tone fractions III and IV in cells in which DNA is not undergoing replication, appears to be a general characteristic of higher organisms, plants, and animals.

It further appears that the inhibition of DNA synthesis in cells in which such synthesis is occurring promptly stops the formation of histones of groups I and II. It is these histones which have been shown to be the most effective in inhibition of DNA-dependent RNA synthesis in reconstituted nucleohistones.[15] The tentative conclusion may be drawn that any portion of the genome which is associated with histones I and II is not available for transcription without replication of DNA.

Summary.—The use of an ion-exchange chromatographic technique for the fractionation of histones has shown that only two classes of histones turn over in cells in which DNA is not undergoing replication. These are histones of groups III and IV, and, in addition, the proteins of the so-called runoff peak which precedes the elution of the histones proper. The lysine-rich histones I and II on the contrary are only formed in cells in which DNA is undergoing replication.

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[1] Huang, R. C. C., and J. Bonner, these PROCEEDINGS, **48**, 1216 (1962).
[2] Allfrey, V. G., V. C. Littau, and A. E. Mirsky, these PROCEEDINGS, **49**, 414 (1963).
[3] Barr, G. C., and J. A. V. Butler, *Nature*, **199**, 1170 (1963).
[4] Busch, H., *Histones and Other Nuclear Proteins* (New York: Academic Press, 1965), p. 69.
[5] Rasmussen, P. S., K. Murray, and J. M. Luck, *Biochemistry*, **1**, 79 (1962).
[6] Filner, P., in press.
[7] Flamm, W. G., and M. L. Birnstiel, *Exptl. Cell Res.*, **33**, 616 (1964).
[8] Reisfeld, R. A., V. J. Lewis, and D. E. Williams, *Nature*, **195**, 281 (1962).
[9] McAllister, H. C., Jr., Y. C. Wan, and J. L. Irvin, *Anal. Biochem.*, **5**, 321 (1963).
[10] Bucher, N. L. R., *Intern. Rev. Cytol.*, **15**, 245 (1963).
[11] Maurer, H. R., and C. W. Raiborn, unpublished.
[12] Ontko, J. A., and W. R. Moorehead, *Biochim. Biophys. Acta*, **91**, 658 (1964).
[13] Starbuck, W. C., and H. Busch, *Cancer Res.*, **20**, 891 (1960).
[14] Davis, J. R., and H. Busch, *Cancer Res.*, **19**, 1157 (1959).
[15] Huang, R. C., J. Bonner, and K. Murray, *J. Mol. Biol.*, **8**, 54 (1964).

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*EVIDENCE FOR VIRUS-SPECIFIC NONCAPSID PROTEINS IN POLIOVIRUS-INFECTED HELA CELLS**

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It is known that poliovirus RNA functions as messenger RNA in virus-specific cytoplasmic polyribosomes.[1, 2] If the entire viral RNA molecule specifies information for protein synthesis and the coding ratio is 3 nucleotides per amino acid,[3] the 6000 nucleotides of polio RNA[4] would encode peptide chains containing 2000

total amino acids, sufficient for 10 chains of 200 amino acids each. A number of events take place in poliovirus-infected cells which may be due to gene products other than the virus capsid protein. For example, an enzymatic activity capable of forming viral RNA develops in the cell cytoplasm,[5] host cell protein synthesis and RNA synthesis are interrupted early in virus infection,[6] and the infected cell has an increased capacity to incorporate choline.[7]

Two developments have made it possible to examine the total virus-directed protein synthesis in infected cells during most of the virus replicative cycle. First, conditions have been established for complete suppression of host protein synthesis prior to any viral RNA replication or most of the virus-controlled protein synthesis.[8] Second, improved techniques for sampling proteins separated by high-resolution acrylamide gel electrophoresis permit identification of radioactive virus-specific proteins in crude extracts from infected cells after exposure to isotopic labels.

The present experiments provide evidence (1) that 12–14 electrophoretically different virus-specific protein chains are formed in poliovirus infected cells, and (2) that many, if not all, of these chains are synthesized during most of the virus cycle, although the rates of synthesis of certain proteins may vary during infection.

Materials and Methods.—Suspension cultures of S-3 HeLa cells grown in Eagle's medium[9] (purchased as a dry powder from General Biochemicals) and a plaque-purified strain of type 1 poliovirus were used throughout the studies. Cell growth, harvesting, infection and labeling procedures have been described.[2] Cells (4–5 × 10^6/ml) were infected with an input multiplicity of 300 plaque-forming units per cell. Actinomycin D (5 γ/ml) was added to all cultures at the time of infection, and guanidine, when present, was 3 mM. Since virus RNA formation in the absence of serum has recently been variable in our laboratory, infection is now initiated in serum-free medium and 5% undialyzed horse serum added 30 min later. As previously described,[1] cells were fractionated by Dounce homogenization after a 5-min period of swelling in hypotonic buffer, termed RSB (reticulocyte standard buffer = 0.01 *M* NaCl, 0.015 *M* $MgCl_2$, 0.01 *M* tris, pH 7.4). C^{14} algal protein hydrolysate (1 mC/mg), C^{14}-uridine (30 μC/μmole), and C^{14}-L-amino acids (30 μC/μmole carbon) were obtained from New England Nuclear; H^3-L-amino acids (1–2 mC/μmole) from Schwarz BioResearch. Radioassay of samples other than gel fractions was performed as previously described.[2]

A procedure was developed for solubilizing proteins in samples to be subjected to electrophoresis based on the susceptibility of poliovirus particles to inactivation by SDS (sodium dodecyl sulfate) at acid pH[10] and the solubilization of lipoproteins of chloroplasts[11] in dilute SDS-urea solutions. Cell cytoplasm in RSB was acidified with $^1/_{10}$ vol of glacial acetic acid and then made 0.5 *M* in urea and 1% in SDS. After incubation at 37° for $^1/_2$–1 hr the samples were dialyzed 16–18 hr at 25° against 2000–6000 vol of 0.01 *M* sodium phosphate buffer, pH 7.1, containing 0.1% SDS, 0.5 *M* urea, and 0.1% 2-mercaptoethanol. After dialysis, samples were mixed with $^1/_6$ vol of 60% sucrose (total vol less than 0.35 ml) and layered under phosphate buffer without sucrose onto the prepared gel. Electrophoresis was performed in glass tubes (0.6 × 27.5 cm) on vertical 20-cm gel columns composed of 10% acrylamide, 0.27% N, N'-bis-methylene acrylamide, 0.1% SDS, 0.5 *M* urea, 0.1 *M* sodium phosphate pH 7.2. Polymerization was catalyzed by final concentrations of 0.05% (v/v) N,N,N'-tetramethylethylene diamine and 0.075% ammonium persulfate. The polymerization step was carried out under a 1-cm layer of water to obtain a smooth gel surface. The buffer used in the electrode vessels was 0.1 *M* sodium phosphate, pH 7.2, containing 0.1% SDS. No temperature control was employed and the gels were warmed to about 30°C. The voltages and times used in each electrophoresis are given in the text with the figures. After electrophoresis the gels were fractionated mechanically by extrusion through a device (to be described in complete detail elsewhere[12]) which pulverized the gel in sequential fashion. The pulverized gel was carried directly into liquid scintillation counting vials by a continuous flow of water. Capped samples of the gels in 1 ml of water were allowed to stand at 25° overnight, and

H^3 and C^{14} radioactivity was determined the next day in a Packard liquid scintillation spectrometer after the addition of 10 ml of Bray's solution.[13]

Results.—Establishment of conditions for observing exclusively virus-specific protein synthesis: Guanidine concentration greater than 1 mM prevents the initiation of synthesis of infectious viral RNA, as well as interrupting already established RNA synthesis during the replicative cycle of guanidine-sensitive strains of poliovirus.[5, 14] Its effect on the synthesis of viral RNA with the virus strain used in these experiments is shown in Figure 1. Viral RNA synthesis was measured by total uptake of C^{14}-uridine into actinomycin-treated poliovirus-infected cells (Fig. 1 *inset*) as well as by the appearance of newly formed cytoplasmic RNA which sediments at 35*S*, the sedimentation coefficient of viral RNA.[6] (The latter test has recently been shown to be sufficiently sensitive to detect the first 0.05% of newly formed progeny viral RNA.[15]) By both criteria guanidine prevented viral RNA formation. Upon the removal of guanidine, virus RNA synthesis was evidenced by an increasing total incorporation of uridine 2-C^{14} within 1.5–2 hr, 30 min in advance of the first detectable increase in untreated infected cells (Fig. 1 *inset*). The same total amount of viral RNA is formed in the guanidine-treated reversed cultures as in untreated samples, but the time course of synthesis (starting from the time of reversal) remains 30 min ahead of the untreated control throughout. This gain of approximately 30 min in the guanidine-treated reversed cultures may represent a "synchronization" of infection due to all infectious particles proceeding through the steps of adsorption, penetration, and release of RNA without RNA replication. Soon after guanidine is removed, viral RNA replication begins synchronously from many particles.

Although guanidine inhibits replication of viral RNA, other events due to viral in-

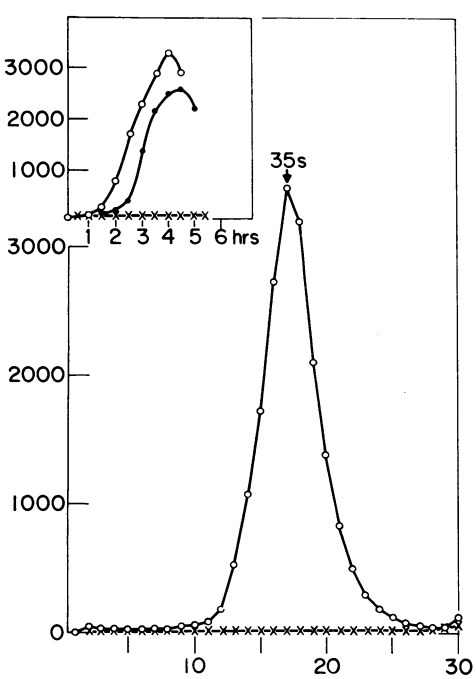

FIG. 1.—Effect of guanidine on poliovirus RNA synthesis. *Major graph:* 50 ml infected actinomycin-treated cells were treated with 3 mM guanidine for 2 hr, at which time guanidine was removed from one culture. Two hr later 15 μC of C^{14}-uridine were added to each culture and after 1 hr viral RNA synthesis determined by sucrose gradient zone sedimentation analysis of SDS released cyoplasmic RNA as previously described.[2] The 35*S* viral RNA peak was determined by reference to OD markers from ribosomal RNA (-O- reversed, -×- unreversed). *Inset:* Two samples of actinomycin-treated infected cells were guanidine-treated for 2 hr. One was reversed as above (-O-), while the other remained in guanidine (-×-). The cultures were labeled with 0.15 μC C^{14}-uridine/ml at the time of reversal and 0.1-ml samples plated at intervals for acid-precipitable radioactivity. Another culture (-●-) not treated with guanidine was similarly labeled from the time of virus addition.

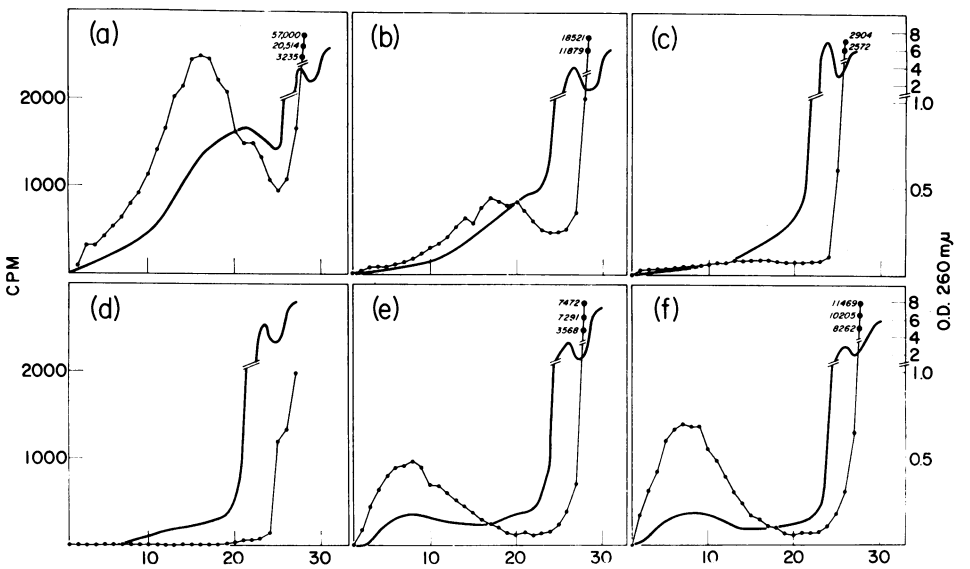

FIG. 2.—Protein synthesis in guanidine-treated poliovirus-infected cells. At intervals during and after a 2-hr guanidine treatment 15 ml of infected actinomycin-treated cells in Eagle's medium[8] minus amino acids plus 5% undialyzed horse serum were pulsed for 5 min with 20 μC algal protein hydrolysate. Cytoplasmic extracts were prepared and analyzed by sucrose gradient zone sedimentation (15–30% sucrose, 4°, 90 min, 25,000 rpm) for incorporation of amino acids into polyribosomes (—●— cpm; solid line absorbance at 260 mμ). Fractions 1–20 contain polyribosomes; fractions 25–29 contain 74S ribosomes. Guanidine-treated: (*a*) 60 min postinfection. (*b*) 120 min postinfection. Guanidine-treated for 2 hr and guanidine removed for (*c*) 60 min; (*d*) 120 min; (*e*) 135 min; (*f*) 150 min. Samples *a,b, c, d* from one experiment; *e* and *f* from a comparable but separate experiment.

fection can proceed. For example, Penman and Summers[8] have recently demonstrated that the interruption of host cell protein synthesis, tested by amino acid incorporation into total cell protein, proceeds in the infected, guanidine-treated cell.

Figure 2 shows that this virus-induced suppression of host protein synthesis in guanidine-treated cells is due to a progessive loss of functioning polyribosomes. Within 2 hr host protein synthesis has declined by at least 75–80 per cent, and when guanidine is removed so that virus RNA can replicate, the remaining traces of host protein synthesis disappear (Fig. 2, *c* and *d*) within about 1 hr. Within 2–2¼ hr after guanidine removal, large virus-specific polyribosomes are observed (Fig. 2, *e* and *f*). These structures contain 35*S* viral messenger RNA[2] together with about 50 host ribosomes engaged in the synthesis of viral proteins.[1, 16]

Nature of proteins found in infected cells: To study the proteins being formed by poliovirus polyribosomes after the complete suppression of host protein synthesis, a technique was needed which would allow reproducible assay of isotopically labeled viral specific proteins after separation by gel electrophoresis in the presence of unlabeled cellular proteins.

It had previously been shown that poliovirus particles, purified by column chromatography and CsCl banding, can be dissociated into four protein components detectable by amido black staining of acrylamide gel electropherograms.[17] However, the use of stains as the only means of locating proteins after electrophoretic separation severely limits the utility of the gel technique for two reasons. Only samples

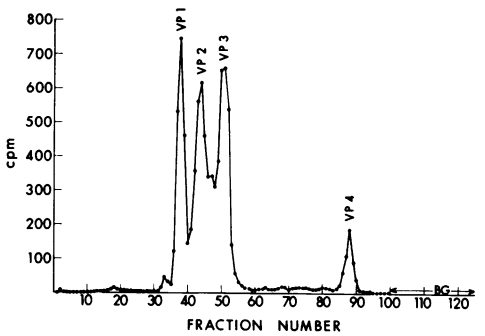

FIG. 3.—Protein chains in purified poliovirus. Poliovirus labeled with C^{14}-leucine, isoleucine, and valine was taken from the virus peak of a sucrose gradient of infected cell cytoplasm,[2] combined with 2 mg purified unlabeled virus, treated with 0.1% SDS, centrifuged (2 hr × 100,000 *g*), and banded in CsCl.[14] After dialysis against 0.02 *M* sodium phosphate buffer, pH 7.2, the virus was treated as described in *Methods*, and about 1/6 of the sample analyzed by gel electrophoresis. The electrophoresis was for 8 hr at 3 v/cm (–●–cpm). The apparatus and basic techniques used in the electrophoresis were modifications of those described by Davis.[20] The anode is to the right in this and subsequent figures.

containing a limited number of proteins can be resolved, since the protein stains are nonspecific; and quantitation of the amount of each protein by the amount of dye it takes up would require standardization for each individual protein. Figure 3 shows that by combining the techniques of protein solubilization, gel electrophoresis, and mechanical fractionation, described in *Methods*, excellent resolution of radioactivity is obtained from a sample of purified virus labeled with C^{14} amino acids. The radioactive profile contains three major components (*VP 1, 2, 3*) plus a smaller "fast-running" component (*VP 4*), just as the stained preparations do. The radioactive peaks for *VP 1, 3*, and *4* are all sharp and symmetrical, while the peak for *VP 2* tends to have an as yet unexplained shoulder on the anodal side. A

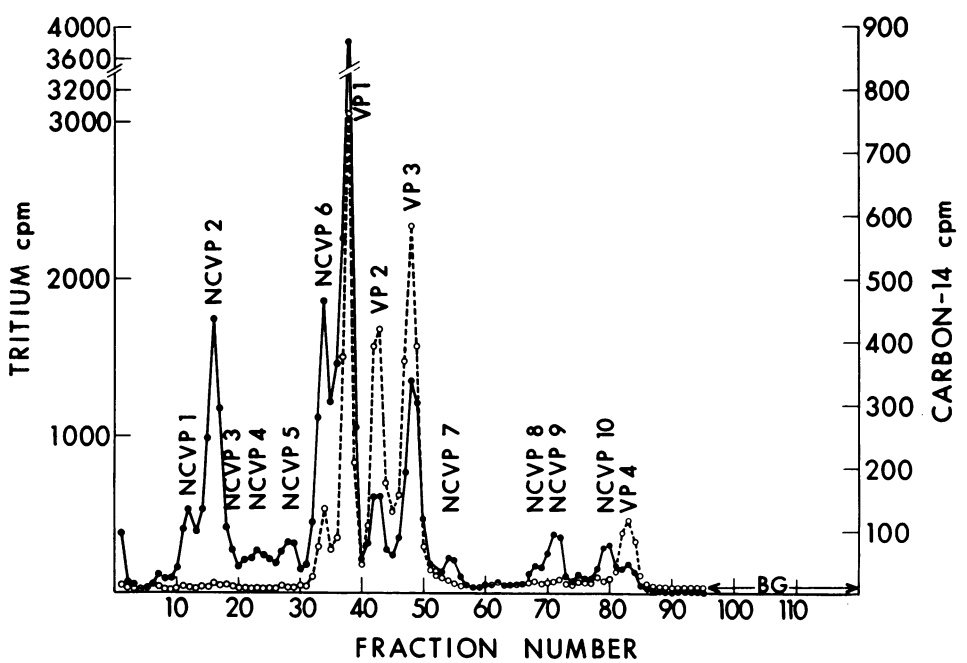

FIG. 4.—Virus-specific proteins in total cytoplasmic extract of infected cells. 25 ml of infected cells were guanidine-treated for 2 hr, reversed, and labeled 1–4 hr postreversal with 30 μC each H^3-leucine, isoleucine, and valine. Cytoplasmic extract was prepared and mixed with virus labeled with C^{14}-leucine, isoleucine, and valine. (The virus was taken from a sucrose gradient without further purification.) The sample was then treated and analyzed by gel electrophoresis (8 hr, 3 v/cm) C^{14} –○–, H^3 –●–.

small fifth peak found just before *VP 1* is detectable in labeled virus preparations taken straight from sucrose gradients of cytoplasmic extracts without additional purification (see Fig. 4, for example). This peak is not considered an integral part of the virus particle.

The virus proteins corresponding to peaks *VP 1*, *VP 2*, and *VP 3* (the major viral peaks) have been recovered after gel electrophoresis in 8 *M* urea.[17] Preliminary analysis has shown that each protein has a distinctive amino acid composition and, in addition, a different peptide map.[12] Poliovirus particles therefore probably contain four different peptide chains. Rueckert has also recently described two major protein components with different amino acid compositions in the capsid of ME virus, a virus similar to polio.[18] Thus the small icosahedral RNA-containing animal viruses, previously assumed to be of simple construction, contain several different peptide chains.

After the electrophoretic pattern of isotopically labeled proteins associated with the virus particle had thus been established, experiments were performed to detect other noncapsid proteins synthesized in poliovirus-infected cells. Cells were infected in 3 mM guanidine and after 2 hr washed and returned to 37°. The infected cells were then labeled with H^3-valine, leucine, and isoleucine 1–4 hr after guanidine removal. Well over 90 per cent of the protein synthesis during this time is virus-specific (see Fig. 2). A cytoplasmic extract of the labeled infected culture was prepared and mixed with a sample of virus labeled with C^{14} amino acids and partially purified in a sucrose gradient. The mixture was then treated and subjected to electrophoresis, as described in *Methods*, with the results shown in Figure 4. The first point to be emphasized is that, even in the presence of all the proteins of the infected cell cytoplasm, the C^{14} virus remains disaggregated, and its constituent chains are separated by electrophoresis. (There is, in addition to *VP 1–4* seen in Fig. 3, a minor component present in virus taken directly from sucrose.) It is evident in Figure 4 that a number of protein chains are made in the infected cell which differ in electrophoretic properties from the capsid proteins. For convenience the virus proteins are labeled *VP 1–4* and the noncapsid virus-specific protein peaks, *NCVP 1–10*.

To determine the time during replication at which the *NCVP* are synthesized, guanidine-treated infected cells were pulse-labeled (8 min pulse plus 5 min chase) with C^{14} amino acids at various times after guanidine removal. The total cytoplasm from these various cultures was then mixed with the cytoplasm of infected cells labeled throughout infection with H^3 amino acids, and gel analysis was performed on each sample. Labeled cytoplasm from uninfected guanidine-treated cells and infected cells which were not removed from guanidine was also examined. Amino acid incorporation in the infected cultures continuously exposed to guanidine was very low (<5%) compared to the uninfected control. Within 70 min after guanidine removal, virus-specific protein synthesis became apparent as specific peaks which were not present in unreversed or uninfected controls (Fig. 5*A–C*). By 90 min the synthesis of virus-specific proteins made up at least 75–80 per cent of the observed protein synthesis and increased further as infection proceeded. Two points about the patterns in Figure 5*D-I* may be emphasized. Most of the characteristic peaks seen in Figure 4 can be distinguished in all patterns beginning as early as 90 min after guanidine reversal when viral RNA synthesis is only 5–10 per

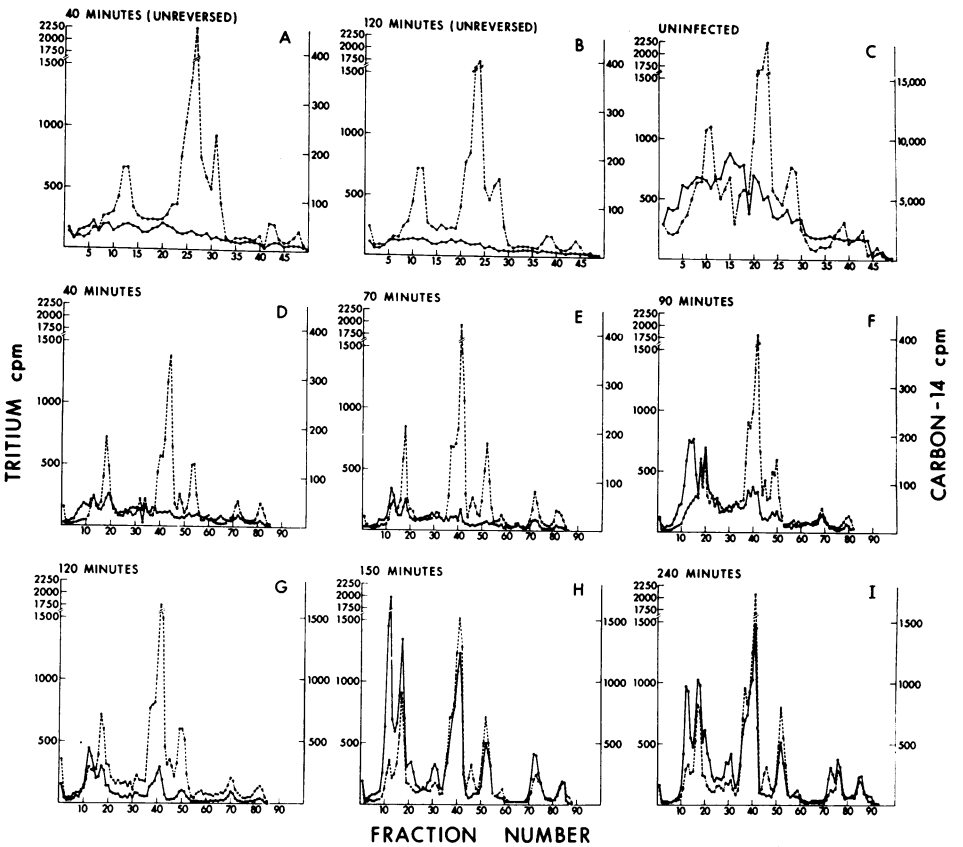

FIG. 5.—Synthesis of virus-specfic proteins during replicative cycle. At various times before and after removal of guanidine 25-ml samples of infected actinomycin-treated cells in amino acid-free Eagle's medium plus 5% serum were labeled with 10 μC each of C^{14}-isoleucine, tyrosine, and valine for 8 min and then "chased" for 5 min with C^{12} amino acids (final concentration valine and isoleucine 4.8 mM, tyrosine 0.8 mM). Sample C was actinomycin- and guanidine-treated but not infected and received only $^1/_{10}$ as much label as the infected samples. Cytoplasmic extracts of the various samples were mixed with H^3-labeled infected cell cytoplasm from a sample similar to that of Fig. 4, treated, and analyzed by gel electrophoresis (*A, B, C,* 10-cm gels, 4 hr, 3 v/cm; *D-I,* 20-cm gels, 8 hr, 3 v/cm) cpm, H^3—○—, left-hand ordinates; C^{14}–●–, right-hand ordinates. Note scale changes for C^{14}. Unreversed cultures: (*A*) 40 min postinfection, (*B*) 120 min postinfection, (*C*) uninfected culture. Cultures reversed after 2 hr: (*D*) 40 min postreversal, (*E*) 70 min, (*F*) 90 min, (*G*) 120 min, (*H*) 150 min, (*I*) 240 min.

cent completed (see Fig. 1). However, the proportions of newly incorporated radioactivity in the various peaks change during the course of infection. Thus, *NCVP 1* and *2* (fractions 10–25) dominate the 90- and 120-min samples while the virus proteins *VP 1* and *3* (fractions 35–55) are relatively more prominent at 150 and 240 min. The data in Figure 5 have been summarized in Table 1, where the apparent fluctuations in synthesis of different protein classes during infection can clearly be seen.

In comparing *NCVP 1* and *NCVP 2* from 8-min labels, it is to be noted that at all time points *NCVP 1* contains as many or more counts (C^{14}) than does *NCVP 2.* After exposure to H^3 amino acids for 3 hr, however, the proportion of *NCVP 1* relative to *NCVP 2* is very small. It would appear, therefore, that *NCVP 1* is

TABLE 1

DISTRIBUTION OF RADIOACTIVITY IN SEVERAL OF THE VIRUS CAPSID AND NONCAPSID PROTEINS LABELED AT VARIOUS TIMES DURING REPLICATION*

Time after reversal (min)	*NCVP 1* †	*NCVP 2*	*VP 1*	*VP 2*
90	897 (22.9)‡	604 (15.4)	260 (6.6)	183 (4.7)
120	1723 (24.4)	934 (13.2)	981 (13.9)	400 (5.7)
150	5616 (22.4)	3904 (15.6)	4011 (15.9)	1817 (7.2)
240	3011 (12.5)	3697 (15.4)	4182 (17.4)	1808 (7.5)
60–240	1096 (5.1)	2633 (12.4)	5357 (25.1)	2643 (12.4)

* Data taken from Fig. 5; 90, 120, 150, 240 are pulse-labeled C^{14} counts; 60–240 is H^3.
† Identification of peaks given in Fig. 4.
‡ The open numbers are counts per minute (cpm); numbers in parentheses are % of total recovered cpm. The recovery of C^{14} label in the gels was 100%; that of H^3 was 90–95% of the amount applied.

rapidly synthesized, but then is either degraded, transformed into one of the other peaks, or lost from the cytoplasm.

Discussion.—The cytoplasm of polio-infected cells contains a number of virus-directed peptide chains differing in their electrophoretic behavior. Although not yet proved, there are reasons to believe that most, if not all, of these electrophoretic peaks represent different proteins with different primary amino acid sequences. For example, purified virus contains at least three major peptide chains with distinct amino acid compositions.[12] Although these chains are approximately the same size, they give three separate electrophoretic peaks,[17] even in the presence of all the infected cell cytoplasmic proteins (Figs. 3 and 4). Under the conditions of the present experiments, therefore, proteins are readily disaggregated into their constituent peptide chains. However, it should be pointed out, it is likely that the basis of separation under these circumstances is not the charge and size of the native protein but the charge and size of the protein-SDS complex. Attempts are in progress to determine the amino acid composition of each electrophoretic peak, in order to provide conclusive evidence of individuality.

If we assume that at least most of the peaks observed represent different proteins, the present experiments provide insight into the problem of translation of a polycistronic messenger RNA. Three basic methods of translating the structural genes of a polycistronic viral messenger RNA may be considered: (1) no regulation whatever, that is, proteins are formed from all genes at equal rates throughout infection; (2) each protein or class of proteins is formed during a specific phase of infection; (3) protein chains from different genes are formed in different numbers, but the ratios at which they are formed remain constant throughout infection.

Two findings in the present experiments indicate that a combination of methods 2 and 3 is operative in the translation of poliovirus RNA. (*a*) Early in the infection, the total radioactivity in *NCVP 1* and *2* is greater than in *VP 1* and *3*, while the reverse is true late in infection. (*b*) The amount of radioactivity in *VP 1* or *VP 3* is at least 20 times greater than that of *NCVP 8* or *VP 2*, and perhaps 100 times greater than *VP 4*. These differences cannot be due simply to a large difference in the size of the protein chains, since the molecular weights of the major capsid proteins *VP 1* and *VP 3* are in the range of 25,000–40,000.[17, 21] The translation of poliovirus RNA then is controlled so that the rate of translation of different genes changes during infection and many more products of certain genes are made than of others.

It should be emphasized that in these experiments we have studied the transla-

tion of RNA which is almost entirely progeny RNA, and whether the initial infecting RNA strand follows the same patterns of translation remains unknown.

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† NIH special postdoctoral fellow.

‡ USPHS Career Development Awardee.

[1] Penman, S., K. Scherrer, Y. Becker, and J. E. Darnell, these PROCEEDINGS, **49**, 654 (1963).

[2] Penman, S., Y. Becker, and J. E. Darnell, *J. Mol. Biol.*, **8**, 541 (1964).

[3] Crick, F. H. C., *Progr. Nucleic Acid Res.*, **1**, 164 (1963).

[4] Schaffer, F. L., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 89.

[5] Baltimore, D., H. J. Eggers, R. M. Franklin, and I. Tamm, these PROCEEDINGS, **49**, 843 (1963).

[6] Salzman, N. P., R. Z. Lockart, and E. D. Sebring, *Virology*, **9**, 244 (1959); Zimmerman, E. F., M. Heeter, and J. E. Darnell, *Virology*, **19**, 400 (1963).

[7] Penman, S., *Virology*, **25**, 148 (1965).

[8] Penman, S., and D. F. Summers, in press.

[9] Eagle, H., *Science*, **130**, 432 (1959).

[10] Mandel, B., *Virology*, **17**, 288 (1962).

[11] Criddell, R. S., and L. Park, *Biochem. Biophys. Res. Commun.*, **17**, 74 (1964).

[12] Maizel, J. V., in preparation.

[13] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).

[14] Crowther, D. L., and J. L. Melnick, *Virology*, **15**, 65 (1961).

[15] Baltimore, D., M. Girard, and J. E. Darnell, in preparation.

[16] Scharff, M., A. Shatkin, and L. Levintow, these PROCEEDINGS, **50**, 686 (1963).

[17] Maizel, J. V., *Biochem. Biophys. Res. Commun.*, **13**, 483 (1963).

[18] Rueckert, R., personal communication.

[19] Levintow, L., and J. E. Darnell, *J. Biol. Chem.*, **234**, 70 (1960).

[20] Davis, B. J., *Ann. N. Y. Acad. Sci.*, **121**, 404 (1964).

[21] Boeye, A., *Virology*, **25**, 550 (1965).

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*PURIFICATION, ELECTRON MICROSCOPY, AND X-RAY DIFFRACTION STUDIES OF THE SATELLITE TOBACCO NECROSIS VIRUS**

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The Rothamsted culture of tobacco necrosis virus was long known to contain spherical particles of two sizes,[1] which were recently established as two serologically unrelated viruses, one called the "satellite virus" (SV) which causes no lesions and multiplies detectably only in the presence of the other virus (TNV).[2] The SV is an unusually small nucleoprotein with a calculated molecular weight of $1.97 \cdot 10^6$, containing 20 per cent of RNA.[3] The genetic information carried by this RNA with a molecular weight of the order of $4 \cdot 10^5$ is very limited and it was recently proposed that SV RNA could code for a maximum of 400 amino acid residues, which is consistent with the number of amino acid residues in the coat protein.[3]