trypsinogène. Néanmoins, étant donné la très forte activité potentielle du mélange (courbe II de la Fig. 1), on peut d'ores et déjà penser que les protéines "ouvertes" sont activables et qu'elles méritent par conséquent d'être appelées néochymotrypsinogènes.

On notera que les résidus terminaux des néochymotrypsinogènes précédents sont justement ceux que les protéolyses additionnelles de l'activation lente[1] font apparaître dans les chymotrypsines du type α. Il est curieux de constater que l'"ouverture" du chymotrypsinogène produit des protéines actives quand elle est faite par la trypsine en un point apparemment bien déterminé de la molécule, et qu'elle produit des protéines inactives mais encore activables quand elle est faite par la chymotrypsine en un autre point.

Il est d'ailleurs intéressant de signaler que la dégradation que comporte cette "ouverture" peut être encore poursuivie sans que l'aptitude à l'activation soit perdue. A côté de la tyrosine C-terminale, la chaîne contient vraisemblablement de la leucine et de la lysine*. Or, l'ablation des trois résidus par la carboxypeptidase n'influe pas de façon sensible sur l'activité potentielle. (courbe III de la Fig. 1). Toute cette région de la molécule ne paraît donc pas essentielle.

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* Nous avons vu que l'attaque du néochymotrypsinogène par la carboxypeptidase engendre 1 mole de tyrosine et des traces de leucine. Si, au terme de cette 1° étape, on dialyse le mélange et on ajoute à nouveau de l'enzyme, on obtient après 6 h d'incubation des quantités substantielles de leucine et de lysine. La séquence C-terminale est donc vraisemblablement — (Lys, Leu)-Tyr. C'est cette séquence qui peut être coupée sans que l'activité potentielle soit diminuée.

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Stimulation by ribonucleic acid of induced β-galactosidase formation in *Bacillus megaterium*

A recent report of REINER AND GOODMAN[1] on the effect of polynucleotides on induced enzyme formation in *Escherichia coli* has prompted us to record some similar results obtained while studying adaptation in *Bacillus megaterium*.

The strain KM of *B. megaterium*, very kindly supplied by Dr. MCQUILLEN, was grown at 30° in a glucose/NH_3/salts medium (MCQUILLEN[2]). The conditions of growth and maintenance of the culture were as used by MCQUILLEN[2], and the organism also grew readily—after a lag period of a few hours—when the glucose in the medium was replaced by an equal weight of lactose (medium C/L). The strain trained to grow on lactose was maintained by daily sub-culture on C/L in the same way as that trained to grow on C/G. It was found that there was always a small amount of β-galactosidase activity present in culture of *B. megaterium* growing on glucose alone, but the amount of enzyme was increased about thirty-fold when 0.5% lactose was present (C/L). The present work was initiated to determine whether the level of β-galactosidase found in cells growing on C/G could be increased by incorporating into the medium nucleic acid obtained from the strain trained to grow on lactose.

The nucleic acid was obtained by a method to be described in more detail elsewhere. Briefly, cells of *B. megaterium* suspended in *M* NaCl were converted into protoplasts by treatment with lysozyme, and then stirred slowly for several hours in the presence of an equal volume of sodium dodecylsulphate (SDS at 0.4% weight/volume). Nucleic acid was precipitated with alcohol from the clear supernatant liquid obtained after centrifuging at 10,000 *g*. Initial attempts to separate the DNA (constituting up to 40% of the mixture) from the RNA by the methods of DUTTA, JONES AND STACEY[3] were unsuccessful, but it was found that the SDS-protein precipitate still contained varying amounts of DNA. DNA free from RNA could be liberated by treatment of this precipitate suspended in *M* NaCl with $CHCl_3$-octanol as in the SEVAG procedure. On the other hand, most of the DNA could be precipitated, together with some RNA, from the mixed nucleic acids dissolved in 0.1 *M* NaCl by high speed centrifugation at 100,000 *g*. It was then possible to precipitate RNA of greater than 90% purity from the supernatant fluid. The RNA

and DNA contents of the various preparations were estimated from the amounts of uracil and thymine detectable after perchloric acid hydrolysis. Further deproteinisation was effected by $CHCl_3$ treatment. The deproteinisation thus followed quite closely the method of JONES AND MARSH[4], although in this case, the nucleic acid was freed from SDS-precipitable protein immediately after its liberation from the cell: the danger of degradation following the action of ribonuclease or deoxyribonuclease was thus reduced to a minimum. The product was further purified by several precipitations from *M* NaCl-alcohol, dialysis to remove NaCl and freeze drying. The final drying was carried out at 30°/0.2 mm in the absence of a desiccant.

For the experiments on the induction of β-galactosidase activity, cells were grown on C/G until well within the exponential phase of growth, having attained a dry weight of 100 to 200 μg/ml. Lactose or the appropriate nucleic acid were then added. Determinations of β-galactosidase activity were carried out at intervals by withdrawing small samples, centrifuging, resuspending in 0.05 *M* phosphate buffer (5 ml, pH 7.1) containing 0.2% *o*-nitrophenyl-β-galactoside, and finally incubating at 30°. The *o*-nitrophenol produced was measured spectrophotometrically at 420 μg, and the unit of β-galactosidase activity was defined as the amount of enzyme necessary to produce 1 μg of *o*-nitrophenol per hour under these conditions. The results from one experiment are shown in Table I: while ribonucleic acid extracted from bacteria grown on C/G had only a very small effect in increasing the amount of β-galactosidase, the addition of ribonucleic ex-

TABLE I

EFFECT OF ADDITION OF RNA AND INDUCER ON FORMATION OF β-GALACTOSIDASE IN *B. megaterium* GROWING EXPONENTIALLY ON C/G (DRY WEIGHT 150 μg/ml)

Time (hours)	*No addition*	*10 mg RNA from strain grown on C/G*	*10 mg RNA from strain grown on C/L*	*10 mg lactose*
0	1.57	1.57	1.57	1.57
2	1.88	1.81	3.30	3.27
4	2.35	2.50	5.26	6.93
6	2.83	3.22	7.23	7.42
8	2.67	3.06	6.60	5.97
24	1.51	1.73	2.58	2.41

tracted from the strain adapted to grow on C/L (LRNA) led to an increase in the amount of enzyme formed to approximately 2.5 times the control level. The LRNA added possessed no β-galactosidase activity itself, and after the several purification steps it seems unlikely that any significant amount of simple carbohydrate inducer could have been carried through with it from the original medium. The increase was in any case of the same order as that produced by a similar amount of lactose added to C/G but of course still much less than the amount developed in the full C/L medium. It probably contained small amounts of protein and DNA, but no inducing activity was ever detected in any of the pure DNA samples tested.

However, it has not so far been found possible to obtain completely reproducible effects, different LRNA samples stimulating the formation of the adaptive enzyme by amounts varying from 100 to 500%, whereas some preparations (about a third) were completely inactive. On two occasions also, more inducing activity than usual has been detected in RNA derived from the strain growing on C/G, stimulation of β-galactosidase production of 40 to 60% being observed. Similar irregularities in the ability of RNA preparations to bring about an acceleration of enzymic induction of *Pseudomonas* have been reported, ODA[5].

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