able to grow on minimal glucose plates. The method for transduction has been described by CHUNG and GREENBERG[8].

Results and discussion. Observations concerning survival and mutation frequencies for maltose negative mutants, using MNG in different media and for various time intervals, are summarised in the Table. According to these results, we find that the fraction of cells surviving decreases while the mutation frequency increases with duration of treatment in all the media, and that the rate of mutagenesis is close to its optimum in both broth and DM-glucose within 30 min, while in buffer, it is reached at 60 min. This implies that MNG takes almost twice the time to attain optimum efficiency in non-growing media, than in growing media.

Mal mutants are realized by mutations at two loci, *malA* and *malB*. Classification of these mutants as *malA* or *malB* was done, while performing these experiments, by transductional studies since the phenotypic expression of these mutants are indistinguishable. The mutants which were able to recombine with AB325 (*malA*) and were unable to recombine with PAM201 (*malB*) to give Mal$^+$ were considered to be *malB*. The inverse was true for those considered to be *malB*. It was found that the loci were almost equally affected.

The results obtained in the present study indicate that MNG treatment is most effective for mutagenesis in DM-glucose medium probably because glucose acts immediately providing an extra source of energy which increases the reactivity. On the other hand, no Mal mutants were recovered immediately after treatment in J.N. broth, indicating that its mutagenic activities are reduced. The slowed down activity of MNG in this complete medium could be due to the complex nature of the broth. Among the buffers, the treatment given to cells in TM buffer was least effective, probably due to the slow rate of growth of the cells. It was observed that the effectiveness of MNG was not altered by the medium in which the cells were grown prior to treatment. However, it was dependent, both as a killing and mutagenic agent, upon the type of medium in which the treatment was given, its constituents, their interaction with MNG and the relative rate of growth of the cells. The results do not indicate a clear correspondence between MNG lethality and mutagenesis. The immediate reactivity of this mutagenic agent suggests that a breakdown product of diazomethan[4] is not the principal agent involved. MNG itself is probably exerting directly both lethal and mutagenic effects on the cells. Further details about the nature of MNG action will be published elsewhere.

Résumé. L'efficacité de la nitrosoguanidine (MNG) dans différents milieux de culture et tampons a été étudiée. Les résultats obtenus montrent qu'avec le temps, la fraction de cellules survivant au traitement diminue progressivement alors que la fréquence de mutation augmente dans tous les milieux. L'activité de cet agent atteint un maximum dans le milieu DM-glucose tandis que dans le tampon TM elle est à son plus bas niveau.

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Preparation of Virus-Like Particles of DNA-IgG

A natural way to introduce nucleic acids into the genome of a cell is by means of viruses. It is known that on the cell membranes of tissues susceptible to virus infection there are specific receptor sites. The virus particles get fixed to these receptor sites – probably by means of secondary forces of the same type that are involved in the tertiary structure of proteins and in antigen-antibody interactions – and finally penetrate into the cell by pinocytosis[1]. This phenomenon suggested to us a method to introduce genetic material into cells of specific tissues: to prepare an inclusion complex between DNA and protein, using DNA extracted from a tissue that has a desirable genetic characteristic and introduce it to the homologous tissue of another animal that lacks this characteristic. The protein necessary to cover and protect this DNA should have the specificity for the tissue chosen as target; we chose an immunoglobulin (IgG) prepared by immunizing animals with lipoproteins of the membrane cells of the target tissue. We also used one of the fragments of this IgG obtained by papain treatment (Fab). Our approach requires that the clathrate should be formed with the DNA as core of the particle and the IgG or Fab proteins as capsomers. In this paper we present some of our results, which were partially presented previously at 2 meetings[2,3].

Material and methods. Preparation of DNA. It was prepared after SZYBALSKI and SZYBALSKA[4] using razored skin of black rabbits. The final purification was attained by means of agarose colums type Bio-Gel A-15 M and Bio-Gel A-150 M.

Lipoproteins. They were prepared by extracting razored skin of white rabbits – previously homogenized in a Ultra-Turrax homogenizer – with 0.8 *M* potasium thiocyanate, stirring 30 min at 4 °C. The mixture was centrifuged first at 2,500 g to separate the gross particles and then at 105,000 g to sediment the lipoproteins. Both centrifugations were performed at 0 °C. The thiocyanate was eliminated by dialyzing against NaCl 0.8 *M* pH 7.0 and the lipoproteins were kept at 5 °C in triton X-100 0.001 *M*[5].

Preparation of antibodies. The lipoproteins mixed with complete Freund's adjuvant, were injected weekly to a goat. The IgG was obtained after KENDALL[6], and it was kept frozen at −23 °C in 0.15 *M* saline solution.

Preparation of the clathrate DNA-IgG. It was prepared by mixing DNA, previously purified as described, with IgG at a ratio of 1/312, both substances dissolved in 0.15 *M* NaCl. The mixture was dialyzed vs sodium citrate

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0.02 *M* for 12 h at room temperature, changing the sodium citrate solution several times. After dialysis, a precipitate was formed. This was dissolved by adding NaCl to adjust the concentration to 0.15 *M*; the insoluble residue was removed by centrifugation at 2,000 g for 15 min. The supernatant was passed through a Bio-Gel P-300 column, and the fraction eluted with the void volume was filtered through a Millipore membrane of 0.45 μm. The filtered solution was passed through a Bio-Gel A-15 *M* column and the fraction eluted with the void volume was passed finally through a column of Bio-Gel A-150 M, separating the fraction of high molecular weight. The clathrate DNA-Fab was prepared in the same way as the DNA-IgG complex, using in this case the Fab fragment prepared after PORTER[7], instead of the IgG[8,9].

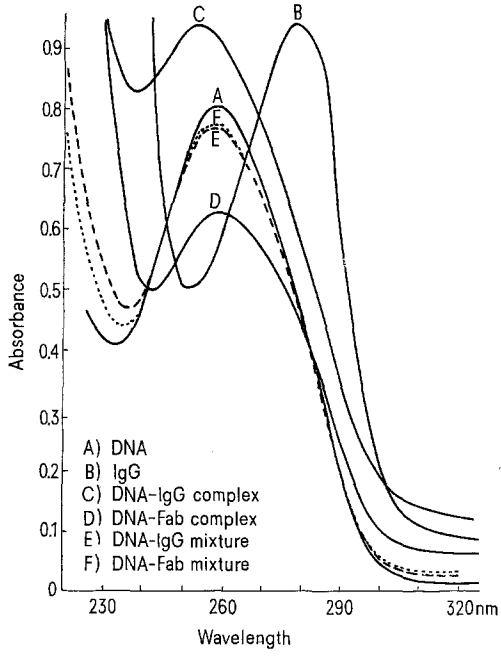

Fig. 1. UV-absorption spectra. A) DNA 36 μg/ml; B) IgG 581.2 μg/ml, C) DNA-IgG complex containing 42 μg of DNA and 23.7 μg of IgG per ml, D) DNA-Fab complex containing 36.2 μg of DNA and 13.1 μg of Fab per ml, E) DNA-IgG mixture containing 42 μg of DNA and 23.7 μg of IgG per ml; F) DNA-Fab mixture containing 42 μg of DNA and 23.7 μg of Fab per ml. A Cary 15 Spectrophotometer was used.

Analytical techniques. DNA was determined using DISCHE's Method[10] modified by BURTON[11]; the protein was determined after LOWRY[12]. The effect of DNase on the DNA-IgG and DNA-Fab clathrates, was studied, using the spectrophotometric method of KUNITZ[13] in a Zeiss PMQ-II.

Results. Figure 1 presents the spectra of the 2 DNA complexes: It can be observed that the spectra of the complexes are different to the isolated components and also to the mixture of them. The spectra of the complexes are similar to that of DNA and to those of some viruses reported in the literature[14]. The spectra of the mixture of DNA plus IgG and of DNA plus Fab, in the same proportions that existed in the particles, exhibited the same maxima (257 nm) as DNA alone and practically the same minima (235 and 234 nm respectively). However, the spectrum of the DNA-IgG complex presented a maximum at 258–259 nm and a minimum at 241 nm). The shifting of the maxima and minima observed in both cases suggests a sort of interaction between DNA and protein, in the particles of the complexes, probably stronger in the DNA-IgG complex than in the DNA-Fab[15].

According to the working hypothesis, the complexes would be formed with the DNA as core and the protein located externally, covering the DNA. As a result of this, the complexes would be resistant to DNase. This was demonstrated, because we saw that DNase degrades the naked DNA and the mixture of DNA plus IgG, but it does not attack the DNA-IgG and DNA-Fab complexes.

The chemical analysis of both complexes (DNA-IgG and DNA-Fab) is in good agreement with the composition calculated by the molecular weight of the DNA used to form the particles. Figure 2 shows an electron micrograph of the particles. The symmetry of the particles seems to be cubic, the size variable, and, apparently, the protein subunits cover the core of DNA.

Discussion. In recent years it has been possible to achieve the introduction of genetic characteristics into mammalian cells by means of natural viruses[16] pseudovirions[17–19] bacteriophage[20] and cell fusion[21]. This paper shows that it is possible to prepare virus-like particles designed to introduce genetic material into specific tissues. We plan these particles with the idea of inducing

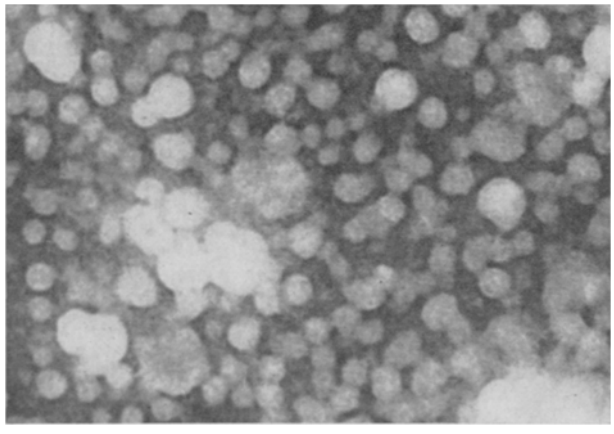

Fig. 2. Electron micrograph of virus-like particles, formed between DNA (MW 17.5×10^6) and IgG. (×225,000). It was obtained using phosphotungstic acid negative stain and a Philips EM-200 electron-microscope by Z. PRICE (UCLA) to whom we owe our thanks for the electron-micrographs.

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genetic transformation of somatic cells, in order to modify the phenotype of cells lacking some characteristic, such as happens in genetic diseases. We believe that this approach, in line with the goals of genetic engineering, could achieve the correction of genetic deficiences. The virus-like particles prepared have immunological specificity and might be capable of transforming cells located in deep tissues of the whole animal.

The particles with Fab were planned with regard to the possibility that the complement fixation could, with the complete IgG molecules, produce cytolysis. It is well known that the Fc fraction of IgG fixes complement. Therefore, the particles formed with Fab could recognize the antigen, but, as they do not fix complement, they would be innocuous to the cell membranes.

Zusammenfassung. Experimentell hergestellte, virusähnliche Partikel, bestehend aus einem Kern von genetischem Material und einer Proteinhülle, werden in Analogie zu einer Virusinfektion in Zellen eingeschleust. Die Komplexe zwischen DNA-IgG und DNA-Fab werden beschrieben und die Partikel enzymatisch, chemisch und elektronenmikroskopisch charakterisiert.

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Orderly Arrangement of G-Chromosome Bands in Interphase Nuclei

It has recently been demonstrated that different treatments, with protelytic enzymes, protein denaturants, detergents, strong salt solutions or heat, produce the appearance of chromosome bands[1] grouped under the name of g-bands to distinguish them from fluorescent or q-bands and from c-bands which are representatives of the constitutive heterochromatin location in the chromosomes. The pattern of g-bands distribution in a given species seems to be constant and characteristic for each chromosomal pair. Hence, the analysis of g-bands has proved to be a iusefultool for a correct chromosome indentification.

Dur ng the analysis of g-band distribution in the chromosomes of the vole mice *Akodon molinae*, we observed a characteristic morphology in the interphase nuclei of preparations treated with trypsin[2]. Accordingly, we started a series of experiments aimed at obtaining further information on this phenomenon.

Material and methods. Chromosome spreads were prepared from bone marrow tissues of *Akodon molinae* and *Akodon azarae* (Rodentia Cricetidae), spleen tissues of normal and leukemic mice (BALB strain), lung and kidney cultures from New Zealand rabbits. 3 to 5 individuals from each species were studied. Chromosome preparations were divided in groups and digested at room temperature for ½,1,2,3,4 and 5 min with trypsin (0.25%) in Hanks saline (without Ca and Mg) at pH 7.2. Slides were afterwards washed in 70% and 100% ethanol and stained for 3–5 min with Giemsa diluted 1 to 5 in Sörensen buffer at pH 6.9.

Results. The results obtained were similar in all individuals analysed. Chromosome banding was clearly noticed in slides treated with trypsin for 2 to 3 min. In $^1/_2$ to 1 min treatments, chromosome digestions was slight or absent and bands did not show. On the other hand, 4 to 5 min digestions were excessive and chromosomes appeared swollen and empty. In slides showing chromosome banding, most interphase nuclei showed a radial array of dark bands starting at the nuclear membrane and converging to form a single or double ring parallel to the nuclear envelope and peripherally located. A series of fine filaments emerged from the inner part of the ring forming a network which in many cases showed a general tendency to become radially oriented toward the center of the nucleus. In perinucleolar areas, the network usually condensed, forming dark masses of chromatin (Figure 1). This characteristic nuclear morphology was not observed in preparations which, as a result of under or overdigestion, did not show chromosome banding. Thus, a correlation between chromosomal g-bands and interphase bands may be reasonably assumed.

Discussion. It has been shown that nuclear membrane preferentially associates with various chromatin types or chromosome regions. Among these associations, perhaps the most widely known is the case of the sex chromatin in female mammals[3]. However, there are several others which have also been well documented. The attachment of telomeric chromosome regions to the nuclear membrane has been demonstrated in several instances[4,5]. It has also been reported that centromeric chromosome regions rich in constitutive heterochromatin lie adjacent to the

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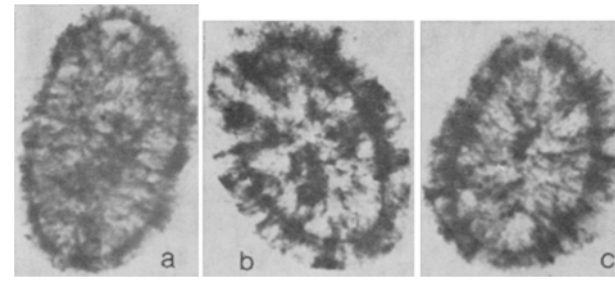

Interphase nuclei treated with trypsin for 3 min. a) cultured lung fibroblast from New Zealand rabbit. b) spleen cell from normal mice, c) bone marrow cell from *A. molinae*. ×1400.