,000).

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Memory in the Japanese Quail: Effects of Puromycin and Acetoxycycloheximide

Abstract. *Intracerebral injections of puromycin produced memory deficits in naive quail trained to discriminate between red and green stimuli. Puromycin aminonucleoside, acetoxycycloheximide, and saline had no such effect. After a single reversal of the visual cues, naive quail treated with puromycin performed better than control birds. Also, puromycin had no effect on performance when injected into previously trained animals. High doses both of puromycin and acetoxycycloheximide inhibited ribonucleic acid and protein synthesis to a similar extent, while low doses of puromycin inhibited only protein synthesis. Since only puromycin inhibited memory, the basis for its effect appears more likely to be mediated by the action of peptidyl-puromycin rather than by the quantitative inhibition of macromolecular synthesis or by some nonspecific toxic action.*

The prevailing theory of memory indicates that it is composed of at least two phases (*1*). The first or short-term phase is thought to be held by neuronal reverberatory electrical activity, which in time gradually gives way to a second more stable phase called the long-term memory or engram. The transition from the transient to the more permanent state is called consolidation and is thought to involve some sort of electrical interaction with the synthesis of macromolecules such as DNA, RNA, and protein. Methods which disrupt or severely reduce electrical activity—such as electroconvulsive shock, hypothermia, and deep anesthesia—have been used to interfere with consolidation and, consequently, the establishment of the engram. A more recent approach has been to inject agents which inhibit the synthesis of a particular macromolecule and, therefore, consolidation, and to observe and correlate the resulting biochemical changes with the concomitant changes in the animal's performance (which serves as an index of memory). The foregoing methods have been found to be effective in producing memory deficits only when employed shortly after (usually minutes to hours) the initial exposure to training.

Of the various inhibitors used to investigate memory only puromycin dihydrochloride has consistently produced deficits in escape behavior in mice and in avoidance responses in goldfish (*2*). Although there is still considerable question concerning the extent and nature of puromycin's action (*2*)—behavioral as well as biochemical and physiological—the focus of this report will be on the interaction of puromycin with macromolecular syntheses to test the macromolecular engram hypothesis.

Adult male Japanese quail (*Coturnix coturnix japonica*) were maintained at 85 percent of their free-feed weight. They were trained in a modified pigeon test chamber to discriminate between a red and a green light by reinforcing with food their responses (pecks) to the green light (*3*). Programming equipment successively presented, in a counterbalanced order, ten color stimuli on the translucent pecking key. Each stimulus presentation lasted for 60 seconds and was followed by an intertrial time-out period of 15 seconds during which time the key was not illuminated. All stimuli were equated for brightness. Correct responses were reinforced on a variable interval schedule of 30 seconds with 6.9 seconds access to food (Purina game bird chow). During each reinforcement, the stimulus light was turned off and the food hopper light was turned on. The total number of responses emitted during each stimulus presentation and the number of reinforcements delivered were recorded on digital counters. At the end of their first day's training (day 1)—which lasted approximately 12 minutes—the animals were placed in a stereotaxic apparatus and intracerebrally injected, with a microsyringe with a 30-gauge needle, at each of four sites with 10 μl of the appropriate solution. Two injections were made into each hemisphere to a depth of 3 mm. Unilateral sites were 3 mm from midline. The rostral sites were 2 mm anterior, while the caudal sites were 1 mm posterior to the interaural line. All injections were made slowly and were complete within 5 minutes after the last training trial. After a 3-day recovery period, training was resumed on day 4 and was continued daily until the criterion of 90 percent correct responses (100 times the responses to the reinforced stimulus divided by the number of total responses) was reached for two consecutive days. All subjects that did not respond on day 1 or day 4 or exhibited a bias by giving a correct response of greater than 90 percent on day 1 were eliminated from the experiment (*4*).

In experiment 1 (Table 1), four experimental groups (*5*) of quail were injected with either of two concentrations of puromycin (45 and 180 μg/site), puromycin aminonucleoside (PANS, 90 μg/site), or acetoxycycloheximide (AXM, 6 μg/site). All agents were prepared in 0.9 percent saline and adjusted to *p*H 7 with 1*N* NaOH. Control animals were injected with 0.9 percent saline solution. The group injected with PANS was used to determine whether

Table 1. Percentage of correct responses and days to criterion for naive control and experimental groups to a successive color discrimination. Green was the reinforced stimulus throughout experiment 1; in experiment 2, the stimulus on day 1 was green, and on day 4 and after the stimulus was red. Injections, in both experiments, were given immediately after training on day 1. Results are expressed as the mean ± standard deviation, with significant *t* scores, based on a one-tailed Dunnett's test, in parentheses. Abbreviations are: PANS, puromycin aminonucleoside; AXM, acetoxycycloheximide; and P, puromycin.

Group (μg/site)	Number	Percentage of correct responses		Days to criterion
		Day 1	Day 4	
		*Experiment 1**		
Saline	39	61 ± 16	78 ± 16	5.3 ± 1.7
PANS(90)	9	57 ± 14	77 ± 17	5.4 ± 1.6
AXM(6)	12	62 ± 15	80 ± 17	6.2 ± 2.8
P(45)	13	60 ± 14	66 ± 14 (2.30†)	6.7 ± 1.8 (2.28†)
P(180)	10	58 ± 20	60 ± 16 (3.27‡)	7.3 ± 1.0 (2.98§)
		Experiment 2 ||		
Saline	17	55.5 ± 18.9	24.9 ± 13.6	13.3 ± 2.57
P(180)	10	50.1 ± 14.8	36.8 ± 21.1	10.0 ± 2.45 (3.01‡)

* d.f. = 78. † $P < .05$. ‡ $P < .005$. § $P < .01$. \|\| d.f. = 25.