

an, das bald nicht mehr von den übrigen kleinen Vesikeln im Zytoplasma der fusionierten Zelle zu unterscheiden ist.

Welcher Art die Wirkung auf Sarkolemm und Myoblastenmembran ist, kann mit strukturellen Befunden allein kaum erklärt werden. Höchstens über die Lokalisation des postulierten Einflusses ist eine gewisse Aussage möglich. Auf Figur 1 fällt auf, dass nur einer der Myoblasten durch Zytoplasmabrücken mit der MAZ verbunden ist, während die beiden Membranen gegen den zweiten Myoblasten hin auf der ganzen Länge intakt sind. Dies lässt vermuten, dass der Ursprung der «membranauflösenden» Wirkung auf der Myoblastenseite zu suchen ist. «Fuzzy coated vesicles», denen eine mögliche Funktion bei der Membranfusion zugesprochen wird¹³, konnten bei *Antheraea* nicht beobachtet werden. Hingegen enthalten Myoblasten vor der Fusion oftmals Gruppen von transparenten Vesikeln von ca. 500 Å Durchmesser, die direkt unter der Membran gegen die MAZ liegen können. Es ist nicht auszuschließen, dass diese gewisse Stoffe enthalten, die eine Wirkung auf die Zellmembran ausüben.

Der Verschmelzungsvorgang scheint nur kurze Zeit zu beanspruchen. Darauf lässt die Seltenheit schliessen, mit der dieser Prozess beobachtet wird. Auffällig ist hingegen, dass die Myoblastenzahl im Laufe der Entwicklung sukzessive zurückgeht, ohne dass Degenerationsspuren festzustellen sind. Doch findet man gelegentlich mehrkernige Zellen, die eine mosaikartige Feinstruktur aufweisen: ein

Teil des Zytoplasmas ist reich an Ribosomen; er entspricht dem ehemaligen Myoblasten^{2, 8, 13}.

LIPTON und KONIGSBERG¹³ haben intensiv Verwechslungsmöglichkeiten zwischen Zellfusionen und Mitoseprozessen diskutiert. Ihre Beobachtungen an Myoblasten konnten sie danach nur als Fusionen interpretieren, da die Kerne stets im Interphasezustand vorlagen und nie Spindelstrukturen festzustellen waren. Dasselbe gilt auch für *Antheraea* (vgl. Figur 2). Mikrotubuli des Spindelapparates konnten nur in sich teilenden Myoblasten (Figur 3) nachgewiesen werden. Auch in der übrigen Feinstruktur unterscheidet sich diese Art Zellkontakt von der oben beschriebenen.

Summary. The fusion of both myoblasts and 'Muskelanlagezellen' during development of the dorsolongitudinal muscles in *Antheraea polyphemus* (Lep.) is shown at the ultrastructural level. Several cytoplasmic bridges are formed simultaneously between the two cells. It is supposed that the effect of breakdown of both myoblast membrane and sarcolemma originates from the myoblast.

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The Effect of Cysteine on the Sedimentation Profiles of DNA of *E. coli* Cells in Alkaline Sucrose

A relationship is suspected between the mortality (reproduction integrity) of the cells and the incidence and recovery of breaks in cellular DNA. Therefore some authors have studied the correlation between the effects of chemical radioprotectors on the reproduction integrity and on the incidence and recovery of breaks in DNA, respectively¹⁻⁵. The activity of cysteamine, which is one of the most effective radioprotector, was investigated most intensively in this respect. It was established that the cysteamine protected the cells not only against the lethal effects, but also against the single-strand break inducing activity of ionizing irradiation. A complicating factor in the investigation of protection against radiation-induced DNA breaks by cysteamine was the fact that due to the compound itself during the lysis of the cells on alkaline sucrose gradients, breaks in DNA were induced^{2, 3}. This effect of cysteamine was found to be dose-dependent².

Investigating the protective activity of cysteine (another good radioprotector from the same chemical group) we found also difficulties in establishing of its radioprotective effect against radiation induced breaks⁶. Therefore it was decided to study the single-strand breaks inducing effect of cysteine (at various concentrations) in *E. coli* K12 rec⁺ cells. These experiments are described in the present paper.

E. coli K12 (AB2497) rec⁺ bacteria⁷ were labelled in their DNA by inoculating stationary-phase cells into prewarmed 'GS' medium⁸ containing ³H-methyl-thymine (20 µCi/ml medium). The size of the inoculum was adjusted to allow 3 to 4 generations of growth in the label-containing media before harvesting the cells in exponential growth (at 2 × 10⁸ cells/ml). To some of the cultures, cysteine and cysteamine (at various concentrations) were added 30 min before the end of cultivation.

Experimental procedures employed for sedimentation are essentially the same as those described by McGRATH

and WILLIAMS⁹. A minor modification used was published elsewhere⁶.

Part A) of the Figure indicates the changes in sedimentation profile of DNA, as plotted against concentrations of cysteine. The above effect of cysteine was tested at concentrations of 0.5, 1, 2, 5, 10, 15, 20, 30 and 50 mM, respectively. Part B) of the Figure shows the effect of cysteamine as a control at concentrations of 30 and 50 mM, respectively, known to induce breaks. For the quantitative analysis of the curve we have used the 'S 1/2' values as described by VEATCH and OKADA in 1969¹⁰. From this value the molecular weight of DNA can be calculated on the basis of the given equation. 'S 1/2' is the distance beyond which 1/2 of mass of DNA sediments. It is expressed in 'fraction number' from the top.

No changes in the sedimentation patterns and in 'S 1/2' of cysteine-treated cells at concentrations 0.5 mM (curve 2), 10 mM (curve 3) and 50 mM (curve 4) could be observed as compared to that of the untreated cells (curve 1). 'S 1/2' values are as follows: curve 1: 17.0; curve 2: 17.8;

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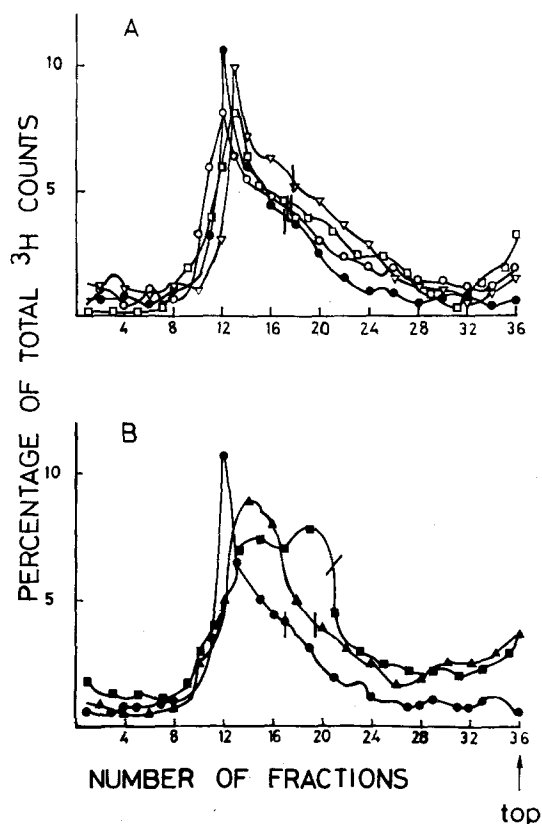
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Effect of cysteine on the sedimentation profiles of DNA of *E. coli* cells in alkaline sucrose. Logarithmic phase *E. coli* K12 cells labelled in their DNA by ^3H -Me-thymine were incubated at 37°C for 30 min in 'GS'-medium with and without cysteine and cysteamine. After incubation the sedimentation profiles were analyzed by the technique described by McGRATH and WILLIAMS⁹. A) ●—●, untreated control; ▽—▽, treated with 0.5 mM cysteine; ○—○, treated with 10 mM cysteine; □—□, treated with 50 mM cysteine. Vertical bars indicate the 'S 1/2' values. B) ●—●, untreated control; ▲—▲, treated with 30 mM cysteamine; ■—■, treated with 50 mM cysteamine.

curve 3: 17.1; curve 4: 17.6. As the sedimentation pattern and the 'S 1/2' were similar at the other concentrations tested, they were not indicated in the figure.

Cysteamine used as a control at concentrations of 30 and 50 mM changed both the sedimentation pattern and 'S 1/2' values. These were found to be: curve 1: 17.0; curve 2: 19.5; curve 3: 21.5.

Present experiments were based on our previous results^{11,12} according to which an asynchronous synthesis of macromolecules was induced by cysteine, i.e. after addition of cysteine, the net synthesis of RNA and of protein stopped immediately whereas the DNA synthesis preceded for about 30 min. This effect of cysteine appeared at a concentration of 0.2 mM and it reached 100% at 10 mM.

It follows from this that the cysteine-treated cells are able to incorporate ^3H -thymine only for about 30 min. This fact becomes a source of error in experiments in which the incubation period takes 60–90 min or more. During the incubation period longer than 30 min, the untreated cells incorporate bigger quantity of radio-protective thymine due to their unaffected DNA synthesis.

The series of our experiments in which the incubation period was 30 min, showed that cysteine at concentrations of 0.5–50 mM did not change the sedimentation pattern of DNA, hence probably did not cause single-strand breaks of DNA in *E. coli* K12.

Zusammenfassung. Mit bekannter Methode wurde die Aktivität des Cysteins in verschiedenen Konzentrationen über das Sedimentationsprofil des DNS von *Escherichia coli* K12 Zellen geprüft und festgestellt, dass Cystein das Sedimentationsprofil des DNS nicht veränderte.

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Two Different Aggregation Principles in Reaggregation Process of Dissociated Sponge Cells (*Geodia cydonium*)

Species specific reaggregation of dissociated sponge cells seems to be restricted to a few binary combinations^{1,2}. In an extensive study, JOHN et al.² demonstrated that aggregation of dissociated sponge cells from different species initiates as a random process. In a later stage, a sorting out of the cells from one species occurs, uniting all homologous cells. In order to understand the basic mechanism of this observation, two different aggregation principles have to be postulated: a non-specific one, responsible for the early phase of aggregation of bi-specific cell mixtures, and another of higher specificity, causing species-specific aggregation in the late phase. In a previous paper³ we were able to show that the aggregation process of dissociated sponge cells from the species *Geodia cydonium* Jam. occurs in 3 discrete steps, starting in an early phase with the initiation of primary aggregates, followed by a subsequent formation of

secondary aggregates, and a late phase with a reconstitution of functional aquiferous systems by rearrangement in the secondary aggregates. This paper deals with the biochemical nature of the first two aggregation phases.

The primary aggregation phase. Washed cells from *Geodia cydonium*, chemically dissociated with calcium- and magnesium-free artificial sea water containing 20 mM EDTA and trypsin³, reaggregate when placed into calcium- and magnesium-containing artificial sea water within 45 min, forming compact primary aggregates of

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