a morphism $\mathbf{H}(\varphi)$ from the ringed space $(V,\mathbf{H}^*(V))$ to the ringed space $(W,\mathbf{H}^*(W))$. The assignment $(V,O_V) \to (V,\mathbf{H}^*(V))$ and $\varphi \to \mathbf{H}(\varphi)$ is a functor from the category of nonsingular varieties defined over k to the category of ringed spaces of graded anticommutative Q_R-algebras.

6. In a subsequent note we shall describe the formal cohomology groups of a nonsingular variety, as well as further elaborations of the theory.

For a special affine variety V with coordinate ring A there is a spectral sequence with

$$E_2^{p,q} = H^p(V,\mathbf{H}^q(V))$$

whose limit is the associated graded module of a canonical filtration on the DeRham algebra $H^*(A)$. The right-hand side of the formula for $E_2^{p,q}$ is the p-dimensional cohomology group of the formal cohomology sheaf $\mathbf{H}^q(V)$ on V (i.e., the pth derived functor of the functor $\Gamma(\cdot,V)$ obtained by using injective resolutions in the category of sheaves of Q_R-modules on V).

We want to mention that if V is a nonsingular curve of absolute genus g, then we have proved that $H^0(V,\mathbf{H}^1(V))$ is a vector space of dimension $2g$ over Q_R. This is our reason for introducing $A\dagger$, since one gets extremely complicated spaces working with A^∞. Moreover, if $g = 1$ and k is a finite field, then the Frobenius mapping F of V (qth power map where q is the number of elements of k) induces an endomorphism on $H^0(V,\mathbf{H}^1(V))$ whose characteristic polynomial is indeed the numerator of the zeta function of the elliptic curve V defined over k.

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[1] *Géométrie Formelle et Géométrie Algébrique,* Exposé 182, *Sem. Bourbaki,* vol. 11, 1958–1959 (Paris).

[2] There are various "tempered" completions; just one of them suffices for the present purposes.

[3] Mimeographed Notes, Math. 529–530, Princeton University, p. 66, 1963.

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*MECHANISM OF ACTION OF COLICINES**

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Study of the mode of action of various colicines has revealed that each colicine has a specific biochemical effect on sensitive bacteria.[1] Thus, colicine K inhibits all macromolecular synthesis in an *E. coli* cell, and this inhibition is reversible after trypsin treatment; colicine E3 inhibits only protein synthesis, not DNA or RNA synthesis; and colicine E2 causes degradation of bacterial DNA (and induction of λ in lysogenic cells) without an immediate inhibition of the synthesis of other macromolecules. Inhibition of all macromolecular synthesis by colicine K may be explained by its inhibition of oxidative phosphorylation[2] similar to the inhibition of oxidative phosphorylation by colicine E1 discovered by Levinthal and Levinthal.[3] However, treatment of sensitive cells with either colicine E2 or E3 did not

cause any such inhibition of energy-producing mechanisms even under conditions in which other effects were immediately observed.[2] In spite of such differences, all colicines have features in common.[1, 4] Therefore, the nature of the mechanism involved in the primary reaction of colicine with sensitive cells may be the same for all the colicines. The reversibility of the killing action and of the biochemical action of some colicines by trypsin[1, 5, 6] suggests that these colicines remain at the bacterial surface and that their primary action is on the bacterial membrane. In this paper, we shall present our current model of colicine action, and discuss some new observations together with the known features of colicine action in terms of the proposed model.

Materials and Methods.—Salmonella typhimurium cys D36 (E2)[25] and *E. coli* strain CA 38,[26] obtained from H. Ozeki, were used to make colicine E2 and E3 preparations, respectively. *E. coli* B, K12 strain W3110 and W4573 (Ara^-, Mal^-, Xyl^-, Mtl^-, Gal^-, Lac^-, S_m^r), were used as strains sensitive to colicine E2 and E3. Colicine E1 and colicine E2 factors were introduced into several *E. coli* strains from *Salmonella typhimurium* cys D36 (E1) and cys D36 (E2), respectively, according to the method described by Stocker, Smith, and Ozeki.[27]

Colicine E2 preparation was made as follows: exponentially growing cells of *Salmonella typhimurium* cys D36 (E2) were treated with mitomycin C (0.5 μg/ml) for 4 hr at 37°C. Chloroform was then added to the culture. The mixture was shaken for a few minutes and then left overnight in the cold. Cells were removed by centrifugation. Colicine E2 was partially purified from the supernatant by fractional precipitations with $(NH_4)_2SO_4$ and ethanol.[2] Most of the preparations had a specific activity of about 1×10^{13} killing particles/mg dried material. About 10-fold purification was achieved over the first crude extract.

Partially purified colicine E3 preparation was similarly made from the induced culture of *E. coli* strain CA 38. The induced culture had a very high colicine E3 activity, but very little colicine I activity. The preparation contained about 2×10^{13} killing particles per mg protein.

The killing activity of colicines was assayed by a method similar to the one described in the legend to Figure 2. A plot of the fraction of cells surviving against the concentration of colicine usually gives a single hit curve. The number of killing particles of colicine contained in the colicine preparation was determined from the curve.[1] In analogy with the practice in phage studies, we use the term "multiplicity," which is the average number of killing particles adsorbed to a cell.

Definition and Explanation of Terms.—Receptor site: The receptor site is the site where a colicine particle attaches, and exerts some effect on a target. Since a sensitive cell can adsorb many killing particles, and since there are different biochemical changes at different colicine multiplicities (see below), there appear to be many receptors on a cell. The receptors are sometimes common for a colicine and a bacteriophage.[4]

Biochemical target: The biochemical target is one which is specifically affected by colicine and leads to the observable biochemical change. Different colicines may have different biochemical targets. One colicine may have more than one biochemical target.

Killing target: The target which is affected by colicine and leads to the death (the loss of colony-forming ability) of a cell is the killing target. This target is not necessarily the same as the biochemical target.

Model Proposed.—The attachment of a single killing particle of colicine to any one of the receptor sites causes some kind of reversible change at the receptor site, but does not necessarily cause a direct effect on a killing or biochemical target. However, the change at the receptor site is transmitted to the killing and/or biochemical target by some specific transmission mechanism, presumably along the cell membrane, and causes eventual death and/or observable biochemical

change. The biochemical target, and hence the observable biochemical change, is different depending on the kind of colicine, but all biochemical targets have a common property in that each is affected by a specific stimulus sent through a transmission mechanism. Immunity in colicinogenic cells is due to an alteration in the mechanism responsible for the initiation and/or transmission of such specific stimulus.

General Features of Colicine Action Which Support the Proposed Model.—(1) *Indirect action on the biochemical target:* Colicine E2 causes DNA degradation, but has no DNase action by itself. DNase activity of the colicine E2 preparation was tested using radioactive *E. coli* DNA as a substrate. About 300 μg of purified colicine E2 preparation containing 10^{13} killing particles did not show any DNase activity when the assay clearly detected the activity of 1 μg crystalline pancreatic DNase. Therefore, the biochemical action, and perhaps the killing action, of colicine E2 does not seem to be due to a direct action of the colicine particle on the target. It was already known that the action of colicines requires the presence of the specific receptors. The loss of the receptors by mutation renders cells resistant to the corresponding colicine.[4]

(2) *Lack of reversibility of the adsorption:* The attachment of a colicine particle to a receptor site was irreversible under the experimental conditions ordinarily used (37°C, in solutions of moderate or high ionic strength). The experiments were done with colicine K, E2, and E3.

(3) *Reversibility of action with trypsin:* The inhibition of macromolecular synthesis, phage growth, and colony formation in colicine K-treated cells was reversed by treatment with trypsin.[5] The inactivation of colony-forming ability by colicine E3 was also shown to be reversed by treatment with trypsin. The reversal of the action of colicines does not seem to be due to destruction of a pre-existing trypsin-sensitive structure necessary for colicine action. The treatment of cells with trypsin before colicine challenge did not change the sensitivity of cells to colicine in the subsequent test (after washing to remove trypsin) as shown in Table 1. These experiments were done in the presence of chloramphenicol (CM) plus, 2,4-dinitrophenol (DNP) to prevent resynthesis of the hypothetical trypsin-sensitive essential component on the cell surface after the removal of the trypsin.

TABLE 1

EFFECT OF TRYPSIN TREATMENT OF CELLS ON THE KILLING ACTION OF COLICINE E3

Cells		Trypsin Pretreated Cells		Untreated Cells			
Concentration of colicine E3		1/24,000	1/8,000	1/24,000	1/8,000	1/24,000	1/8,000
% Survivors	At 30 min	31	1.3	31	0.7	31	0.7
	At 60 min	30	0.7	21	0.3	—	—
	At 60 min (trypsin added at 30 min)	—	—	—	—	112	76

Log phase cells of *E. coli* W3110 Sm^r were harvested, washed, and suspended in tris-S containing CM (50 μg/ml). Half of the cell suspension was treated with trypsin (250 μg/ml) for 30 min at 37°C, and the other half was incubated without trypsin. Cells were then collected by centrifugation, washed twice with cold tris-S containing CM (50 μg/ml) + DNP (10^{-3} *M*), resuspended in tris-S containing CM (50 μg/ml) + DNP (10^{-3} *M*). Colicine E3 was then added (time 0) as indicated. At 30 min, trypsin (final 250 μg/ml) was added to a part of untreated cells. Number of survivors was determined at 30 and 60 min. The composition of tris-S is the same as tris-glycerol medium[19] except that glycerol and NH_4Cl are omitted.

If, as suggested, the reversal of colicine action by treatment with trypsin is due to digestion of the colicine particle, the colicine must remain at the bacterial surface.

(4) *Multiplicity effect:* There is a multiplicity effect (or a dosage effect) in the action of colicines. The adsorption of a single killing particle causes some observable biochemical effect (DNA degradation in the case of E2, inhibition of β-galactosidase synthesis in the case of E3, and inhibition of oxidative phosphorylation and macromolecular synthesis in the case of K), but such changes appear rather slowly, especially in the case of E2 and E3. However, when the multiplicity of colicine is increased, the observable changes appear faster. An example of such a multiplicity effect is shown in Figure 1. The observed long lag before the complete

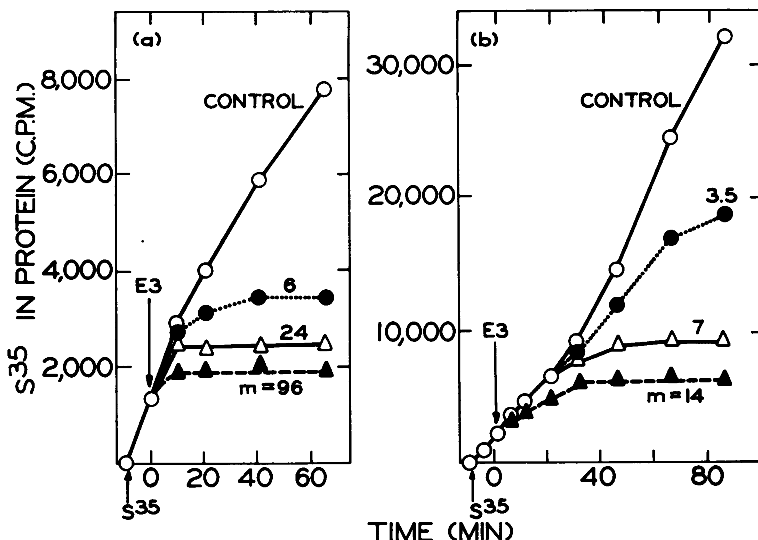

FIG. 1.—Inhibition of S^{35}-sulfate incorporation into protein by colicine E3 at various multiplicities. *E. coli* K12 strain W3110 was grown in tris-glycerol medium[19] to a cellular density of 1-3 × 10^8/ml; S^{35}-sulfate (5 μc/ml) was then added. Ten min after the addition of S^{35}-sulfate, the culture was treated with colicine E3 at the multiplicities indicated in the figure (time 0). The multiplicity values, 6 in (*a*) and 3.5 in (*b*), were calculated from the colony-forming survivor fraction assayed at 15 min. Other higher multiplicity values shown were calculated from the above value and the relative concentrations of the input colicine. Samples (50 μl) were taken at various times, and the radioactivity in the acid-insoluble fraction was determined. The results of two independent experiments are shown in (*a*) and (*b*), respectively.

inhibition of protein synthesis with E3 is not due to slow adsorption. The calculated multiplicity shown in Figure 1 was obtained from the survivors measured 15 min after colicine addition. The adsorption of colicine is nearly complete by this time under the experimental conditions used.

There are many receptors for adsorption of colicine on an *E. coli* cell. The observed multiplicity effect shows that many of them are in fact sites where colicine not only attaches, but also affects the biochemical (and killing) target. Although a single killing colicine particle can exert an eventual effect on a biochemical target by virtue of the proposed transmission mechanism, the same effect can be obtained very fast with high multiplicity of colicine as described. It seems that either the initiation of the transmission is inefficient or the transmission process itself is slow at a low multiplicity.

(5) *A single-hit killing process:* As first shown by Jacob, Siminovitch, and Wollman,[7] the killing action of most colicines is a single-hit process.[1] Thus, the

irreversible attachment of a single particle to one of a large number of receptors causes, with a certain probability, a change of state, which leads to an eventual death of a cell. If the target exists in large numbers in a cell, like ribosomes, or oxidative phosphorylation systems, the change caused by the attachment of a colicine to a receptor must clearly be transmitted to many of these targets. Even if there is only a single target in a cell, as we may suppose with respect to a DNA molecule as a target for E2, the effect caused at the primary receptor site must be transmitted to the target, unless we assume that such a target has a physical connection to all the receptor sites and is affected by a colicine at any one of these receptor sites. This assumption is unlikely. Therefore, we conclude that there is a mechanism which transmits a change from the receptor site to the target.

(6) *Nature of target:* Final proof of the nature of the killing target and of the biochemical targets involved in the action of the different kinds of colicines must await further studies. DNA has been shown to be a biochemical target for colicine E2. The DNA in a bacterial cell has presumably a physical connection to the cell membrane.[8, 9] A biochemical target for E3, the protein-synthesizing machinery, may also have a physical connection to the bacterial membrane.[10, 11] The oxidative phosphorylation system, a biochemical target for El and K, also resides in the membrane.[12] Thus, we are led to formulate a hypothesis that all the biochemical and killing targets have a physical connection to the cell membrane or are a part of the membrane. In addition, the transmission of the initial change, discussed in the previous section, could be along the membrane.

(7) *Immunity:* It was previously shown that immunity to E2 is not due to alteration in the properties of DNA, the biochemical target.[1] Immunity is also distinct from resistance to adsorption; immune cells retain receptors,[4] and in fact adsorb the corresponding colicine.[13] Therefore, the alteration in immune cells seems to be in some process after the adsorption step, but not in the nature of the target itself. Our hypothesis is that immunity is due to an alteration in the mechanism responsible for the initiation and/or the transmission of the specific stimulus which eventually affects the target in sensitive cells. It is known that immunity to colicines is seldom complete and that colicinogenic cells are sensitive to a very high concentration of the homologous colicine.[4, 13] According to the present hypothesis, this breakdown of immunity at high multiplicities would be either the result of a repetition of a local action at many individual receptor sites or the result of an increase in the number of colicine particles per cell, each one with low probability of the initiation of the primary change.

(8) *The resistance of colicinogenic cells to heterologous colicine:* Immunity in colicinogenic cells is specific and distinct from resistance to adsorption. E2-colicinogenic cells are sensitive to El and E3.[4] According to our hypothesis, immunity is due to a change in some component or structure, perhaps in the membrane, which is important for the initiation or the transmission of the initial event to the target. Such a change might affect the sensitivity of colicinogenic cells to a heterologous colicine. Accordingly, the sensitivity of E2-colicinogenic cells to E3 was examined quantitatively, and compared with that of parental noncolicinogenic cells. The suspected change was, in fact, observed. Figure 2*a* shows the results of one such experiment using W4573 $T4^r$ and its E2-colicinogenic derivative, strain 75 [W4573$T4^r$ (E2)]. The presence of colicine E2 factor rendered cells

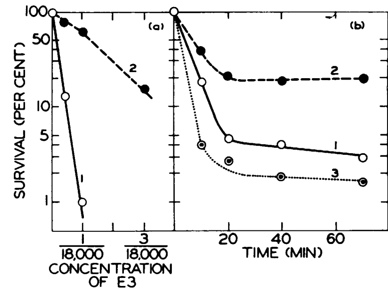

FIG. 2.—(*a*) The difference in the sensitivity to colicine E3 between a noncolicinogenic strain and its E2-colicinogenic derivative. *E. coli* K12 strain W4573T4^r (curve 1) and its E2-colicinogenic derivative (strain 75) (curve 2) were grown in TB medium to a concentration of 2×10^8/ml, harvested, washed by centrifugation, and resuspended in tris-S solution containing CM (20 μg/ml). Cells were treated with various concentrations of colicine E3 for 30 min at 37°C. The number of viable cells was then determined. Both control tubes without E3 showed the same viable counts, 1.3×10^8/ml. (*b*) Kinetics of adsorption of E3 to a noncolicinogenic strain and to an E2-colicinogenic derivative. The experimental conditions are similar to those in (*a*). Samples are taken at various times after colicine E3 addition, as indicated, diluted with warm (37°C) tris-S solution 100-fold, and incubated further at 37°C. After 70 min incubation, all the tubes were cooled in ice, and the number of viable cells was determined. Curve 1: W4573T4^r with colicine E3 (multiplicity 3.5). Curve 2: strain 75 [W4573T4^r(E2)] with E3 (multiplicity calculated on the basis of killing of noncolicinogenic strain, 4.2). Curve 3: strain 75 with E3 (multiplicity as above, 13).

about 8-fold more resistant to colicine E3. (The degree of increase varied from 3 to 10-fold depending on the strains and the experiments.)

A kinetic analysis of adsorption presented in Figure 2*b* shows that the partial resistance is not due to slow adsorption. Somehow, the presence of colicine E2 factor in the cell decreases the efficiency of killing action of adsorbed E3 particle. This suggests that the change in colicinogenic cells responsible for immunity affects, though to a lesser degree, the efficiency of initiation and/or transmission of the primary event caused by heterologous colicines.

Preliminary experiments have shown that the presence of colicine E1 factor also causes partial resistance to colicine E2 and E3.[14] Therefore, the phenomenon described above seems to be of wide occurrence like the phenomenon of interference with heterologous phage multiplication in lysogenic cells (prophage interference).[15]

(9) *A special class of resistant mutants:* A single receptor seems to be shared by colicines in the E group and phage BF23. All the mutants selected by resistance to one of the colicines in the E group or to phage BF23 have been thought to be resistant because of the lack of a receptor.[4] However, the present model predicts the existence of mutants which are resistant to a colicine due to an alteration of the system responsible for the initiation and/or the transmission of the primary event. Such mutants may adsorb a colicine and yet be resistant to its action. It might also show resistance only to some of the colicines in the group, not to all of them.

The predicted class of mutants was found. Mutants were selected for resistance to colicine E2, after the treatment of a sensitive strain W3110$S_m{}^r$ with various mutagens (ultraviolet light and ethylmethane sulfonate). Many of them showed the same sensitivity pattern as that of a conventional resistant mutant (strain ER343 in Table 2), but others showed various patterns different from a conventional one. The ability of those special resistant mutants to adsorb colicine E2 was tested. Among 50 of those special resistant mutants so far isolated, 46 adsorbed E2 (examples: strains ER454 and ER447 in Table 2). The remaining 4 showed or very weak no adsorption. Quantitative study showed that strains ER454 and ER447 adsorbed E2 to the same extent as the parental strain W3110$S_m{}^r$ (see the legend to Table 2). Since, in the mutants of strain ER447, presumably a single-step mutation caused the resistance to both E2 and E3, the resistance does not seem to be due to alteration of the properties of the targets. Somehow these

TABLE 2

ADSORPTION OF COLICINE E2 BY VARIOUS E2-RESISTANT MUTANTS OF *E. coli* K12 AND THEIR SENSITIVITY TO VARIOUS COLICINES

Strains*	Sensitivity to†				Adsorption of‡	
	E1	E2	E3	Phage BF23	E2	E3
W3110S_m^r	s	s	s	s	+	+
ER454	s	r	s	s	+	+
ER447	s	r	r	s	+	+
ER343	r	r	r	r	−	−

* Mutants, ER454 and ER447, were obtained after the treatment of W3110S_m^r with ethylmethane sulfonate. Mutant ER343 is a spontaneous mutant. Fifty mutants showing the resistance pattern different from a conventional one (mutant ER343) were isolated. Among them, 46 adsorbed E2 and 4 did not. Among those 46, 22 showed the same resistance pattern as ER447, 14 showed the same resistance pattern as ER454, and the remaining 10 showed other patterns.

† s: sensitive. r: resistant. Sensitivity to colicines and phage BF23 was examined by cross streaking on the plate with colicines or phage. The results were also confirmed by assaying the survivors after the treatment of washed cells with various concentrations of colicine.

‡ A washed cell suspension (5×10^9/ml) was mixed with a colicine E2 or E3 preparation (about 5×10^9 killing particles/ml). After 30 min incubation at 37°C, the mixture was centrifuged, and the killing action of the supernatants was assayed. No significant decrease of the killing activity was observed with the supernatant treated with strain ER343. Supernatants treated with strain W3110S_m^r, ER454, and ER447 showed only a very weak killing action. Calculation showed that these three strains adsorbed more than 80% of the colicine E2 and E3 activity. The tests were repeated with higher concentrations of colicine E2 preparations. When W3110S_m^r showed a partial adsorption, mutants ER454 and ER447 showed a partial adsorption of about the same degree.

mutants prevent adsorbed colicines E2 and E3 from affecting their corresponding targets.

Discussion.—Studies on colicine action have led to the hypothesis that a primary event at a receptor site on the cell surface affects several different targets in a specific way. This implies a specific connection of several important cellular components, such as DNA, protein-synthesizing systems, and oxidative phosphorylation systems, to the surface structure, and the possible significance of this connection in cellular regulation. In relation with this, it is interesting to note that a hypothesis developed in the study of animal cells states that all membrane systems—cytoplasmic, nuclear, endoplasmic reticular, mitochondrial, and Golgi—may have different properties, but are topologically continuous, either permanently or at least during their genesis.[16]

The significance of the proposed transmission mechanism in normal physiological processes is not clear at the present time. However, the observation of similar phenomena in other systems, e.g., T-even phage infection,[17–20] virus action on animal cells,[21] bacterial conjugation,[22] and fertilization,[24] suggests some possible significance. These biological phenomena involve membranes and are difficult to understand in molecular terms. Elucidation of the mechanism in any of these systems may contribute to our understanding of many apparently complex biological regulatory systems.

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RNA CODEWORDS AND PROTEIN SYNTHESIS, III. ON THE NUCLEOTIDE SEQUENCE OF A CYSTEINE AND A LEUCINE RNA CODEWORD

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Previous studies utilizing randomly ordered synthetic polynucleotides to direct amino acid incorporation into protein in *E. coli* extracts indicated that RNA codewords corresponding to valine, leucine, and cysteine contain the bases (UUG).[1-4] The activity of chemically defined trinucleotides in stimulating the binding of a specific C^{14}-aminoacyl-sRNA to ribosomes, prior to peptide bond formation,[5] provided a means of investigating base sequence of RNA codewords and showed that the sequence of a valine RNA codeword is GpUpU.[6]