The action of ribonuclease, thiouracil and azaguanine on the synthesis of phage proteins by an induced lysogenic bacterium

The theory has been advanced for cellular organisms that the deoxyribonucleic acid (DNA) carrying the genetic information might not exert its influence until after such information has been transmitted to ribonucleic acid (RNA)[1]. It is of interest to know, in the case of phage multiplication where genetic information is carried primarily by DNA, whether various agents know to interfere with the synthesis of RNA would block the synthesis of the virus proteins.

GROTH[2] has shown that ribonuclease inhibits growth in *Bacillus megatherium*, and HAMERS[3] found that thiouracil is incorporated into RNA in the same organism. The purine analogue, azaguanine, is known to be massively incorporated into RNA in several bacteria while scarcely at all into their DNA[4]. The action of these three agents on a lysogenic *B. megatherium* induced by ultra-violet light or H_2O_2 was therefore studied.

Each of these agents inhibits the lysis of induced cells, without preventing the growth that usually precedes their lysis (Fig. 1). Determinations of the total protein content and of the quantity of specific phage proteins[5] per unit volume of the culture were made at several times after induction, in the presence or absence of inhibitors. These show that the synthesis of phage proteins is always inhibited by ribonuclease, thiouracil and azaguanine to a much greater extent than the synthesis of bacterial proteins. The synthesis of phage proteins can indeed be totally suppressed while synthesis of bacterial proteins continues at a reduced rate.

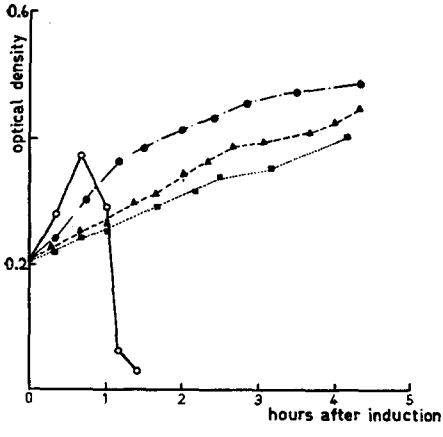

Fig. 1. Evolution of the optical density of lysogenic cultures of *B. megatherium* induced by ultra-violet light, 2537 A.

○——○ without addition.
●——● addition of thiouracil (2 mmole/ml) 30 min before induction.
△-----△ addition of ribonuclease (100 μg/ml) 10 min after induction.
□-----□ addition of azaguanine (0.065 mmole/ml) 30 min before induction.

This selective inhibitory action on the synthesis of phage proteins is not the result of a reversion of the state of activation into which the prophage has been placed by induction. To lift the inhibition and allow phage to be produced in abundance, it is, in fact, only necessary to add uracil or guanine to an induced culture blocked for several hours by thiouracil or azaguanine respectively.

The three inhibitors act appreciably on the synthesis of phage protein only if they are brought into contact with the bacteria before the phage proteins begin to be synthesized[5].

It would be tempting to consider the observed effect in the three cases as related to an alteration in the structure of a specific RNA necessary for phage-protein synthesis. This RNA would be synthesized rapidly after induction, under the influence of the DNA of the phage. This would explain why the described inhibition functions only before the onset of phage-protein synthesis, and also why the inhibition is stronger for phage proteins, which are dependent upon the newly formed, abnormal RNA, than for bacterial proteins, which depend on the normal RNA that existed before the addition of inhibitors.

An indication that the effect of the inhibitors is due to a structure alteration in the RNA is the fact that ultra-violet light, which under certain conditions prevents the incorporation of thiouracil into RNA, at the same time suppresses the inhibiting action of thiouracil on phage-protein synthesis.

If this interpretation is correct, our results would indicate that the RNA with a rapid turnover, which appears after infection of *Escherichia coli* by T_2[6], is a specific product of the infection, and plays a role in the synthesis of phage protein.

Other conjectures could be made to explain the action of each of the inhibitors used. Since an effect on RNA is probably their only common property, however, it seems most plausible that it is through this channel that they exert their inhibition.

R. JEENER

Laboratory of Animal Physiology, University of Brussels (Belgium)

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Received December 9th, 1957

A new partition agent for use in the rapid separation of fatty acid esters by gas-liquid chromatography*

The standard methods for the separation of long-chain fatty acids by reversed-phase partition chromatography or paper chromatography have been supplemented or replaced by the procedure of gas–liquid chromatography recently developed by JAMES AND MARTIN[1]. By utilizing a paraffinic hydrocarbon (Apiezon "M") as a partition liquid, these investigators were able to separate the methyl esters of saturated and certain unsaturated acids of chain lengths up to 20 C atoms in 3–4 h. The separation of the C_{18} unsaturated acids was difficult. Frequently, the separation of linoleate from oleate was incomplete and, further, it was not possible to distinguish linolenate from linoleate under their experimental conditions.

With the intention of obtaining quicker, sharper, and more complete resolution, particularly of the aforementioned polyunsaturated acids, a series of different types of partition agents was investigated in this laboratory. The adipate polyester of diethylene glycol** proved to be an extremely efficient phase for the analysis of fatty acids. Due to the more polar nature of this material in comparison to the hydrocarbons previously used[1], two significant changes were noted. First, there was the complete elution of a standard mixture of methyl esters of fatty acids up to C_{22} from a column operating at a temperature of 186° within 85 min (Fig. 1). Second, a sufficient increase in retention times occurred (Fig. 2) as the degree of unsaturation increased, giving for the first time the complete resolution of each member of the C_{18} series from its preceding, less funsaturated member. ORR AND CALLEN[2] working with another relatively polar phase also noted avorable separations of this class of compounds.

The present investigation was carried out with a thermal conductivity device employing a split stream of helium through the reference and detection sides of the sensing element which

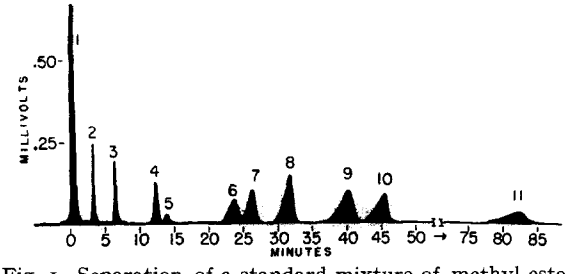

Fig. 1. Separation of a standard mixture of methyl esters from fatty acids. Load 2.5 mg. 1, air; 2, methyl laurate; 3, methyl myristate; 4, methyl palmitate; 5, methyl palmitoleate; 6, methyl stearate; 7, methyl oleate; 8, methyl linoleate; 9, methyl linolenate; 10, methyl arachidate; 11, methyl behenate.

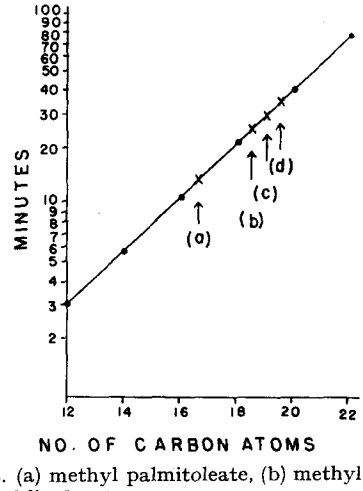

Fig. 2. Semi-logarithmic plot of retention time against number of C atoms for a standard mixture of methyl esters of fatty acids. ● – saturated esters, × – unsaturated esters. (a) methyl palmitoleate, (b) methyl oleate, (c) methyl linoleate, (d) methyl linolenate.

* This work was supported by grants from the National Heart Institute, U.S. Public Health Service, the National Dairy Council and the U.S. Atomic Energy Commission.

** No. LAC-1-R296, obtainable in the 1 lb. containers from Cambridge Industries Company, Inc., 101 Potter Street, Cambridge 42, Mass., U.S.A.