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# Biosynthesis of Host and Viral Deoxyribonucleic Acid During Hyperplastic Fowlpox Infection In Vivo[1]

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The synthesis of deoxyribonucleic acid (DNA) during in vivo infection of chick epithelium with fowlpox virus was examined by incorporation of tritiated thymidine into the acid-insoluble fraction. The proportion of precursor incorporated into host and viral DNA at various times after infection was determined by chromatography on columns of methylated albumin-kieselguhr. The first 60-hr period of infection was characterized by the synthesis of predominantly host DNA, the rate of production of which increased markedly over the control between 36 and 48 hr postinoculation (PI). Although the replication of viral DNA began between 12 and 24 hr PI, the rate of synthesis was very low during the first 60 hr. In contrast, an abrupt increase in the rate of viral DNA synthesis occurred between 60 and 72 hr PI, concomitantly with a sharp decline of host DNA synthesis. Subsequently, between 72 and 96 hr, the ratio of synthesis of viral DNA to host DNA progressively increased to a maximum of greater than 2:1. The temporal relationship of this biphasic pattern of host and viral DNA synthesis to hyperplasia and viral replication is discussed.

Infection of the squamous epithelium of chickens with fowlpox (FP) virus is characterized by a pronounced transitory hyperplasia, with development of classical intracytoplasmic inclusions (3, 8, 22). These features are not evident by in vitro methods (Randall and Gafford, *unpublished data*), which have largely supplanted animal studies on the biochemistry of virus infection, and inherent difficulties of the in vivo model do not ordinarily permit application of the techniques of quantitative virology. The study of concomitant hyperplasia and viral replication induced by poxviruses, therefore, does not appear feasible in vitro, as the complete expression of the phenomena is apparent only in the intact host. However, quantitative in vivo studies of this important problem have received little attention. This situation is probably the result of lack of proper methodology, as any quantitative approach depends on the availability and methods of isolating homogeneous, uniformly infected tissue. Toward this end, techniques utilizing chick skin which permit the separation of control and approximately 100% infected squamous epithelium from underlying connective tissue have been devised by Randall et al. (15) and Randall and Gafford (13).

[1] Taken from a thesis submitted by W. P. Cheevers in partial fulfillment of requirements for the Ph.D. degree.

To establish the rationale for the present study, it is necessary to re-emphasize that FP infection of chick skin is associated with characteristic cytopathic changes, the most conspicuous markers being hyperplasia and the presence of enduring inclusion bodies by 60 hr in the affected cells. In preliminary studies (Cheevers and Randall, Proc. Soc. Exptl. Biol. Med., *in press*), we have examined the relationship of the sequential development of hyperplasia to viral replication. Briefly, FP virus induces proliferation of cutaneous epithelium, first evident after 48 hr of infection and terminating in a 2.5-fold increase in cell number at 72 hr. On the other hand, viral replication, initiated after a latent period of approximately 24 hr, is exponential through 96 hr. It is significant that, although viral replication begins prior to induction of hyperplasia, greater than 99% of the titer is attained (between 72 and 96 hr) after cell proliferation has ceased.

Thus, it appears that periods of hyperplasia and viral replication are relatively distinct and are amenable to experimentation. To this end (in the present communication) the biosynthesis of deoxyribonucleic acid (DNA) during FP infection in vivo is analyzed, and a temporal relationship of host and viral DNA synthesis to hyperplasia and viral replication is established. The nature of virus-cell interaction operative

during periods of hyperplasia and viral replication is discussed.

## MATERIALS AND METHODS

*Virus and inoculation of animals.* The strain of virus, preparation of inoculum, and collection of tissue have been described (13, 15, Cheevers and Randall, *in press*). Briefly, the scalp and neck of 1-day-old cockerels were plucked and (excepting the controls) inoculated 2 hr after removal of feathers. The inoculum contained $2.5 \times 10^8$ pock-forming units (PFU)/ml, and approximately 0.2 ml applied by light rubbing with a cotton swab insured a confluent infection.

As FP infection is characteristically epithelial, it was desirable to restrict analyses to more accessible cutaneous epithelium (referred to as surface epithelium) and to disregard follicular epithelium which, if infected for fewer than 5 days, cannot be effectively separated from connective tissue. For quantitative comparison of control and infected tissue, all preparations of surface epithelium for analyses were obtained from sections of skin measuring approximately 5 cm long $\times$ 1.5 cm wide (7.5-cm$^2$ surface area). The results of all experiments were expressed relative to this constant sample. The thickness of epithelium was variable, as this dimension increases during infection (Cheevers and Randall, *in press*).

*Tissue fractionation and chemical procedures.* In experiments on the incorporation of thymidine (TdR) into total DNA of control and infected epithelium, tissue was separated into acid-soluble and acid-insoluble fractions by the procedure of Schneider (16). Where applicable, DNA was determined by the colorimetric method of Burton (5).

*Isotopic procedures.* Each animal received 50 $\mu$c ($2.85 \times 10^{-2}$ $\mu$mole) of TdR-methyl-$^3$H (Schwarz Bio Research, Orangeburg, N.Y.) as an intraperitoneal injection of 0.25 ml. The frequency of injections and labeling intervals are described with the procedures employed for each experiment. Samples for assay of radioactivity were dispensed in scintillator fluid prepared as described by Gentry et al. (7) and were counted in a Packard Tri-Carb liquid scintillation spectrometer.

*Extraction of tritium-labeled DNA.* Purified DNA for density gradient and chromatographic analyses was prepared as previously described (6). The concentration of DNA was estimated from the ultraviolet (UV) absorbance at 260 m$\mu$ with the use of a conversion factor of 0.02 $A_{260}$ for 1 $\mu$g/ml of native DNA (19). FP DNA was extracted and purified from viral inclusions isolated from epithelium after 6 days of infection, by the trypsin procedure of Randall and Gafford (13). Each animal had received isotope at 24, 48, 72, and 96 hr postinfection (PI). Cellular DNA was extracted from epithelium obtained from chicks 12 hr after a single injection of isotope and from liver nuclei isolated according to Bracken and Randall (4).

*Analytical density gradient centrifugation.* The conditions for CsCl equilibrium density gradient centrifugation were essentially those described by Soehner et al. (17). DNA (approximately 2 $\mu$g/ml final concentration) was mixed with 0.01 M tris(hydroxymethyl)aminomethane (Tris)-buffered (*p*H 7.0) CsCl solution to a final buoyant density of 1.700 g/cm$^3$ and was centrifuged in analytical cells with 4° sector Kel-F centerpieces at 44,770 rev/min for 24 hr at 22 C by use of a Spinco An-F rotor. Banding patterns were traced on a Beckman Analytrol film densitometer. The procedures used for determination of buoyant density and base composition have been described in detail (17, 18).

*Chromatography of DNA on methylated albumin-kieselguhr (MAK) columns.* Viral and cellular DNA, purified as described, were analyzed by chromatography on three-layer MAK columns (liquid displacement volume, approximately 7.5 ml) prepared by the procedures of Mandell and Hershey (11). DNA was applied to washed columns at a concentration of 10 $\mu$g/ml in phosphate-buffered NaCl (*p*H 6.7) of the starting molarity. The gradient technique (11) was employed for elution of DNA. Reproducible conditions of gradient elution were maintained as follows. A 100-ml amount of starting buffer in the mixing vessel (lower NaCl concentration) was mixed with the solution of higher NaCl concentration (reservoir) at a constant rate of 0.4 ml/min. The eluent from the column was collected in 2-ml portions (5 min/tube). The molarity of NaCl in eluent fractions was calculated as described (11). Fractions containing radioactivity were collected into a solution of carrier protein (albumin bovine fraction V) at 400 $\mu$g/ml final concentration; acid-insoluble material was precipitated with 5% trichloroacetic acid, collected and washed by centrifugation, and counted as previously described. Unlabeled fractions were collected without addition of carrier protein and monitored directly by UV absorbance at 260 m$\mu$.

## RESULTS

*Incorporation of $^3$H-TdR into acid-precipitable fractions of control and FP-infected epithelium.* Overall DNA synthesis in control and FP-infected epithelium was compared during each of eight 12-hr intervals between 0 and 96 hr PI by examining the incorporation of $^3$H-TdR into acid-precipitable nucleic acid fractions. At specified times, control and infected chicks received isotope; epithelial preparations were fractionated and analyzed as described.

As shown in Fig. 1, incorporation into DNA of both control and infected tissue increased markedly between 12 and 24 hr PI. The possibility that this initial stimulation was related to the removal of feathers in a manner similar to hair-plucking in mammals (12) was studied. The incorporation of TdR into epithelial DNA of chicks treated as described was compared with that in feathered controls. The results (Fig. 1) indicated that the removal of feathers was responsible for early increased incorporation of precursor into DNA.

Following the stimulative effect of removal of feathers during the initial 24 hr, incorporation

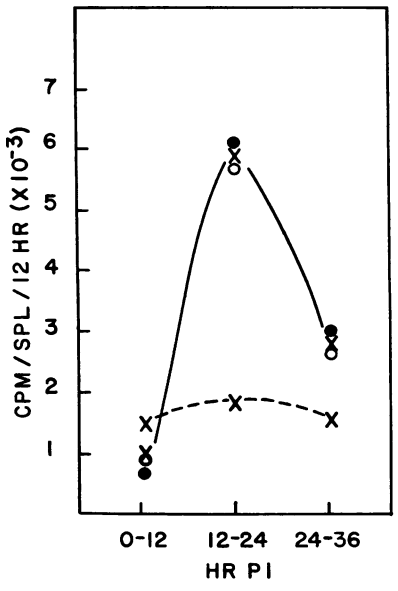


FIG. 1. *Nonviral stimulation of $^3$H-TdR incorporation into DNA of fowlpox-infected epithelium in vivo. Incorporation of precursor into acid-insoluble fractions of control and infected epithelium was measured after each indicated 12-hr postinfection interval. At specified times, chicks received an intraperitoneal injection of 0.25 ml of isotope solution (50 μc in 2.85 × 10⁻² μmole). Epithelial preparations were fractionated and analyzed as described in Materials and Methods. Control, ●; Infected, ○. Values are the average of three experiments. To analyze the effect of feather removal, incorporation into epithelial DNA of uninfected defeathered chicks was compared with feathered controls. Scalp and neck feathers were not plucked from feathered controls until after termination of each indicated labeling interval. Unfeathered during labeling periods, ×—×; feathered during labeling periods, (×- -×). Values are the average of two experiments.*

into DNA of uninfected tissue began to decline (Fig. 1). As shown in Fig. 2, the decline was arrested between 48 and 60 hr, and subsequently a slight sequential increase of acid-insoluble radioactivity was noted. No significant difference from the control was apparent in the incorporation of TdR into DNA of infected epithelium during the first 36 hr (Fig. 1). Subsequently, in infected tissue, however, incorporation increased markedly to reach a maximum over the control of greater than fourfold after 72 hr of infection (Fig. 2).

It is pertinent to comment on the reliability of these data as an accurate index of DNA synthesis. It was recognized that increased incorporation into DNA during infection could have been due to increased availability of precursor rather than to a stimulation of DNA synthesis. As shown in Fig. 2, however, the period of increased incorporation in infected epithelium was not accompanied by a significant change in acid-soluble activity, indicating that utilization rather than distribution of TdR was increased as a result of infection. Furthermore, as expected, the termination of further increase of incorporation after 72 hr was accompanied by a slight increase in acid-soluble radioactivity, probably as a result of the accumulation of phosphorylated derivatives of TdR. In addition to this evidence, the sequential incorporation of $^3$H-TdR (Fig. 2) reflects a pattern of cumulative DNA synthesis in infected tissue consistent with that previously obtained in a preliminary study by colorimetric techniques (Cheevers and Randall, *in press*).

*Characterization of viral and cellular DNA.* Although the data given in Fig. 2 indicate that FP virus induces a stimulation of DNA synthesis in chick epithelium infected in vivo, a distinction

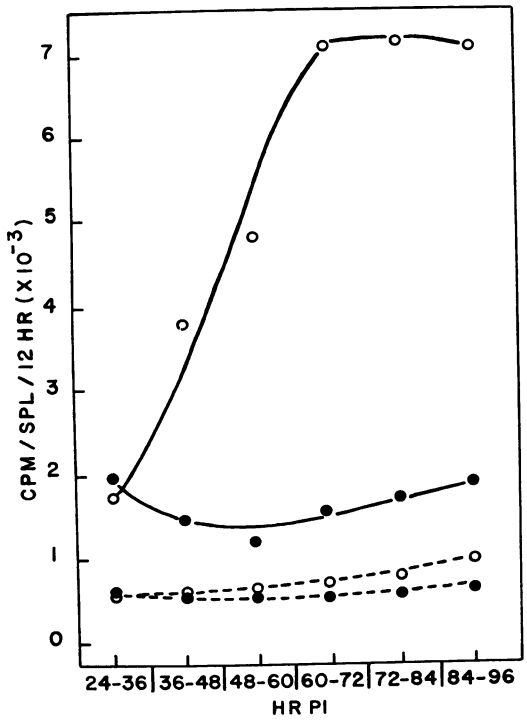


FIG. 2. *Incorporation of $^3$H-TdR into DNA of control and fowlpox-infected chick epithelium in vivo. The distribution of acid-soluble (dashed line) and acid-insoluble (solid line) radioactivity was measured in control (●) and fowlpox-infected (○) epithelium after each indicated 12-hr postinfection interval. The conditions of labeling were the same as those described in Fig. 1. The acid-insoluble counts for 24 to 36 hr were corrected for the effect of feather removal (Fig. 1). Values are the average of three experiments.*

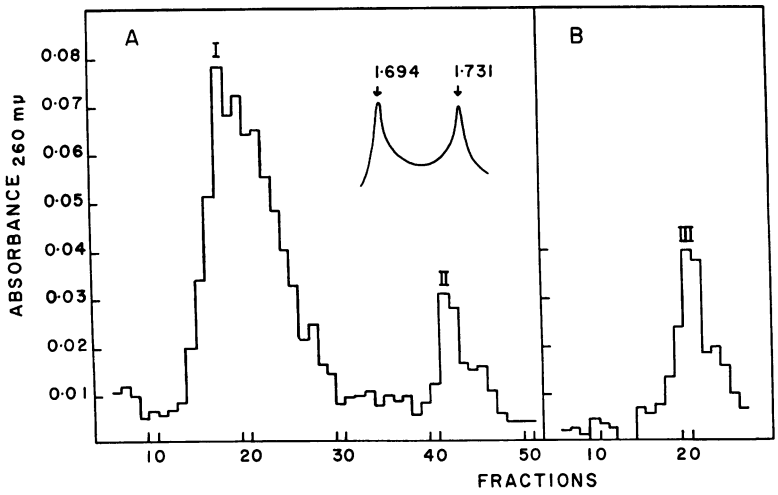


FIG. 3. *MAK chromatography of fowlpox-inclusion DNA. (A) Elution profile of DNA (89.4 μg) chromatographed in 0.4 M NaCl with 0.8 M NaCl employed in the reservoir. Peaks I and II contained 65.9 and 12.1%, respectively, of recovered absorbance. The NaCl concentration of fractions 18 and 41 are approximately 0.49 and 0.57 M, respectively. Inset: Densitometer tracing showing buoyant density distribution in CsCl equilibrium density gradient of viral DNA (ρ = 1.694 g/cm³) used for chromatography. Micrococcus lysodeikticus DNA (ρ = 1.731 g/cm³) was used as the density standard. (B) Elution profile of DNA (89.4 μg) chromatographed in 0.6 M NaCl with 1.0 M NaCl employed in the reservoir. Peak III contained 82.1% of recovered absorbance. The NaCl concentration of fraction 20 is approximately 0.7 M.*

between the synthesis of host and viral DNA is not possible. To examine the nature of DNA synthesized during FP infection, it was necessary to achieve a physical separation of viral and host DNA. To this end, studies were undertaken to compare their buoyant density and MAK chromatographic properties.

A buoyant density of 1.694 g/cm³ [35% guanine plus cytosine (GC)] was obtained for viral DNA extracted and purified from inclusions. This value is in agreement with the result of Szybalski et al. (21), and it reflects a base composition closely corresponding to the figure of 35.2% GC previously determined by direct chemical analysis (14). A value of 1.701 g/cm³ (42% GC) was common to cellular DNA extracted from chick epithelium, liver, and erythrocytes (erythrocyte DNA was prepared by L. G. Gafford), and this value is in close agreement with the value of 1.7008 g/cm³ previously determined for erythrocyte DNA (21). As expected from the buoyant density distribution of each DNA when centrifuged separately (*see* Fig. 4A, inset), these molecular species could not be entirely resolved from a mixture by density gradient centrifugation.

Figure 3B shows the elution pattern of FP DNA (peak III) extracted from inclusions and applied to a MAK column in 0.6 M NaCl. Under these conditions, the sample eluted in a pattern similar to that previously described for high molecular weight viral DNA (6); however, only about 10% of the DNA could be recovered in this form. In contrast, when inclusion DNA was applied in 0.4 M NaCl, the bulk was retained and eluted as shown in Fig. 3A. The predominant fraction (peak I), mobilized at approximately 0.49 M NaCl, was present in all preparations of viral DNA; electron microscopic examination (courtesy of J. M. Hyde) revealed predominantly short fragments, confirming the comparatively low molecular weight of fraction I DNA suggested by its position of elution. When present, the peak II fraction, like high molecular weight DNA, constituted a very small percentage of the total preparation. The chromatographic properties of viral inclusion DNA shown in Fig. 3 are the result of extensive fragmentation of the majority of molecules into pieces of approximately uniform size during the extraction or purification process. Gafford and Randall (6) and Hyde et al. (9) have previously shown that, despite the relative ease with which DNA may be liberated from FP virus, the very long molecules are quite susceptible to mechanical fragmentation, and only a portion can be purified intact.

The data presented in Fig. 4 illustrate the chromatographic separation of chick epithelial DNA and FP DNA I. Host DNA, when chromatographed alone (Fig. 4A), was eluted in fractions 10 to 17 at concentrations of NaCl from

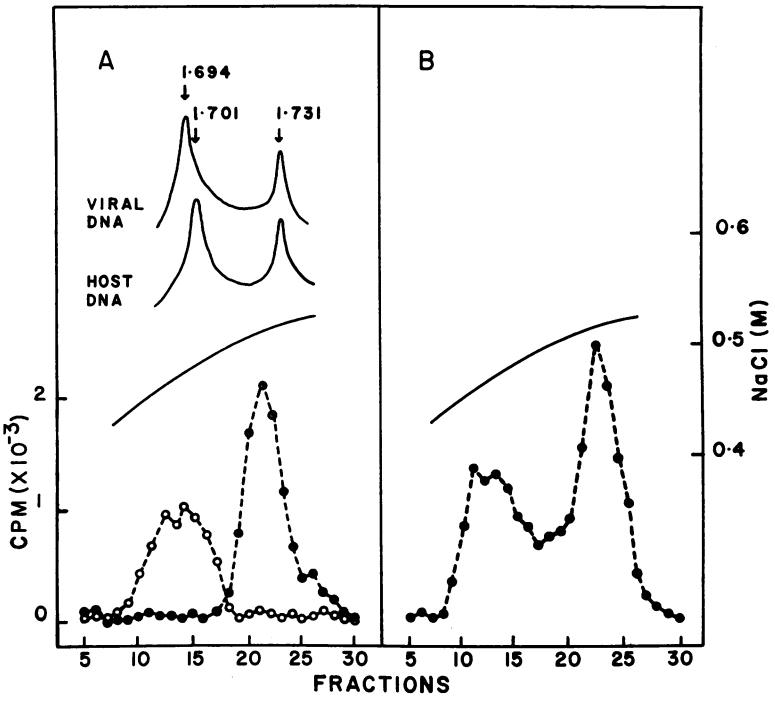


FIG. 4. *Differentiation of chick epithelial DNA and fowlpox DNA I by MAK chromatography. (A) Elution profiles of tritium-labeled cellular DNA (35.1 μg) and fowlpox DNA (23.2 μg) chromatographed in 0.4 M NaCl on separate columns with 0.8 M NaCl employed in the reservoir. Fractions 10 to 17 (0.43 to 0.49 M NaCl) contained 95.5% of recovered cellular DNA (○). Fractions 18 to 26 (0.49 to 0.53 M NaCl) contained 89.4% of recovered viral DNA (⊙). Inset: Densitometer tracings showing buoyant density distribution in CsCl equilibrium density gradients of viral DNA (ρ = 1.694 g/cm³) and cellular DNA (ρ = 1.701 g/cm³) used for chromatography. Micrococcus lysodeikticus DNA (ρ = 1.731 g/cm³) was used as the density standard. (B) Elution profile of tritium-labeled cellular DNA (43.9 μg) and viral DNA (29.0 μg) chromatographed together in 0.4 M NaCl with 0.8 M NaCl employed in the reservoir. Fractions 8 to 26 contained 98.1% of recovered radioactivity. NaCl concentration is shown by solid line.*

approximately 0.43 to 0.49 M. FP DNA I was mobilized from a second column under identical experimental conditions of gradient elution at concentrations of NaCl from approximately 0.49 to 0.53 M. Figure 4B illustrates the degree of resolution attained when tritiated viral and host DNA were chromatographed together. As shown, acid-insoluble tritium counts were eluted from MAK in two peaks; fractions 9 to 17 corresponding to cellular DNA, and 18 to 26 corresponding to viral DNA (Fig. 4A).

*MAK chromatography of cellular and viral DNA synthesized during infection.* It is clear from the results of Fig. 4 that host DNA can be separated from viral DNA by MAK column chromatography. The nature of DNA synthesized in chick epithelium during each of the eight 12-hr intervals between 0 and 96 hr after inoculation with FP virus was analyzed by this technique. At specified times, infected chicks received isotope as previously described; DNA extracted from epithelium after each interval was purified and chromatographed. Since only the DNA replicated during the labeling period is radioactive, the relative amounts of viral and cellular DNA synthesized during this time are proportional to the amount of label incorporated into each. Samples were chromatographed in 0.4 M NaCl, with 0.8 M NaCl employed in the reservoir, to determine the percentages of host DNA and fractions I and II of viral DNA; 0.65 M NaCl, with 1.0 M NaCl in the reservoir, was used to check for the presence of viral DNA III.

The DNA extracted from control epithelium after any labeling interval and that synthesized during the first 12 hr of FP infection eluted from MAK columns after application in 0.4 M NaCl in a profile similar to that previously shown for cellular DNA (Fig. 4A). The elution characteristics of labeled DNA extracted from infected epithelium after subsequent PI intervals and chromatographed in 0.4 M NaCl depended upon

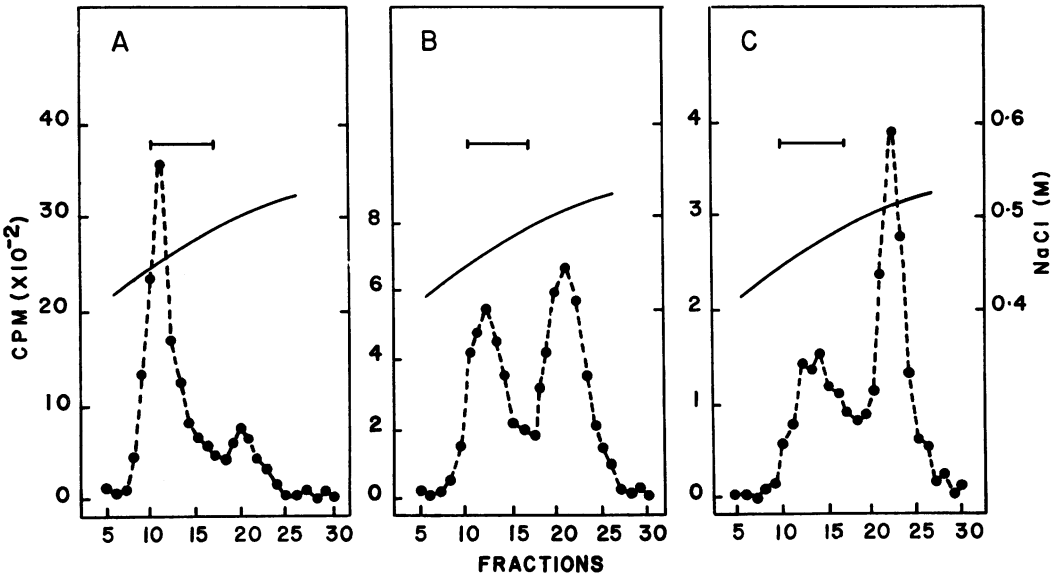


FIG. 5. *Typical elution profiles from MAK chromatography of DNA synthesized in chick epithelium infected in vivo with fowlpox virus during various postinfection intervals. After specified labeling intervals as described in the text, DNA was extracted from infected epithelium and chromatographed in 0.4 M NaCl with 0.8 M NaCl employed in the reservoir. (A) 48 to 60 hr postinfection (61.6 μg chromatographed); 83.7% of recovered radioactivity eluted fractions 8 to 17 and 16.3% in fractions 18 to 26. (B) 60 to 72 hr postinfection (18.8 μg chromatographed); 48% of recovered radioactivity eluted in fractions 8 to 17 and 52% in fractions 18 to 26. (C) 72 to 84 hr postinfection (14.9 μg chromatographed); 36.4% of recovered radioactivity eluted in fractions 8 to 17 and 63.6% in fractions 18 to 26. The horizontal bar denotes the range in which standard chick epithelial DNA was eluted under these conditions. NaCl concentration is shown by solid line.*

the time at which this newly synthesized DNA was examined. Three types of elution patterns were found (Fig. 5). DNA isolated after any of the four intervals between 12 and 60 hr, eluting in a profile similar to that shown for 48 to 60 hr in Fig. 5A, was mostly cell-like. The chromatographic behavior of DNA synthesized between 60 and 72 hr is illustrated in Fig. 5B; the label was associated about equally with each DNA. DNA newly synthesized during both 12-hr intervals between 72 and 96 hr PI was predominantly viral in nature, exhibiting chromatographic properties similar to the elution profile shown for 72 to 84 hr in Fig. 5C. Only one sample (84 to 96 hr PI; chromatographic profile not shown) contained FP DNA II, and, as expected, the percentage of total viral DNA recovered from this column was unchanged from that recovered after chromatography of an 84 to 96 hr sample containing only fraction I (see Table 1). Peak III of viral DNA (Fig. 3B) could not be detected in preparations extracted after any of the labeling intervals studied.

Chromatographic elution profiles of labeled DNA extracted after each of the eight intervals studies were used to calculate the percentage of radioactivity associated with host and viral DNA. The results of calculations of all experiments are given in Table 1. It can be seen that DNA synthesized between 0 and 12 hr PI was exclusively cellular in nature. Between 12 and 60 hr, cellular DNA synthesis was predominant, averaging approximately 80 to 90% of DNA newly synthesized during any 12-hr interval. However, the

TABLE 1. *MAK chromatographic properties of DNA synthesized during fowlpox infection in vivo*

| Labeling interval (hr PI) | Percentage of radioactivity exhibiting chromatographic elution properties typical of | | | | | |
|---|---|---|---|---|---|---|
| | Cellular DNA | | | Viral DNA | | |
| | I | II | Avg | I | II | Avg |
| 0–12 | 100.0 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| 12–24 | 80.9 | 77.4 | 79.2 | 19.1 | 22.6 | 20.8 |
| 24–36 | 83.8 | 83.8 | 83.8 | 16.2 | 16.2 | 16.2 |
| 36–48 | 87.9 | 90.8 | 89.4 | 12.1 | 9.2 | 10.6 |
| 48–60 | 83.7 | 80.3 | 82.0 | 16.3 | 19.7 | 18.0 |
| 60–72 | 48.0 | 47.0 | 47.5 | 52.0 | 53.0 | 52.5 |
| 72–84 | 36.4 | 35.0 | 35.7 | 63.6 | 65.0 | 64.3 |
| 84–96 | 32.4 | 32.4 | 32.4 | 67.6 | 67.6 | 67.6 |

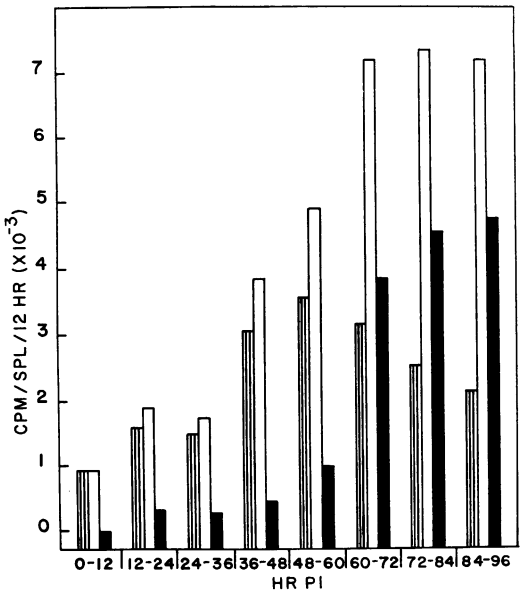


FIG. 6. *Kinetics of cellular and viral DNA synthesis during fowlpox infection in vivo. Trichloroacetic acid-insoluble radioactivity (total DNA) in infected tissue, open bar. The values plotted for 12 to 24 hr and 24 to 36 hr labeling intervals were corrected for the effect of feather removal (Fig. 1). The data for host (lined bar) and viral (solid bar) DNA synthesis were calculated from trichloroacetic acid-insoluble radioactivity of infected epithelium and percentage from MAK chromatography (Table 1) as described in the text.*

proportion of newly synthesized DNA which was viral increased abruptly to approximately 50% between 60 and 72 hr and progressively increased to almost 70% between 84 and 96 hr.

*Kinetics of cellular and viral DNA synthesis.* Since the data of Fig. 2 on the sequential increase of incorporation of TdR into total DNA and that of Table 1 on the type of DNA synthesized were obtained under identical experimental conditions, the two may be related to show the pattern of host and viral DNA synthesis during infection. This relation was computed for each time period by multiplying the percentage given for each DNA in Table 1 by the counts per minute incorporated into total DNA of infected epithelium (Fig. 2) for each 12-hr interval. A typical example of these calculations is as follows. The trichloroacetic acid-insoluble fraction of infected epithelium from chicks labeled between 60 and 72 hr PI averaged 7,059 counts/min (Fig. 2). From Table 1, it is seen that $^{3}$H-DNA extracted after this labeling period and chromatographed on a MAK column averaged 47.5% cellular DNA and 52.5% viral DNA. Thus, by multiplying 7,059 counts/min by these percentages, it was determined that during the interval between 60 and 72 hr PI 3,353 counts/min were incorporated into cellular DNA and 3,706 counts/min were incorporated into viral DNA. The results of all calculations are shown in Fig. 6; incorporation of TdR into trichloroacetic acid-insoluble fractions (total DNA) of infected epithelium is also plotted for comparison.

The increase of incorporation of TdR into total DNA between 36 and 60 hr PI was accompanied by a similar increase of incorporation in cellular DNA. The replication of viral DNA began between 12 and 24 hr after inoculation, but synthesis was insignificant and did not appreciably contribute to total DNA synthesis during the first 60 hr of infection. Between 60 and 72 hr, however, increased incorporation into total DNA was accompanied by a decline of incorporation into host DNA and a pronounced

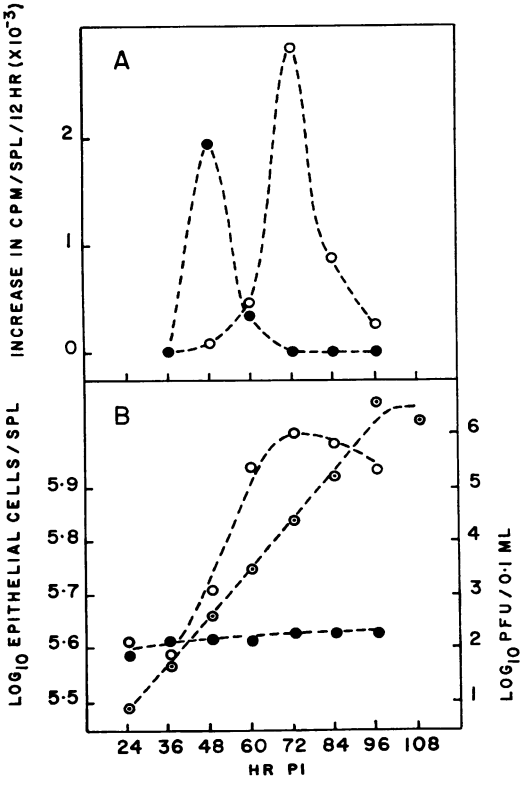


FIG. 7. *Correlation of the rate of host and viral DNA synthesis with epithelial hyperplasia and viral titer during fowlpox infection in vivo. (A) Rate of increase of incorporation of TdR into host DNA (●) and viral DNA (○) during infection was calculated as described in the text. (B) Data on enumeration of epithelial cells (control, ●; infected, ○) and growth curve of fowlpox virus (⊙) were derived from Cheevers and Randall (in press).*

stimulation of almost fivefold in incorporation into viral DNA. Subsequently, between 72 and 96 hr, there was a continued increase of viral DNA synthesis and a concomitant decrease of host DNA synthesis.

The rise periods of host and viral DNA synthesis (Fig. 6) were examined for rate of increase; for example, 3,706 counts/min for viral DNA at 60 to 72 hr minus 799 counts/min for viral DNA at 48 to 60 hr equals 2,907 counts/min increase in incorporation/12 hr. It is clearly evident from these data (Fig. 7A) that development of the in vivo lesion of FP virus is characterized by a biphasic pattern of DNA synthesis, involving initial induction of cellular DNA synthesis between 36 and 48 hr PI and subsequent maximal viral DNA synthesis between 60 and 72 hr.

It is pertinent to correlate the pattern of DNA synthesis during FP infection with previous studies on viral and cellular growth (Cheevers and Randall, *in press*). As shown in Fig. 7B, viral replication was initiated (24 hr PI) prior to evidence of hyperplasia (48 hr PI), but less than 1% of the maximal titer had been attained when hyperplasia was complete (72 hr PI). Thus, it is evident that the biphasic pattern of host and viral DNA synthesis associated with the development of FP infection in vivo (Fig. 6 and 7A) is temporally related to hyperplasia and viral replication (Cheevers and Randall, *in press* and Fig. 7B).

## DISCUSSION

These studies show that the biosynthesis of DNA during hyperplastic FP infection in vivo proceeds in two relatively distinct phases. The first 60 hr of infection is characterized predominantly by the synthesis of cellular DNA at a rate markedly increased over the control between 36 and 48 hr PI. Although the replication of viral DNA begins between 12 and 24 hr PI, the rate of synthesis is very low during the first 60 hr. In contrast, an abrupt enhancement of almost fivefold in the rate of viral DNA synthesis occurs between 60 and 72 hr, concomitantly with a sharp decline of cellular DNA synthesis. Subsequently, during the 72 and 96 hr period, the ratio of synthesis of viral DNA to cellular DNA progressively increases to a maximum of greater than 2:1.

These findings provide a biochemical basis for previous results (Cheevers and Randall, *in press*) which indicated that the pathogenesis of in vivo FP infection was divided into two relatively distinct biological phases. While all morphological evidence of hyperplasia of epithelium resulting from infection was confined to the period between 48 and 72 hr PI, greater than 99% of the maximal virus titer was attained between 72 and 96 hr.

The temporal relationship of host and viral DNA synthesis to hyperplasia and viral replication suggest the following sequence of events during infection. The replication of viral DNA, initiated between 12 and 24 hr PI, is followed closely by the first appearance of infectious virus at 22 to 24 hr. During the first 60 hr of infection, however, the rate of viral DNA synthesis is very low, and only about 0.1% of the maximal virus titer is attained. A stimulation in the rate of host DNA synthesis, beginning between 36 and 48 hr, is accompanied by induction of epithelial hyperplasia which ends in a 2.5-fold increase in cell number at 72 hr. Concomitantly with a decline in the rate of host DNA synthesis is an abrupt and marked increase in the rate of synthesis of viral DNA between 60 and 72 hr. Between 72 and 96 hr, the synthesis of viral DNA becomes progressively more predominant, no further hyperplasia is seen, and synthesis of the remaining infectious virus (greater than 99%) occurs.

A fundamental question concerning the effect of poxvirus infection in cell proliferation remains unanswered by the present study. It is impossible with the morphological data at hand to define the specific nature of the virus-host cell interaction operative during the period of hyperplasia. The induction of cell division may involve a direct response, resulting from primary action of the virus or viral products, such as infectious DNA, on the genome of infected host cells. Alternatively, as suggested by Joklik (10) as a possible explanation of tumor formation by pox-viruses, the hyperplastic response may involve stimulation of cells by an unknown indirect mechanism, such as the passage of virus-specific products from cell to cell.

There is some circumstantial evidence (2, 20) that the proliferative response may result from a direct action of the virus or viral products on the replicative mechanism of infected host cells. Swallen (20) demonstrated by autoradiography that chick epidermis infected for 48 hr with FP virus shows a threefold higher percentage of labeled nuclei than do controls, suggesting that infection is associated with an increased incidence of intranuclear DNA synthesis. Swallen's experiments are significant in that infection was detectable by cytoplasmic labeling 12 hr prior to the earliest time it was detected in the present study (60 hr) by histological means, and the increased incidence of intranuclear labeling was confined to infected cells. Arhelger and Randall (2) have shown by electron microscopy that FP virus particles, presumable progeny virus, are present in the nucleus as well as cytoplasm of chorioal-

lantoic ectodermal cells after 48 hr of infection. It is possible that induction of cellular DNA synthesis occurs in cells infected with FP virus, conceivably by a mechanism involving a nuclear phase of viral replication; however, the presence of virus or viral products within cells destined to divide has not been definitively shown. This problem is being approached by a sequential study of virus-specific immunofluorescence, with monitoring of nuclear and cytoplasmic nucleic acid synthesis.

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