Uptake of Bacterial H^3-DNA into Fish Embryos

Since the experiments with bacteria which have shown that genetic transformation[1] can be produced by adding extraneous DNA to bacterial cultures[2], many attempts have been undertaken to achieve transformation in higher organisms, such as ducks, *Drosophila*, *Ephestia*, *Bombyx*, rats, mice, rabbits, amphibians, and tissue culture cells of various objects, including man[3].

In our laboratory, we have begun transformation experiments with fish embryos. Since most of the high-molecular DNA taken up by mammalian cells is usually rapidly degraded[4], the fate of bacterial H^3-DNA injected into fish embryos was first investigated.

Materials and methods. About 0.3 µl H^3-DNA of *Salmonella typhimurium* (300 µg/ml, specific activity 5×10^4 dpm/µg) was injected into the yolk of embryos of *Platypoecilus maculatus*. The fate of injected H^3-DNA was followed by determination of acid-insoluble and acid-soluble radioactive material in both yolk and embryo at various time intervals. At each time interval, 3 samples including 4 embryos were taken. The yolk separated from the embryos was precipitated with cold trichloroacetic acid, while the embryos themselves were homogenized in cold trichloroacetic acid. Both preparations were centrifuged. Radioactivity in sediment (acid-insoluble material) and supernatant (acid-soluble material) of yolk and embryos, respectively, was measured using a Philips liquid scintillation counter. During the entire experiment, no radioactivity has been found to diffuse through the extra-embryonic membranes into the culture medium. Thus, all values obtained could be calculated in per cent of the total injected H^3-DNA radioactivity.

Results. The distribution of radioactive material in sediment and supernantant of both yolk and embryos at various times after injection is illustrated in the Figure 1. During the first few hours, nearly all radioactivity injected (=100%) is associated with acid-insoluble material in the yolk. Then it decreases, reaching a level of about 40% at 40 h. During the same period, there is an accumulation of radioactivity in the yolk supernatant, reaching a maximum of 45% at 45 h. During the following hours, this radioactive acid-soluble material decreases rapidly to about 30% between 45 and 75 h and then levels off to about 20%.

When the acid-soluble material in the yolk has reached its maximum, there is a rapid ascent of radioactive acid-insoluble material in the embryos to 40% at 75 h. This rapid ascent is followed by a slow one to 50% at 140 h. During the entire experiment, there is no significant radioactivity in the acid-soluble fraction of the embryos. Our most recent results reveal that the radioactive material is incorporated into the nuclear fraction prepared from the embryos.

Discussion. These results show that part of H^3-DNA injected into the yolk of fish embryos is degraded. The degradation products are taken up from the acid-soluble fraction of the yolk into the embryo, where they are used for DNA synthesis. A relative high percentage of the radioactivity injected is still present in the acid-insoluble fraction of the yolk. Since it has not yet been shown that DNA synthesis takes place in the yolk, one can assume that this fraction still contains fairly high-molecular bacterial DNA. Thus, there might be a good chance that this high-molecular DNA could be incorporated into the embryo cells. Further experiments to clarify whether high-molecular DNA can be incorporated are now in progress[5].

[1] F. Griffith, J. Hyg., Camb. *27*, 113 (1928).

[2] O. T. Avery, C. M. MacLeod and M. McCarty, J. exp. Med. *79*, 137 (1944).

[3] For last review see L. Ledoux, Progr. nucl. Acid Res. molec. Biol. *4*, 231 (1965).

[4] K. G. Bensch, G. Gordon and L. Miller, J. Cell Biol. *21*, 105 (1964).

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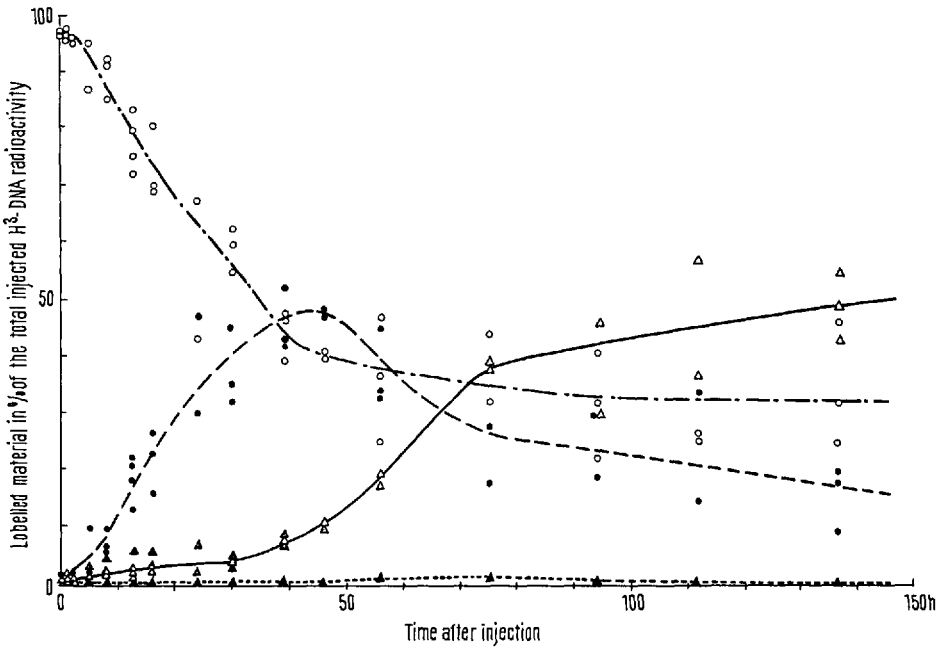

Fate of bacterial H^3-DNA injected into the yolk of fish embryos. ○—○, acid-insoluble material in the yolk. ●---●, acid-soluble material in the yolk. △—△, acid-insoluble material in the embryo. ▲---▲, acid-soluble material in the embryo.

Zusammenfassung. H^3-DNA von *Salmonella typhimurium* wurde in den Dotter von *Platypoecilus maculatus*-Embryonen injiziert und ihr Verbleib untersucht. Nach 45 Stunden findet man 45% der injizierten H^3-DNA-Radioaktivität in der säurelöslichen Fraktion des Dotters. Von diesem Zeitpunkt an nimmt die Radioaktivität in der säureunlöslichen Fraktion der Embryonen ständig zu, bis nach 140 Stunden ein Wert von 50% erreicht ist. Der hohe Anteil an radioaktivem säurenunlöslichem Material im Dotter, der 140 Stunden nach der Injektion noch ca. 35% beträgt, lässt vermuten, dass hier noch hochmolekulare Bakterien-DNA vorhanden ist. Somit erscheint die Möglichkeit einer Aufnahme dieser hochmolekularen DNA in die Embryozellen nicht ausgeschlossen.

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Proteins of Venezuelan Equine Encephalomyelitis Virions

Proteins of several arboviruses were studied by means of polyacrylamide gel electrophoresis[1–3]. Two or three proteins were found in group A arboviruses, one of which was specified as internal protein and another as capsid protein. Nothing is known about protein(s) of the inner membrane in which viral ribonucleoprotein is encapsulated. Proteins of Venezuelan equine encephalomyelitis (VEE) virus, which belongs to group A arboviruses, have not yet been studied. This paper describes proteins of VEE virions including a protein of the inner membrane of the virions.

The methods of propagation of VEE virus in chick embryo fibroblasts and purification of the virus were described earlier[4,5]. Purified virus preparations labelled with ^{14}C amino acids[5] were solubilized by the method of SUMMEERS et al.[6] and studied by means of electrophoresis in 5% polyacrylamide gel. Figure 1 presents an electrophoregram of proteins of VEE virions. It is seen that VEE virions contain 3 major proteins with different electrophoretic mobility. In some experiments the fourth peak with low electrophoretic mobility was found, which can be considered as a product of polymerization of the first major protein.

To specify the proteins in respect to virion substructures, experiments were conducted with fractionation of VEE virus by treatment of purified virus with Tween 80 and ether. This method disrupts the virions and releases hemagglutinin, ribonucleoprotein and also viral cores which contain ribonucleoprotein enveloped into inner membrane[5]. All these components were fractionated by centrifugation in Caesium chloride equilibrium density gradient. Figure 2 shows the results of such experiment. It is seen that ribonuclein bands at $\varrho = 1.43$ g/cm³,

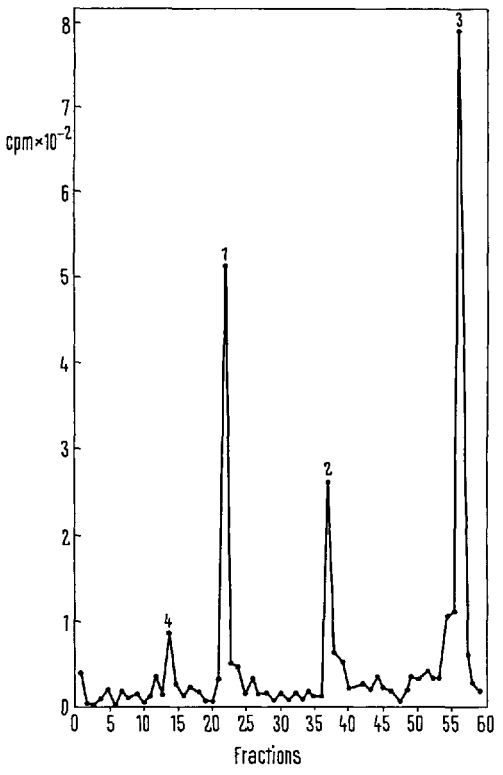

Fig. 1. Electrophoretogram of ^{14}C labelled proteins of VEE virus. The virus was labelled with ^{14}C amino acid mixture, purified, solubilized and subjected to electrophoresis in 5% polyacrylamide gel in a Polyanalyst (USA) apparatus at 5 mA/tube for 6 h. Migration is from the left (cathode) to the right (anode).

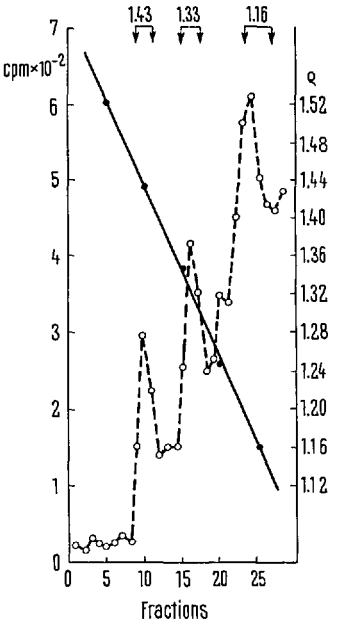

Fig. 2. Equilibrium density centrifugation in Caesium chloride gradient at 30,000g for 16 h of VEE virus labelled with ^{14}C amino acid mixture, purified and disrupted by treatment with Tween 80 and ether. The bottom of the gradient is to the left.