Types 3, 7, 8, 11, 14, 16, and 21 have somewhat higher G + C contents, 50–53 per cent; of these, types 3, 7, and 21 appear to be weakly oncogenic. The remaining adenoviruses contain 56–60 per cent G + C; none of these have been reported thus far to be oncogenic.

* Supported by USPHS grant AI-01725 and USPHS contract PH43-64-928.

† Research Career Awardee (5-K6-AI-4739).

[1] Trentin, J. J., Y. Yabe, and G. Taylor, *Science*, **137**, 835 (1962).

[2] Huebner, R. J., W. P. Rowe, and W. T. Lane, these PROCEEDINGS, **48**, 2051 (1962).

[3] Green, M., and M. Piña, these PROCEEDINGS, **50**, 44 (1963).

[4] *Ibid.*, **51**, 1251 (1964).

[5] Girardi, A. J., M. R. Hilleman, and R. E. Zwickey, *Proc. Soc. Exptl. Biol. Med.*, **115**, 1141 (1964).

[6] Green, M., and M. Piña, *Virology*, **20**, 199 (1963).

[7] Rouse, H. C., V. H. Bonifas, and R. W. Schlesinger, *Virology*, **20**, 357 (1963).

[8] Green, M., and M. Piña, unpublished data.

[9] Green, M., P. Laipis, M. Piña, J. Vinograd, and R. Watson, unpublished data.

[10] Schildkraut, C. L., J. Marmur, and P. Doty, *J. Mol. Biol.*, **4**, 430 (1962).

[11] Huebner, R. J., personal communication.

---

*KINETICS OF X-RAY AND HEAT INACTIVATION OF φX174 RF-DNA**

BY W. GINOZA AND R. C. MILLER

BIOPHYSICS DEPARTMENT, PENNSYLVANIA STATE UNIVERSITY

Communicated by Bruno Zimm, June 22, 1965

Studies of Sinsheimer and his co-workers have shown that the replicative factor (RF) of coliphage φX174 is a double-stranded circular DNA, formed soon after the virus invades its host, *Escherichia coli* C.[1] Our interest in this material stems from our desire to understand (1) the basis for the greater resistance of double-stranded over single-stranded DNA to X rays, and (2) the relationship between its molecular behavior and infectivity as may be revealed through studies of its thermal inactivation kinetics. In our concurrent work, to be reported elsewhere, we find the killing efficiency per X-ray hit, α, for T4 bacteriophage is 0.05.[2] The α for RF-DNA to be reported here is 0.25, and that for single-stranded φX-174 DNA is 1.0.[3,4] In this paper we present a discussion of the contrast in the values of α and a possible mechanism by which RF-DNA survives X-ray and heat-induced sublethal lesions. The results of our thermal studies are consistent with the idea that either strand of the RF double helix can be infectious.

Materials and Methods.—RF-DNA is assayed in *E. coli* C protoplasts in the same way as is φX174-DNA. The preparation method and the properties of protoplasts used here are described elsewhere.[5,6] All RF-DNA samples in X-ray and heat experiments were assayed in a concentration range where the plot of the log of plaque count versus the log of DNA concentration is linear. The concentration of DNA during the usual irradiation and heat-denaturation treatments was 0.01–1/μg/ml. The details of the X-irradiation procedure used here are described in reference 3.

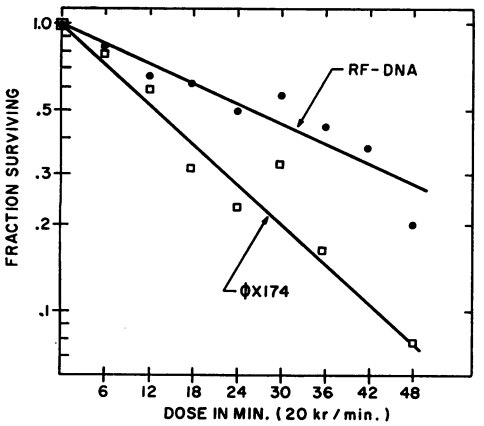

FIG. 1.—X-ray survival curves of RF-DNA and ϕX174. Both samples were suspended in 1% tryptone broth, frozen at −60°, and irradiated together in the same irradiation chamber over an atmosphere of air. The D_{37} dose for ϕX174 is 3.9×10^5 r, which gives a target molecular weight of 1.7×10^6.[3] Using these figures as references, the D_{37} dose and target molecular weight of RF-DNA are 7.8×10^5 r and 0.85×10^6, respectively, and the dose rate for this experiment is estimated to be 20 kr/min. Target mol. wt. = (6×10^{23} molecules /mole)/(D_{37} dose, r, $\times 9 \times 10^{11}$ primary ionizations/gm/r). The value 9×10^{11} in the equation is derived from two figures: 87 ergs, the energy absorbed by 1 gm of nucleic acid per r[10], and 60 ev, the average energy per primary ionization.[3, 11]

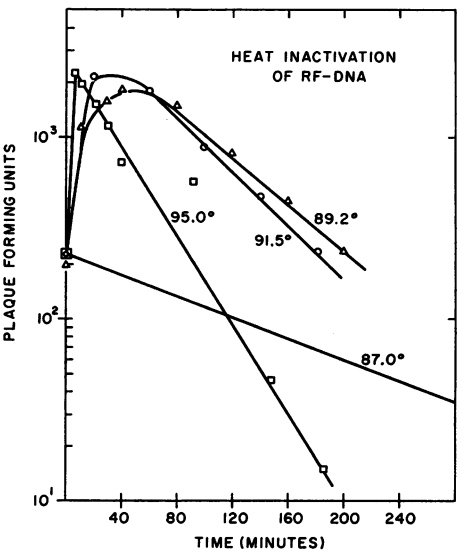

FIG. 2.—Heat-inactivation survival curves of RF-DNA in 0.1 *M* potassium phosphate buffer, pH 7.0 (standard phosphate buffer) at various temperatures. DNA concentration was about 0.1 μg/ml during heating.

ϕX174 served as internal calibration for the radiation dosimetry. Agar layer technique of Adams[7] was used to assay viral activity of DNA.

Results.—X irradiation: The X-ray survival curve of RF-DNA compared with that of the parent virus is shown in Figure 1. It was shown earlier that the D_{37} dose for the intact ϕX174 was 3.86×10^5 r, from which is calculated a radiosensitive molecular weight of 1.7×10^6 daltons.[3] Since its free infectious nucleic acid also leads to the same target mass, it was concluded that the DNA is the radiosensitive target. The present results show that RF-DNA has one half the sensitivity and therefore appears to have one half the radiosensitive target mass of its single-stranded counterpart. With the reasonable assumption that the X rays release their energy in the double- and single-stranded DNA in the same way, then RF-DNA on the average receives three nonlethal hits for each lethal hit. The efficiency of killing per primary ionization event is thus 0.25 which compares with one for the parental single strand and with 0.05 for T4 phage. Recently, Yarus and Sinsheimer demonstrated that RF-DNA is also one half as sensitive as the single strand to ultraviolet radiation if the survival assay is conducted in a strain of *E. coli* which is not capable of dark repair of UV-lesions.[12]

If RF-DNA is first heat-denatured and then X-irradiated, its X-ray sensitivity more nearly approximates that of the single-stranded viral DNA.

Heat inactivation kinetics: The loss of plaque-forming ability of RF-DNA as a

function of time of heating at different temperatures is shown in Figure 2. The main features of the kinetics are as follows: (1) The biological melting transition begins at some temperature intermediate between 87° and 88°. T_m, as determined by optical density measurements at 260 mμ, is 86.5°.[8] (2) At subcritical temperatures (<87°) the inactivation proceeds by simple exponential kinetics, as it does for the transforming activity of pneumococcus. Subcritical heat inactivation is due to random depurination events.[14, 15] From the radiosensitive target size of 8.5×10^5 daltons estimated for RF-DNA and 3×10^5 for the streptomycin resistance marker of pneumococcus estimated by the rate of heat depurination at 87°,[14] the rate constant of 1.2×10^{-4}/sec for RF-DNA at this temperature is a reasonable expectation. The ratio of the size of RF-DNA to that of the transforming marker = 2.8, while the ratio of their rate constants = 2.2. The latter value would in fact be closer to the former, if one takes into account the fact that the guanine-cytosine content of pneumococcal DNA is 5 per cent lower than that of RF-DNA. (3) At melting temperatures there is, in marked contrast to the behavior of transforming DNA, a sharp initial rise in the plaque-forming ability due, in some way, to denaturation of the double-stranded circular helix. The rate of rise increases with increasing temperature. A similar rise has been observed in polyoma DNA by Weil and Vinograd who ascribed the rise to increased efficiency of binding or uptake of the denatured DNA by the host cells.[16] (4) The initial rise in the activity curve is followed by a plateau, indicating the attainment of maximum denaturation and plaque-forming ability. The time to reach the plateau is decreased with increasing temperature. The lag in the decay after this suggests the possibility that the denatured RF-DNA may have more than one heat-sensitive target (see below). (5) The plateau is followed by an exponential decrease in surviving activity with rates similar to those of the single-stranded φX174-DNA at comparable temperatures.[13]

In the case of interlocked heat-denatured helices of RF-DNA, it would appear possible to demonstrate by thermal inactivation kinetics whether both strands are infectious. The inactivation process would be expected to follow the kinetics expressed by the relationship,[23] $s = 1 - (1 - e^{-kt})^n$, where s = surviving fraction of plaque formers, k = the frequency of depurination, t = time of heating, and $n = 2$. But at any temperature in and above the melting transition (see Fig. 2), the simultaneous reactions, (1) the time-dependent melting, which raises the infectious efficiency, and (2) depurination, which inactivates, preclude accurate measurement of the extrapolation number, n. Thus, our RF-DNA preparation was first gently denatured (to minimize depurination) with 70 per cent formamide[17] at 50° to obtain its maximum potential infectivity, and then at 0 time it was diluted directly into standard phosphate buffer held at 95° in a water bath. The inactivation curve at this temperature is shown in Figure 3 with the results of a similar test at 92.5°. Graphs show that n is close to the expected 2, suggesting that both loops of the double helix must be hit in order to inactivate denatured RF-DNA.

To show that the extent of annealing is minimized under our condition of assay, heat-denatured DNA samples were quick-cooled as usual and then allowed to warm to room temperature. At various times thereafter, samples were withdrawn and assayed. We see no measurable drop in activity within a time course of 1–3 min which is the normal span between sampling and assaying. Thus, the reformation

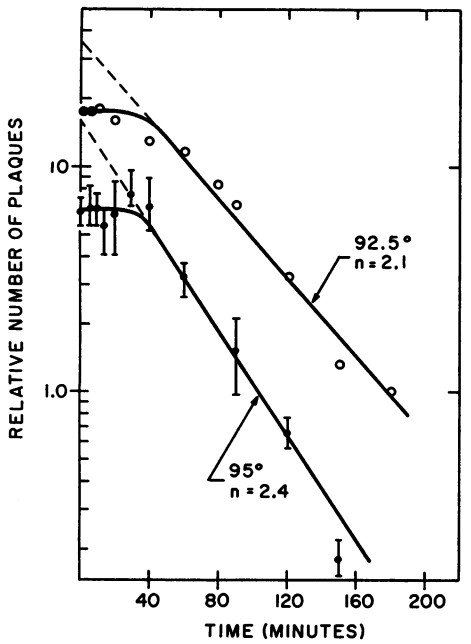

FIG. 3.—Heat-inactivation survival curves of denatured RF-DNA at above-melting temperatures. The DNA was first denatured by exposure to 70% formamide in standard phosphate buffer at 50° for 10 min. The denatured DNA was directly diluted 30-fold in standard phosphate buffer maintained at the experimental temperatures of 92.5° and 95°, and samples were taken at various times indicated, quick-cooled, and mixed with the assay protoplasts within 3 min. Concentration of DNA of these temperatures was 0.1 μg/ml.

of a native helix, which has a lower infectivity than the denatured DNA, does not occur to any appreciable extent in our routine quick-cool procedure.

X irradiation and heat experiments above indicate that an RF-DNA received, on the average, three sublethal hits per lethal hit. To demonstrate that RF-DNA bearing sublethal lesions may still be infectious, it was heat-depurinated at the subcritical temperature of 87° to about e^{-1} survival. It was then diluted into standard phosphate buffer maintained at 94.5° in a water bath, and samples were withdrawn, quick-cooled, and assayed for the survival curve at this melting temperature. The result is shown in Figure 4. We note that despite the melting temperature there is no increase in infectivity due to melting as might be expected if the 87° survivors were lesion-free. Instead there is an immediate drop in activity. This indicates that sublethal depurination hits had taken place in the double-stranded state during the 87° heating and that upon subsequent melting the otherwise sublethal hits are expressed as lethal hits, as any hit is lethal in the single-stranded state. At e^{-1} survival only 2 per cent of the population have received 0 hits, assuming a Poisson distribution of 4 hits per RF-DNA molecule. The fractions receiving one and two hits are 7 per cent and 14 per cent. Of the latter, one half will have two hits in one chain and none in the other. Since the three fractions can still contribute to increased activity on melting, their presence explains why the rate at 94.5° is not more precipitous than observed.

The experiment was repeated but X rays were used instead for inflicting the sublethal lesions, followed by heating at 93°. Here again, no rise in activity due to melting was observed.

In our concurrent work on T4 phage, the evidence obtained for sublethal damages is low burst size and increased latent period.[2]

Discussion.—Single-stranded ϕX174-DNA is X-ray- and heat-inactivated by a single-hit process. Its radiation target molecular weight is equal to the physical molecular weight;[9] therefore, it would appear that one localized X-ray lesion occurring anywhere on the DNA can inactivate the molecule. Its entire mass is thus considered a critical target. Its double-stranded counterpart, RF-DNA, is inactivated by these agents by the same simple kinetics, but while its mass is doubled, its target sensitivity ($\propto$ radiation target molecular weight) is only one half that of the

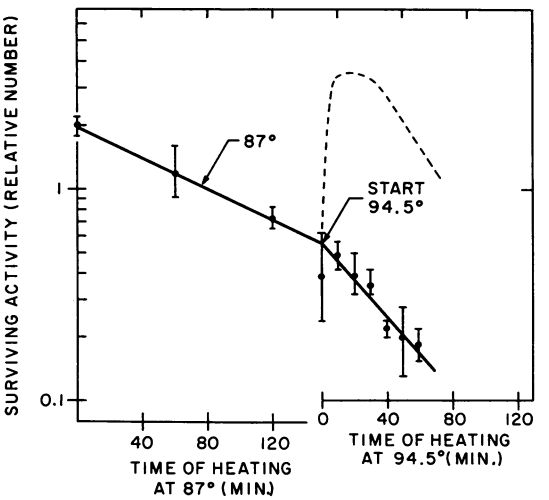

FIG. 4.—Survival curve of RF-DNA at above-melting temperature following heat inactivation at 87° (below-melting temperature). Stippled curve is expected for the population of RF-DNA surviving 87°, if they had no sublethal lesions. Concentration of DNA = 0.1 μg/ml.

single strand. The killing efficiency per primary ionization event is 0.25. The heat-depurination kinetics of RF-DNA at temperatures below the melting transition also leads to the same estimate of the efficiency of lethality per depurination event. This figure indicates that heat or X-ray survivors can bear sublethal lesions, and evidence for this is provided by experiments summarized in Figure 4.

There are three possible explanations for the X-ray and heat resistivity of the RF-DNA relative to the single-stranded ϕX174-DNA: (1) α is an expression of the probability that the infected host can repair lesions in RF-DNA in some still unknown manner. (2) Although a single hit inactivates, each hit has but one chance in four that a certain kind of physicochemical lesion will occur that is lethal, e.g., double-strand scission or base-pair damage per single-hit event. (3) There are two classes of targets, critical and noncritical. A hit in the critical target inactivates. If unhit, the replicative process can be initiated, and the hits that might have occurred in the noncritical target are bypassed (not necessarily repaired) during replication with the ultimate emergence of a complete copy of the genome to continue the infectious process. α of 0.25 means that the critical target is one fourth the size of the RF-DNA.

Regarding (1), it seems unlikely that the cell can repair heat-induced apurinic lesions and heterogeneous X-ray lesions with the same efficiency. On the other hand, any DNA alteration may suffice as a signal to initiate whatever repair mechanism the cell may possess.

Regarding (2), the assumption that only a certain kind of physicochemical lesion is lethal and that it is induced at a certain frequency by X ray should also bear the assumption that the frequency should be the same for all double-stranded DNA. We note, however, that α for T4 phage using the same X ray is only 0.05 compared to 0.25 for the RF, a disturbing fivefold disparity in value.

The third explanation, the critical target hypothesis, appears most plausible to us.

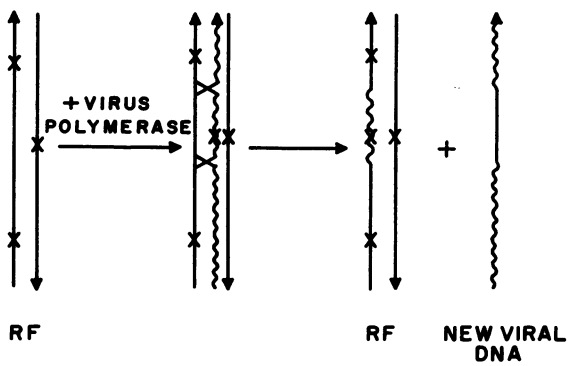

FIG. 5.—Schematic representation of fragmentary semiconservative replication of ϕX174-DNA. Wavy lines represent newly synthesized viral DNA. Open model of the DNA is used for convenience. X = X ray or heat lesion.

Although more involved, its rationalization is not difficult. The concept of the critical target stems in part from studies of T-even bacteriophage for which the term critical target is taken to mean some early enzymatic function of the phage preceding DNA replication.[18] Let us assume that the critical target in RF is the segment of the DNA specifying the virus polymerase which synthesizes single-stranded viral DNA from the RF. This segment is in the strand complementary to the viral DNA strand.[8] Since only this strand can be transcribed for the polymerase function, a hit within this single working copy alone can prevent further function of the RF in virus replication. This describes the situation of the RF-DNA hit in its critical target. Now for the case of RF-DNA still possessing its capability of infection because it has been hit only in the noncritical target, the virus polymerase is expected to be produced in a normal fashion; but it then encounters its RF-DNA template which bears a number of lesions. The problem now is to produce one lesion-free replica of the viral strand from the damaged template.[19] Excluding repair mechanism, the only solution to the problem is to postulate that virus polymerase synthesizes the viral DNA strand on the RF template by a breakage-reunion process; i.e., the replica chain during its synthesis occasionally joins in a covalent linkage with the viral strand of the RF, which is broken in such a way that the nucleotide sequence is held in register. Chain polarity is maintained also in the strand interchange. The breakage reunion may be repeated at random in the course of completing one good replica. The observation that in normal infection the input of ϕX174-DNA is dispersed among its progeny particles makes the postulate plausible.[20] A model of the breakage-reunion replication of viral DNA from the damaged RF-DNA template is shown in the diagram (Fig. 5). It is to be noted that a strict semiconservative replication cannot restitute an exact replica and viability from an RF-DNA that has even a minimum of one random hit in each of two chains. Moreover, if this mode of replication is to operate, a two-hit X-ray inactivation kinetics should be observed, which is contrary to the experimental results.

The above discussion pertains to the kinetic behavior of RF-DNA in the double-stranded state. If the molecule is first denatured by formamide, it is converted to two infectious units, which are bound by some linkage not yet understood. The

attainment of this configuration is shown by the heat-inactivation kinetics which are clearly indicative of a two-hit process. This finding is not surprising in view of the concentration independence of the annealing reaction (denatured → native) demonstrated in polyoma DNA,[16] and RF-DNA is known to share many properties in common with this DNA.[21]

The results of our concurrent work on the X irradiation of T4 phage show an interesting parallel with the RF-DNA results in that they suggest to us common solutions to the same questions: (1) what target must be hit to inactivate double-stranded viral DNA, and (2) what is the mechanism by which apparently normal phage genomes are restituted from X-irradiated (and heat-depurinated) survivors bearing sublethal lesions in their DNA? We believe that the concept of critical target and the evidence for fragmentary semiconservative mode of replication in T4 phage[22] and ϕX174 should be taken into account for a satisfactory solution to these problems. Whatever the final answer, it is clear that the increased resistivity of the double- over the single-stranded DNA is not simply a matter of its possessing an extra copy of genetic information.

Summary.—RF-DNA of ϕX174 is inactivated by X rays and heat with a killing efficiency of about 0.25, suggesting that the genome is made up of critical and noncritical targets. It appears that one lesion alone in the critical target kills the viral activity, while surviving RF bearing several lesions only in the noncritical target can still restitute a normal replica of the viral genome. Restitution mechanisms are discussed.

The authors gratefully acknowledge the capable technical assistance of Mrs. Kristin Vessey.

* Based on work performed under contract AT(30-1)-3116 with the U.S. Atomic Energy Commission.

[1] Sinsheimer, R. L., B. Starman, C. Nagler, and S. Guthrie, *J. Mol. Biol.*, **4**, 142 (1962).

[2] Ginoza, W., and K. Vessey, in *Abstracts*, Biophysics Society Meeting, San Francisco, February 1965.

[3] Ginoza, W., *Nature*, **199**, 453 (1963).

[4] Tessman, I., *Virology*, **7**, 263 (1959).

[5] Guthrie, G. D., and R. L. Sinsheimer, *J. Mol. Biol.*, **2**, 297 (1960).

[6] Ginoza, W., and K. Vessey, in *Abstracts*, Biophys. Soc. Meeting, Chicago, February 1964.

[7] Adams, M. H., *Bacteriophages* (Interscience Publishers, 1959).

[8] Hayashi, M., M. N. Hayashi, and S. Spiegelman, *Science*, **140**, 1313 (1963).

[9] Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 43 (1959).

[10] Andrews, H. L., *Radiation Biophysics* (Prentice Hall, 1961), p. 194.

[11] Rauth, A. M., and F. Hutchinson, *Biological Effects of Ionizing Radiation at the Molecular Level* (Vienna: International Atomic Energy Agency, 1962), p. 25.

[12] Yarus, M., and R. L. Sinsheimer, *J. Mol. Biol.*, **8**, 614 (1964).

[13] Ginoza, W., C. H. Hoelle, K. Vessey, and C. Carmack, *Nature*, **203**, 606 (1964).

[14] Ginoza, W., and B. H. Zimm, these PROCEEDINGS, **47**, 639 (1961).

[15] Roger, M., and R. D. Hotchkiss, these PROCEEDINGS, **47**, 653 (1961).

[16] Weil, R., and J. Vinograd, these PROCEEDINGS, **50**, 730 (1963).

[17] Marmur, J., and P. O. P. Ts'o, *Biochim. Biophys. Acta*, **51**, 32 (1961).

[18] Sinsheimer, R. L., in *The Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1960), vol. 3, p. 191.

[19] Zimmerman, F., H. Kröger, U. Hagen, and K. Keck, *Biochim. Biophys. Acta*, **87**, 160 (1964). Zimmerman *et al.* show that the *in vitro* priming ability of DNA for DNA polymerization is 20 times more radio-resistant than that for RNA polymerization. Thus, in the present discussion it is conceivable that DNA polymerization can occur on damaged template.

[20] Kozinski, A. W., and W. Szybalski, *Virology*, **9**, 260 (1959).

[21] Kleinschmidt, A. K., A. Burton, and R. L. Sinsheimer, *Science*, **142**, 961 (1963).

[22] Kozinski, A. W., *Virology*, **13**, 124 (1961).

[23] The expression is derived from the Poisson probability theory to fit a biological system which contains n units, each of which must be "hit" once to inactivate the system.

---

*C-REACTIVE PROTEIN: A MOLECULE COMPOSED OF SUBUNITS**

BY E. C. GOTSCHLICH AND G. M. EDELMAN

THE ROCKEFELLER INSTITUTE

Communicated by Maclyn McCarty, May 13, 1965

In the course of infectious diseases and various other pathological conditions there appears in the serum of man[1] a protein not normally detectable. This protein is called C-reactive protein (CRP)[2] because it has the curious property of precipitating with the C polysaccharide derived from the cell wall of the pneumococcus; the polysaccharide is composed of bacterial mucopeptide constituents and N-acetylgalactosamine 6-phosphate.[3] Proteins analogous to CRP have been isolated from sera of other species.[4, 5] Hurlimann, Thorbecke, and Hochwald[6] have recently identified the liver as the site of synthesis of CRP. As yet, however, no biologically significant function has been ascribed to this protein.

CRP can be obtained in crystalline form.[7] By immunologic criteria, it is unrelated to any other serum protein.[7, 8] Physicochemical studies by Wood, Slater, and McCarty[7] have shown that CRP migrates in the β-globulin region in moving boundary electrophoresis, and that it has a sedimentation coefficient of 7.5S.

In the present communication we provide evidence that human CRP consists of an aggregate of probably identical subunits having molecular weights of 21,500 and held together by noncovalent interactions. Models for the protein are proposed on the basis of results obtained by amino acid analyses, peptide mapping, molecular weight determinations, and serological studies. Some of the properties of CRP are discussed in terms of these models.

Materials and Methods.—Preparation of CRP: Human CRP was isolated and crystallized from pooled pleural and peritoneal fluids by the method of Wood, McCarty, and Slater.[7] The only significant modification was that the chloroform extraction was performed on the redissolved CRP-C-carbohydrate complex, rather than on the albumin fraction. The pneumococcal C polysaccharide employed was prepared according to the procedure of Liu and Gotschlich.[3] Selected preparations of CRP were tested for their phosphorus,[9] hexosamine,[10] and hexose[11] contents.

The CRP was readied for the various procedures by dissolving the crystals in tris(hydroxymethyl)aminomethane buffers (Tris buffers). After centrifugation, the solutions were dialyzed and concentrated by ultrafiltration in collodion bags to about 20 mg of CRP per ml.

Gel filtration: Sephadex (Pharmacia, Uppsala) was employed in columns measuring 1 cm × 115 cm at 21°C. The buffers used for elution were filtered through Millipore membranes to prevent clogging of the columns. The protein concentration of the effluents was determined by measuring the absorbancy at 280 mμ. Protein concentration was calculated from the absorbancy value of 2.0 for a 0.1% solution and a 1-cm path length as determined by Wood and McCarty.[12]

Starch gel electrophoresis: Starch gel electrophoresis was performed as described by Poulik.[13] Preparation and electrophoresis in starch gels containing urea in formate buffer was performed by the method of Edelman and Poulik.[14]

Serological properties of CRP: Specific reactions with antiserum to CRP were tested by the