amino-oxytocin and one thousandth of the activity of oxytocin. Also, all these compounds had some antidiuretic activity in the ethanol-anesthetized hydrated rat.

One must conclude that biological effects typical of the neurohypophysial hormones can be induced by structural analogues which do not contain a disulfide group and therefore lack the ability to establish a disulfide bond with the receptor by thiol-disulfide interchange. Regardless of the specific activity of these analogues, the results indicate that a disulfide interchange reaction is not obligatory in the formation of the hormone-receptor complex or in its functional role.

The earlier evidence for the functional significance of the disulfide group of neurohypophysial hormones and the thiol-disulfide interchange hypothesis will need reassessment in the light of these results.

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*STREPTOMYCIN AND THE BIOSYNTHESIS OF FUNCTIONAL RNA AND PROTEIN**

BY BARBARA L. BROWNSTEIN

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY, PHILADELPHIA, PENNSYLVANIA

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Studies on the mode of action of streptomycin (SM) indicate that an alteration in the protein-synthesizing mechanism is the primary effect of the antibiotic on sensitive bacteria. Considerable evidence suggests that the SM-sensitive component of the system is the ribosome,[1-3] more specifically, the 30S component of the ribosome.[4,5] A recent report by Davies, Gilbert, and Gorini[6] postulates that an error in the reading of messenger ribonucleic acid (RNA) may result in the formation of aberrant proteins and be responsible for the lethal action of SM. On the other hand, Stern and Cohen[7] have suggested that SM may stimulate the synthesis of a particular type of RNA which is either directly or indirectly responsible for the lethal effect of the antibiotic.

There has been no report, as yet, on whether functional protein or RNA can be made in bacteria inhibited by SM. In the present work we have attempted to determine whether such biologically active protein and RNA are made; in particular, whether the substantial amount of RNA and the small amount of protein synthesized in the presence of SM in SM-sensitive bacteria infected with RNA phage R17 are functional. The results show that phage protein synthesis is at least as sensitive to inhibition by the antibiotic as host protein synthesis, but that the production of infectious RNA continues for a considerable period after both phage and host protein synthesis have stopped and after uninfected bacteria have lost the capacity to divide.

Materials and Methods.—Bacteriophage R17 and its host, *Escherichia coli* Hfr1 (a methionineless mutant of *Escherichia coli* K12 supplied by Dr. A. Garen), are described by Paranchych and Graham[8] as are the methods of preparing and titrating the phage. Tris-maleate medium[8] supplemented with 0.1 per cent glucose and 0.5 per cent casamino acids (C-TMM) was used throughout these experiments. Infectious RNA was extracted with sodium dodecyl sulfate and phenol and used to infect protoplasts of Hfr1.[9]

Streptomycin was streptomycin sulfate purchased from Calbiochem, Inc. Leucine-1-C^{14} (0.14 mc/mg), valine-C^{14} (uniformly labeled, 2 mc/mg), uracil-2-C^{14} (0.42 mc/mg) were products of New England Nuclear Corp.

The incorporation of radioactive amino acids into protein was determined by adding the labeled amino acid to cultures of infected or noninfected cells and removing samples at intervals into equal volumes of 10 per cent trichloroacetic acid (TCA). After 15 min at 90°, the precipitates were collected on glass fiber filters (type D, Gelman) and washed with 10 ml TCA. The filters were dried, covered with 5 ml Bray's solution,[10] and the radioactivity was determined in a Packard Tri-Carb liquid scintillation counter. Uracil-C^{14} incorporation into RNA was determined as above except that the TCA precipitation and the washing of the precipitates was carried out at 4°.

Synthesis of phage coat protein was measured by exposing samples of infected cells to valine-C^{14} for the times indicated in the text. Samples were removed, rapidly chilled to stop further synthesis, and lysed by addition of three drops of chloroform and 20 μg lysozyme/ml. Cell debris was removed by centrifugation at 20,000 $\times$ g for 20 min, and the supernatant fluid was incubated for three hr with serum prepared in rabbits against disrupted *E. coli*. The precipitate formed was discarded and the supernatant fluid incubated for three hr with phage antiserum prepared in rabbits. This precipitate was collected, washed and resuspended in 0.1 N NaOH, dried on a glass fiber filter, and counted, using Bray's liquid scintillation system.

Results.—*Killing of E. coli Hfr1 by SM:* The strain of *E. coli* obtained from Dr. Garen was tested for sensitivity to SM. Viability, as measured by colony formation, was already greatly depressed by addition of 2 μg/ml of SM to the culture.

Loss of viability was determined by adding various concentrations of SM to exponentially growing cultures. At a concentration of 100 μg/ml, loss of viability began 3 min after addition of SM, with 50 per cent survival at 5 min (Fig. 1). This concentration of SM was used throughout the remainder of these studies.

Effect of SM on protein and RNA synthesis in uninfected Hfr1: Protein and RNA

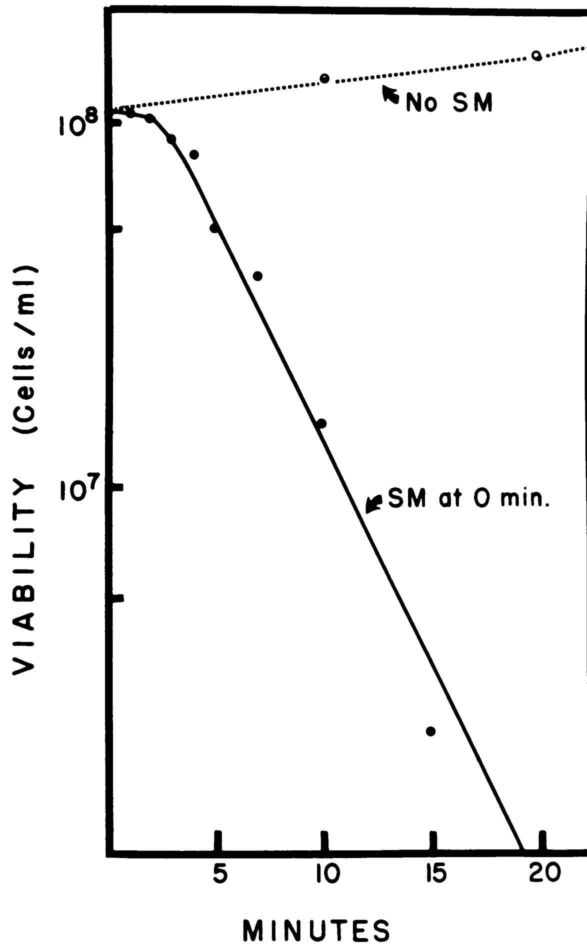

FIG. 1.—Loss of viability of *E. coli* Hfr1 in presence of streptomycin. Bacteria were grown with shaking at 37° in C-TMM medium to a cell density of 10^8/ml. At time 0, 100 μg SM/ml was added to the exponentially growing culture. Samples were removed, diluted in buffer, and 0.1-ml aliquots of suitable dilutions were spread on nutrient agar plates. The agar plates were incubated overnight at 37° and scored for number of colonies formed.

synthesis were measured as incorporation of leucine-C^{14} or valine-C^{14} into hot TCA-insoluble material, and uracil-C^{14} into cold TCA-insoluble material, respectively. Twenty min after addition of the labeled amino acid or pyrimidine, the cultures were divided, and SM was added to half of each culture. Samples were precipitated with TCA and treated as described in *Materials and Methods*.

The uptake of amino acids came to a halt 5–7 min after addition of SM (upper curves, Fig. 2), while incorporation of uracil into RNA continued at approximately the same rate as the control for at least 30 min after the addition of the antibiotic (lower curves, Fig. 2). There appears to be a correlation between loss of viability and cessation of protein synthesis. By 10 min after addition of SM, only 10 per cent of the bacteria are viable, and protein synthesis in the culture has stopped. RNA synthesis continues at an essentially unaltered rate even when viability is reduced to 0.01 per cent. This unaltered rate of RNA synthesis may, in part.

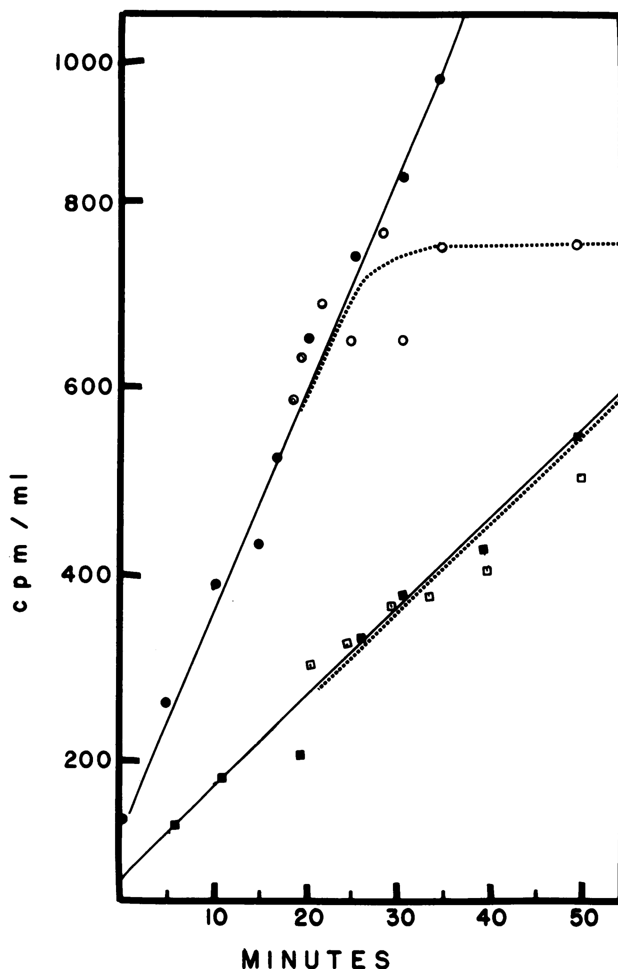

FIG. 2.—Effects of streptomycin on protein and RNA synthesis in Hfrl. *Upper curves:* Leucine-C^{14} (10 μg/ml) was added to exponentially growing bacteria when the culture reached a destiny of 10^8 cells/ml. One-ml samples were added to one ml 10% TCA and kept at 90° for 15 min. Precipitates were collected on glass fiber filters, washed, dried, and counted in Bray's solution in Tri-Carb liquid scintillation counter. The culture was divided 20 min after addition of leucine-C^{14}. To half (solid circles) no additions were made; to remainder SM (100 μg/ml) was added (open circles). At 50 min the control culture had utilized 10% of the added leucine. *Lower curves:* To another culture grown under identical conditions uracil-C^{14} was added at time 0. SM was added to half the culture at 20 min (open squares); on additions were made to control culture (solid squares). Samples were precipitated with TCA at 4° and treated as above. The label incorporated into acid-insoluble material at 50 min represented 5% of the added label.

reflect the nature of the RNA control mechanism in this strain of *E. coli.* Hfrl is "relaxed" in RNA control.[11] "Stringent" strains of *E. coli* (Flaks, personal communication) continue to synthesize RNA in the presence of SM, but at a slower rate than in the absence of the antibiotic.

Effect of SM on replication of phage R17: Brock[12] reported that RNA phage, MS2, underwent a transient period of SM sensitivity shortly after adsorption to SM-resistant hosts, but that phage replication was insensitive to SM in such a

TABLE 1

EFFECT OF STREPTOMYCIN ON PHAGE YIELD

Time of addition of SM (min)	Phage yield[a] at time of addition of SM (pfu/ml)	Final phage yield[b] (pfu/ml)
0	—	1.0×10^9
10	3.0×10^9	2.0×10^9
20	7.0×10^9	1.5×10^{10}
30	2.0×10^{10}	3.0×10^{10}
40	3.0×10^{10}	4.0×10^{10}
50	7.0×10^{10}	1.0×10^{11}
No SM	—	7.8×10^{11}

Hfrl (2×10^8/ml) were infected with 10 R17/bacterium at time 0. At designated times, samples (*a*) were removed, lysed with chloroform and 20 μg lysozyme/ml, and assayed for phage. At each time a second sample (*b*) was incubated with 100 μg SM/ml. All of the SM-treated samples and an untreated control were lysed 120 min *after infection*, and the lysates were assayed for phage.

host when the antibiotic was added five min after adsorption. This finding was confirmed using RNA phage R17 and a SM-resistant host. We then studied the replication of this phage in a SM-sensitive host.

Hfrl was infected with R17. Samples were removed at the intervals stated in Table 1, a portion lysed to determine the number of phage plaque-forming units (pfu) formed at that time, and the remainder of each sample incubated with SM. All SM-treated samples and one untreated control were incubated at 37°, lysed 120 min after they had been infected, and the phage yield was determined by plaque assay (Table 1). SM had no effect on phage already formed at the time of addition. It did, however, markedly inhibit further phage formation (Table 1). The amount of phage in any sample increased no more than twofold after the addition of SM, although an untreated culture had, at 120 min, synthesized 200 times the number of phage present at ten min and almost ten times the number present at 50 min. The small increase in number of mature phage after SM treatment of infected cells may reflect maturation of phage from protein synthesized prior to the addition of SM. Studies in this laboratory on the kinetics of synthesis of phage protein (A. Hagopian, unpublished) suggest the formation of a small pool of phage coat protein containing no more than one equivalent of coat protein for each mature particle.

Synthesis of phage protein in the presence of SM: Having noted that the production of mature phage was sensitive to SM in a SM-sensitive host, experiments were undertaken to determine whether all phage-controlled synthetic activities or only some were sensitive to the action of the antibiotic.

Reports by Loeb and Zinder[13] and Paranchych and Graham[8] show that protein synthesis continues throughout the infectious cycle of RNA phages. From 15 to 50 min after infection there is at least a fivefold increase in phage coat protein in the infected cells (Hagopian, unpublished). This time interval was chosen to study the effect of SM on synthesis of phage coat protein. At 15 min after infection, valine-C^{14} was added to a culture. Two min later the culture was divided and SM added to one part. Samples were removed from the SM-treated culture and from the control at subsequent intervals. One portion of each sample was treated with TCA at 90° (Fig. 3*A*). Another portion was lysed and treated first with antiserum against *E. coli* to remove host proteins and then with serum made against purified phage. The precipitate obtained with phage antiserum was washed and the radioactivity determined (Fig. 3*B*). The same pattern of protein synthesis and inhibition by SM was observed in infected cells (Fig. 3*A*) as was

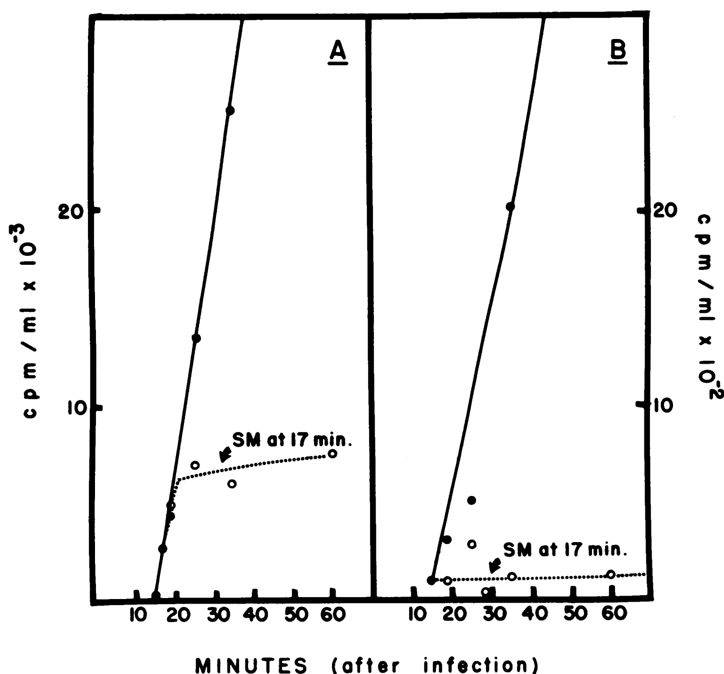

FIG. 3.—Effect of streptomycin on the synthesis of phage coat protein in Hfr1 infected with R17. Bacteria were infected at time 0 with 10 pfu R17/bacterium. Valine-C^{14} was added 15 min after infection; at 17 min the culture was divided. To one part (solid circles) no additions were made; to other (open circles) 100 μg SM/ml was added. Samples were removed at intervals, and the radioactivity in hot TCA-precipitable material (*A*) and in material precipitable with antiphage serum (*B*) was determined.

seen in uninfected cells (Fig. 2). Protein was synthesized in the SM-treated cells at the same rate as in untreated control cultures for approximately five min after the addition of SM, but subsequent synthesis in the SM cultures was sharply inhibited (Fig. 3). However, the protein synthesized in the infected cells in the presence of SM was not an antigenic phage protein as can be seen from Figure 3*B*, since no increase in protein precipitable with phage antiserum was noted in cells treated with SM.

In the cultures without SM, the radioactivity in the phage antiserum precipitate represented about 10 per cent of the total radioactivity found in the TCA precipitates. The remaining 90 per cent of the radioactivity was either in cell debris or in precipitates obtained with antibacterial serum. Host protein synthesis was not substantially inhibited during this time period by infection with RNA phages.[8, 13]

Synthesis of infectious phage RNA in the presence of SM: The availability of a technique for assaying the biological activity of an RNA offers a unique opportunity for deciding whether functional RNA can be made in the presence of SM. As shown in Figure 2, the incorporation of uracil-C^{14} into TCA-insoluble material was not altered by addition of SM for at least 30 min. A similar pattern was observed in phage-infected cells, SM having little or no effect on total RNA synthesis for 30 min after addition.

SM was added to cultures of infected bacteria at 5, 10, and 20 min after infection. At intervals RNA was extracted from each of the cultures and assayed for

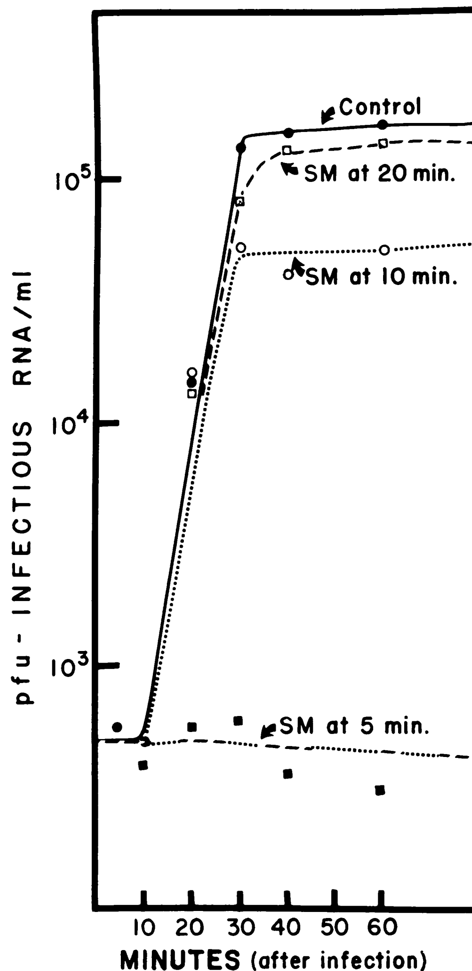

FIG. 4.—Synthesis of infectious RNA in Hfrl infected with phage R17. Bacteria in the logarithmic phase of growth were infected at time 0 with R17 (multiplicity of infection = 10). After 5 min the culture was divided into 4 parts. No additions were made to control culture (solid circles); to other cultures SM was added at 5 min (solid squares), 10 min (open circles), and 20 min (open squares). Aliquots were removed from each culture at intervals, and RNA was extracted and assayed in protoplasts of Hfrl.

infectivity in protoplasts of Hfrl. Solid circles of Figure 4 show the appearance of infectious RNA in phage-infected cells in the absence of SM. Addition of SM at 5 min after infection completely inhibited formation of infectious RNA (solid squares, Fig. 4). When SM was added 10 min after infection, the synthesis of infectious RNA continued for 20 min and stopped, reaching about 30 per cent of the control level (open circles). SM added at 20 min after infection (open squares) had little effect on the yield of infectious RNA, the treated culture reaching the same level as the control. Thus it seems that as long as time is allowed for an early phage function, production of a functional RNA can continue in the presence of SM.

Discussion.—In SM-resistant hosts, once the short initial SM-sensitive period is

passed, the replication of RNA phages is insensitive to the action of the antibiotic. This sensitive period may reflect a unique penetration process during which the RNA of the virus is free of its protein coat and is sensitive to attack by agents which inactivate or precipitate RNA. The infectious cycle of phage R17 is sensitive to the action of SM in SM-sensitive hosts, but this sensitivity manifests itself in different ways and to differing degrees depending upon the time at which SM is added during the cycle. The ease with which the biological activity of the phage components can be assayed permitted analysis of the SM-sensitive steps in the phage replication cycle in a sensitive host.

The results indicate that the synthesis of phage antigenic protein is almost immediately halted upon addition of 100 μg SM/ml, although the incorporation of amino acids into acid-insoluble material continues for 5–7 min. Little or none of this material synthesized in the presence of SM is phage antigen, at least as far as the presently available methods can detect such an antigen. The synthesis of infectious RNA is not inhibited for at least 20 min after addition of SM, provided a short (5–10-min) period of protein synthesis is allowed before the addition of the antibiotic. This suggests that early phage-induced protein synthesis is also inhibited by the antibiotic. The interpretation of data on sensitivity of proteins synthesized very early after infection is complicated by the sensitivity of the phage itself to SM for a brief period after adsorption.

It seems clear, however, that once an early protein synthesis takes place (presumably the synthesis of the phage-induced RNA polymerase), the production of infectious RNA can continue in the presence of SM. Whether cessation of further RNA synthesis after 20 min in SM reflects a direct action of SM on the RNA-synthesizing activity of the infected cell cannot be determined from these data. However, the fact that the initial rate of RNA synthesis is identical in the presence or absence of SM suggests that perhaps there is an asynchrony in the infectious process and that at the time of addition of SM only 30 per cent of the infected cells had made polymerase.

The RNA of the infecting phage must serve as template for its own replication as well as carry the genetic information for making phage-specific polymerase and coat protein. The sensitivity of the phage replicative process to SM cannot be a function of inactivation of this RNA, but rather reflects the selective sensitivity of the protein-synthesizing steps to the antibiotic. Wherever the block in protein synthesis exists, the phage RNA is free to replicate, forming functional copies.

These data do not preclude the possibility of formation of a lethal RNA (presumably a DNA-primed RNA rather than the RNA-primed RNA of the phage) as hypothesized by Stern and Cohen.[7] They do show that biologically active RNA can be synthesized in the presence of SM at the same time that protein synthesis, presumably coded by this same RNA, is completely inhibited. While the synthesis of phage RNA polymerase seems to be inhibited by SM, any enzyme in the infected cell replicates phage RNA to the same extent and with complete fidelity in the presence or absence of SM.

The author wishes to thank Dr. A. F. Graham, in whose laboratory this work was done, and Dr. J. G. Flaks for helpful discussions. I am indebted to Miss Arpi Hagopian for making available her unpublished data and for assistance with the precipitin reaction, and to Miss Clare Kirk for her excellent technical assistance

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*BIOCHEMICAL STUDIES ON ADENOVIRUS MULTIPLICATION, VII. HOMOLOGY BETWEEN DNA'S OF TUMORIGENIC AND NONTUMORIGENIC HUMAN ADENOVIRUSES**

BY SISTER S. LACY† AND MAURICE GREEN‡

INSTITUTE FOR MOLECULAR VIROLOGY AND DEPARTMENT OF MICROBIOLOGY, SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE

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The finding that adenovirus types 12 and 18 induce tumors in newborn hamsters[1-4] has generated a great deal of interest in these viruses. Since this is the first case of a "human" virus known to possess carcinogenic properties, it was of obvious importance to determine the molecular basis of this potentiality. Green and Piña[3, 4] have chemically analyzed and characterized the DNA's of purified tumorigenic adenoviruses by means of sedimentation velocity, thermal denaturation, and buoyant density measurements. These studies have revealed striking similarities between the DNA's of tumorigenic adenovirus types 12 and 18, while similar studies on the DNA's of nontumorigenic adenovirus types 2 and 4 have revealed remarkable differences between these DNA's and those of the tumorigenic adenoviruses (e.g., the DNA's of the latter contain 48–49% guanine-cytosine, while those of the nontumorigenic adenoviruses contain 56–57% guanine-cytosine).

The similar base composition and the carcinogenic activity of adenovirus types 12 and 18 suggest that they may be closely related genetically. The DNA-agar technique of Bolton and McCarthy[5-11] has been employed successfully to measure the degree of genetic relatedness among the enterobacteria,[6] among several mammalian and other vertebrate types,[7] and to demonstrate homology between bacteriophage lambda and the *E. coli* chromosome.[8] Using this technique in the studies reported here, the ability of the DNA's of adenovirus types 12 and 18 to hybridize with each other *in vitro* was measured. The degree of genetic relatedness as reflected by nucleotide sequence homology was estimated by quantitative comparison of the heterologous reaction (e.g., type 12 DNA × type 18 DNA) with the homol-