Incorporation of Heterologous Deoxyribonucleic Acid into Mammalian Cells

Abstract. Particles containing large molecular heterologous deoxyribonucleic acid have been incorporated into strain L cells in suspension tissue culture. The particles are Feulgen-positive, they are fluorescent under ultraviolet light when stained with acridine orange, and they appear in both the nucleus and cytoplasm in radioautographic preparations.

The phenomenon of transformation in bacteria is well established (*1*). At the present time there is no definite evidence of transformation in mammalian cells. The recent work with purified deoxyribose nucleic acid of polyoma virus may be an example of this phenomenon, although it remains to be proved whether viral nucleic acid is incorporated into the genetic apparatus of cells or whether it merely acts as a stimulus to cell division (*2*). The incorporation of labeled nucleic acid into cells has been reported by several investigators (*3*). In all of these experiments it has remained uncertain whether degradation occurred at the cell membrane and whether the label really represented intact nucleic acid or nucleotides and simple base components.

Previous work in this laboratory has shown that strain L cells grown in suspension tissue culture are able to phagocytize large particles and continue to divide in logarithmic growth (*4*). We have found that soluble deoxyribonucleic acid (DNA) does not enter these cells in appreciable amounts under these conditions. Therefore, we have incorporated particles containing high molecular DNA and protein into cells by phagocytosis. Initial attempts at particle formation involved the adsorption of nucleic acids onto resin and activated charcoal particles. Eventually, a system of particle formation by coacervation of protein and nucleic acid was devised, as follows: DNA was extracted from a variety of bacterial and mammalian cells by the salt extraction method of Zamenhof (*5*), the chloroform method of Hotchkiss (*6*), and the phenol method of Kirby (*7*). Dilute aqueous solutions of DNA (0.5 to 10 mg/ml) were coacervated by the addition of an equal volume of a gelatin solution (0.25 to 1 percent) after adjustment of the *p*H to 3 to 4 in a water bath at 50°C. The size of the resulting individual particles depends on the concentration of both the DNA and the gelatin and may vary between 0.5 and 50 μ. The progressive increase in the size of the particles with continued incubation in the water bath may be inhibited by denaturation or fixation of the protein component. Preparations used in these experiments were fixed by the addition of a small amount of a 2.5 percent solution of glutaraldehyde. The particles were dialyzed for 48 hours against distilled water to remove the excess glutaraldehyde, and thereafter they could be stored indefinitely at 4°C (*8*). The protein, determined by the phenol reagent (*9*), measured 88 percent, and DNA, determined by the indole method (*10*), measured 12 percent.

The DNA particles (0.5 ml concentrated by centrifugation) were incubated with 13 ml of strain L cells (5×10^5 cells per milliliter) in a 50-ml erlenmeyer flask on a rotary shaker at 37.5°C for 2 hours. The cells were then washed free of particles and grown as monolayers on cover slips in Leighton tissue-culture tubes. The cover slips were removed at intervals and stained with Feulgen's stain for DNA. In Fig. 1 (top left) Feulgen-positive particles may be clearly seen, distributed throughout the cytoplasm.

In other experiments, DNA was extracted by the phenol method and bound to acridine orange by incubation, for 5 minutes at 23°C, of the dye (20 μg/ml) and the nucleic acid (200 mg/100 ml).

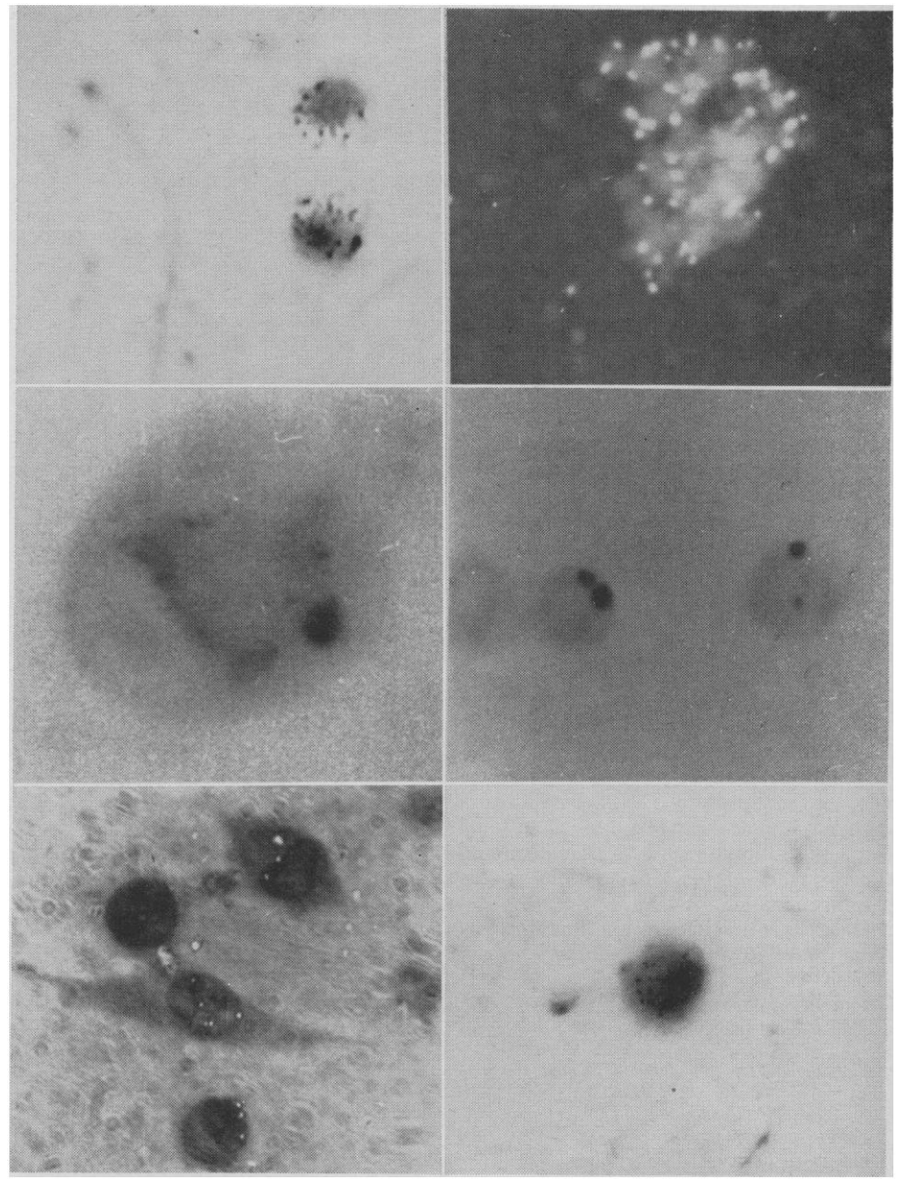

Fig. 1. The incorporation of heterologous DNA-protein particles into the nucleus and cytoplasm of strain L cells by phagocytosis (about × 508). (Top left) Feulgen-positive particles appearing in cytoplasm. (Top right) Radioautograph of a centrifuged clump of DNA-protein particles. (Middle, left and right) Particles of DNA and protein previously complexed with acridine orange, as shown by fluorescence microscopy. (Bottom, left and right) Radioautographs of cells grown on cover slips, with particles in the cytoplasm and nucleus, as shown by light- and dark-phase microscopy after staining with Kernechtrot.

Particles were subsequently made by coacervation with gelatin. Examination of cells by fluorescence microscopy showed the presence of DNA particles in the cytoplasm (Fig. 1, middle).

Since the nuclear DNA obscured the possible presence of particles in the nucleus when the cells were examined after staining with both Feulgen and acridine orange, a third method of following particles was devised. A mouse with sarcoma 180 in the ascitic form was inoculated with tritiated thymidine (total, 1.4×10^6 count/min; specific acitivity, 1.9 c/mmole). After 2 hours the cells were removed from the peritoneal cavity and carefully washed free of extracellular thymidine, and the DNA was extracted by the phenol method. Dialyzed particles (0.5 ml, with a total of 30,000 count/min), prepared by coacervation of protein and DNA labeled with tritiated thymidine, were incubated with the cells, as described above, in the presence of unlabeled thymidine (10 mg/100 ml). This relatively enormous concentration of thymidine was considered sufficient to act as a metabolic trap which would prevent any labeled thymidine, released as a result of membrane degradation of the large nucleic acid molecule, from reaching either the cytoplasm or nucleus. The cells were fixed in a solution of acetic acid and alcohol (1:3) and NTB_3 emulsion used in the preparation of radioautographs. Radioautographs showed the presence of many particles over both the nucleus and cytoplasm (Fig. 1, bottom).

It is generally accepted that when cells are grown in a spread fashion on cover slips, the amount of cytoplasm overlying the nucleus is too thin to contain particles or detectable quantities of labeled thymidine.

It has been reported that fibroblasts in vivo incorporate DNA by phagocytosis (*11*), as do white blood cells in the lupus phenomena (*12*). Determination of whether this has any biological significance and whether true transformation is possible in mammalian systems must await the development of genetic markers similar to those used so successfully with bacteria. An abstract describing our work has previously been published (*13*, *14*).

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14. The work discussed in this report was supported by grant No. C2928 (C3) from the U.S. Public Health Service and the Jane Coffin Childs Memorial Fund.

19 September 1960

Time-out from Positive Reinforcement

Abstract. When an organism can itself impose extinction during fixed-ratio food reinforcement, the duration of the extinction period is a function of the number of responses required for reinforcement. Typically, the subject imposes extinction at the start of the usual fixed-ratio run.

When a response is reinforced, or rewarded, in the presence of a given stimulus, then that stimulus becomes the occasion for more responses. Another stimulus, in the presence of which no reward is obtainable, may be used as a sort of "time-out" condition. It has been shown that time-out can function either to reward or to punish behavior (*1*). Typically, the time-out condition has been introduced at infrequent intervals and for fixed durations. The subject's tendency to initiate or prolong a period of time-out has not been continuously measured. To overcome this limitation, a procedure has been devised in which the organism may initiate, or terminate, a period of time-out at any time. The procedure makes it possible to discover when an organism will initiate a time-out period and how long it will allow the time-out to continue, as functions of the underlying schedule of reinforcement.

A pigeon, at 80 percent of the weight it maintains when allowed to feed freely, is conditioned to peck a plastic disk through reinforcement with food immediately after each peck. Food is then delivered only after every 50 responses —a so-called fixed-ratio schedule of reinforcement. Simultaneously, a second key, the time-out key, is made continuously available to the subject. A single response on this key changes the color and intensity of the ambient illumination, as well as of the light projected on the two translucent response keys. Under the changed illumination, all responses on the food key are ineffective in producing food. As a result, responding on the food key soon drops to zero. However, a second response on the time-out key restores the original conditions of illumination as well as the possibility of reinforcement. Thus, the organism is free at any time to terminate or to restore the stimulus situation which has been differentially associated with positive re-

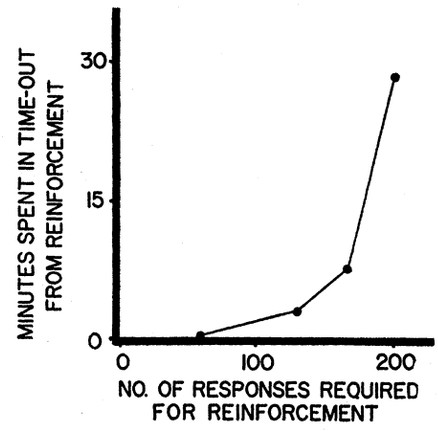

Fig. 1. Self-imposed periods of extinction as a function of the number of responses required during fixed-ratio reinforcement.

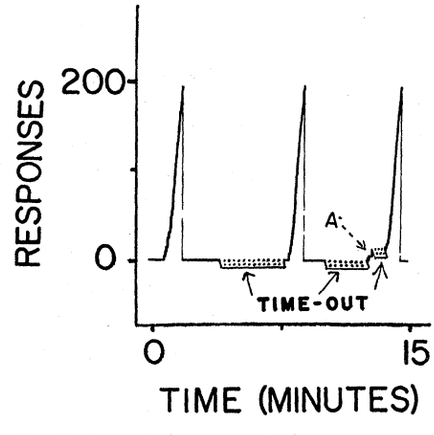

Fig. 2. Cumulative record of responses on a schedule of food reinforcement for every 200th response. The vertical reset line indicates the delivery of the reinforcement. Initiation of extinction is indicated by the downward deflection of the recording pen, and period of extinction, by the dotted area.