group of 6 mice was measured simultaneously in the same activity cage for a period of 3 h after they were given saline, i.p. During the same time on the next day, the same mice were given D-amphetamine, 5 mg/kg, i.p. before their activity was measured again. The numbers of beam interruptions registered on the impulse counters were recorded every 30 min. No other activity was allowed in the room during the testing period. Amphetamine was dissolved in saline just prior to injection.

Results and discussion. The stimulation of locomotor activity of *ob/ob* and normal mice by amphetamine is shown in Figure 1; that of *db/db* and normal mice is presented in Figure 2. The basal activity of both *ob/ob* and *db/db* mice was much lower than that of the corresponding normal mice. It should be pointed out that this difference is probably not only due to the difference in body weight but also due to the difference in the interactions among mice. In our previous study[1], only 1 mouse was tested at a time in order to avoid interactions among mice. In the present study the effect of amphetamine over a period of 3 h was monitored, making it impractical to test each mouse separately.

The response of both *ob/ob* and *db/db* mice to amphetamine is better than that of normal mice of the same strain when the data are expressed on the basis of percentage of the basal level. This indicates that the center that regulates motor activity in these mice is not defective. This observation is compatible with our previous conclusion that in these mice, there is no genetic predisposition for inactivity and that inactivity in these mice is secondary to obesity[1].

Zusammenfassung. Die motorische Aktivität von genetisch obesen (*ob/ob*) und diabetischen (*db/db*) Mäusen wurde durch D-Amphetamin stärker gesteigert als bei normalen Mäusen, wobei das Regulationszentrum für die motorische Aktivität intakt zu sein scheint.

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Mutagenecity in the Mal Regions of *Escherichia coli*

N-methyl-N′-nitro-N-nitrosoguanidine (MNG) is a powerful mutagen for several kinds of bacteria[1–4]. ADELBERG et al.[2] have reported that treatment of cells in *Tris*-Maleate (TM) buffer instead of a broth reduced the lethal effects of MNG without reducing mutagenecity. OLMEDO and HANAWALT[4] found that bacteria are equally sensitive to MNG mutagenesis whether grown in minimal or complex medium. The present investigations were undertaken to find the relative efficiency of MNG as a mutagen for *E. coli*, when used in growth media and various buffers. Forward mutations from the ability to ferment maltose (Mal^+) to inability (Mal^-) were examined.

Materials and methods. Strain B 251 of *E. coli*, a Mal^+ derivative of strain B (gift of Dr. A. ARBER)[5], was used throughout. The media used have been previously described[6,7]. The bacteria were grown overnight in different media (J.N. or DM or DM-maltose), diluted 1:25 in 5 ml of fresh medium, and then incubated until log phase ($\sim 5 \times 10^8$ viable cells/ml) was reached. Only cells in log phase of growth were used. Cells were centrifuged, washed, and resuspended in the appropriate medium for the treatment with MNG. MNG was used at a concentration of 50 µg/ml. Mal mutants were visualized by spreading the treated bacterial suspension on Penassay-tetrazolium medium (TTC), supplemented with maltose[6], and confirmed, after reisolation, by their inability to grow on minimal (DM)[6] maltose plates, though they were

Survival and mutagenesis[a]

Treatment in (MNG-50µg/ml)	Time of treatment (min)	Survival fractions	Maltose negative mutants per 1000 survivors
DM-glucose	Before MNG addition	1.1×10^9	1.7
	Immediately after	2.8×10^{-1}	8.8
	15 after	4.4×10^{-5}	55.3
	30 after	2.6×10^{-7}	50.3
J. N. Broth	Before MNG addition	3.6×10^9	0.76
	Immediately after	1.3×10^{-1}	—
	15 after	2.4×10^{-3}	29.1
	30 after	1.3×10^{-4}	42.8
	60 after	3.0×10^{-5}	46.8
Phosphate-buffer (DM)	Before MNG addition	5.8×10^8	—
	Immediately after	6.3×10^{-1}	9.3
	15 after	3.3×10^{-4}	20.3
	30 after	7.5×10^{-5}	30.1
	60 after	3.7×10^{-5}	89.8
Tris-buffer	Before MNG addition	2.1×10^8	—
	Immediately after	3.5×10^{-1}	—
	15 after	1.1×10^{-1}	9.0
	30 after	2.0×10^{-2}	20.9
	60 after	1.3×10^{-2}	21.3
Citrate-buffer	Before MNG addition	4.2×10^8	—
	Immediately after	4.3×10^{-1}	4.3
	15 after	1.3×10^{-2}	27.7
	30 after	3.6×10^{-3}	32.8
	60 after	3.3×10^{-4}	60.0

[a] Mean of 3 replications.

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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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