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*STUDIES ON LABILE DEOXYCYTIDYLATE HYDROXYMETHYLASES FROM ESCHERICHIA COLI B INFECTED WITH TEMPERATURE-SENSITIVE MUTANTS OF BACTERIOPHAGE T4**

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The present studies were undertaken as an attempt to demonstrate altered properties in the enzyme, dCMP[1] hydroxymethylase, formed on infection of *E. coli* B by genetically altered bacteriophage T4. An earlier report from this laboratory[2] demonstrated that this enzyme is undetectable in extracts of *E. coli* B infected with a T4 mutant, *am* N122;[3] the site of mutation of this strain has been located by genetic mapping in gene 42.[4] Among the temperature-sensitive (*ts*) mutants of T4 isolated by Edgar and Lielausis[5] are some that map in this same gene. The dCMP hydroxymethylases formed in *E. coli* B by two of these, *ts* G25W and *ts* L13, have been studied in some detail. The results demonstrate that each of the two mutant enzymes is more temperature-sensitive than the wild type, and that the sensitivity of one differs qualitatively from that of the other. An interesting property of the wild-type enzyme is reported, namely, its ability under certain conditions to regain most of its activity after heat inactivation at 40°C.

Methods and Materials.—The sources of the following materials were: folic acid (C grade), d-cytidine, and dCMP, California Corp. for Biochemical Research; C^{14}-labeled formaldehyde, New England Nuclear Corp. and Volk Radiochemical Co.; 2-mercaptoethanol (Eastman grade) and the disodium salt of EDTA, Eastman Kodak Co.; Tris, Sigma Chemical Co. The preparation of dHMP has been described.[2] Reagent-grade hydroxylamine hydrochloride was obtained from Baker Chemical Co.

The method of isolation of the *ts* mutants has been described.[5] T4D, a revertant[2] of *am* N82 (gene 44),[4] was used as the wild-type phage and is referred to as T4*ts*$^+$. Mutant *ts* A41 was included in most experiments as a second reference phage; this mutant maps genetically in cistron 39,[4] the same cistron in which *am* N116[2, 4] maps, and presumably contains the genetic information

for wild-type dCMP hydroxymethylase. Its inclusion in these studies was prompted by the possibility that T4*ts*$^+$, as a revertant, might be a slight variant of the wild T4D from which the *ts* mutants, including *ts* A41, were derived; thus the T4*ts*$^+$ hydroxymethylase conceivably could have slightly different properties than the A41 hydroxymethylase. No such difference in properties was revealed, however, in these studies.

Mutant *ts* G25W was picked as a plaque-size variant of *ts* G25 and is even "leakier" than the latter since the plaques it makes at 44°C, in contrast to the tiny ones formed by *ts* G25, are large enough to be indistinguishable from those of T4*ts*$^+$. Infection of *E. coli* B at 42.5°C by either *ts* G25 or *ts* G25W results in very little DNA synthesis; however, at 30° C the rate of DNA synthesis is nearly normal with *ts* G25W and very low with *ts* G25. Preliminary studies on the *ts* G25 hydroxymethylase indicated that it was the same altered enzyme seen with *ts* G25W.

Stocks of all phage were grown on *E. coli* B at 30°C by standard techniques.[6] Purification and assay of phage were carried out as described previously,[2] except that phage pellets were resuspended in a 1:1 dilution of glycerol casamino acids medium[7] and stored at 4°C for 1 week before use.

dCMP hydroxymethylase assay: The assay for dCMP hydroxymethylase was a modification of one described previously.[8] The reaction mixture contained, usually in 0.5 ml: dCMP, 2.0 μmoles (acid form adjusted to pH 7.5 with NaOH); EDTA, pH 8.0, 1 μmole; 2-mercaptoethanol, 50 μmoles; tetrahydrofolate, 0.45 μmole; C^{14}-labeled formaldehyde, 1 μmole with a specific activity of 0.62 μcurie (440, 000 cpm) per μmole; buffer, phosphate or Tris, as indicated, 25 μmoles, except where otherwise specified. For all assays a mixture at pH 7.5 of the tetrahydrofolate, formaldehyde, and half of the mercaptoethanol was heated[9] at 40°C for 10 min and then stored on ice until used (within 3 hr). Temperature and duration of incubation of enzyme with reagents are indicated with each experiment. The pH values of reaction solutions buffered with Tris were measured at the temperatures indicated. This was necessary because of the large temperature coefficient of its pK value.[14,15] For example, a 0.05-*M* solution of Tris acetate that is pH 8.0 at 25°C has a pH of 8.75 at 0°C. No such effect was seen with the phosphate buffers. The reaction was stopped by addition of 1 ml of 0.003 *M* hydroxylamine hydrochloride followed by heating the contents of the tubes to 100° for 2 min.[16] The reaction mixture was applied at room temperature to a 3.3-×-50-mm column of Dowex-1-8X-formate ion exchange resin (200–400 mesh). The column was then washed with 2 ml of water followed by 2.5 ml of 0.03 *M* formic acid. The dHMP was then eluted with 2 ml of 0.10 *M* formic acid and collected on stainless steel planchets[17] on a hot plate. The residue was spread evenly by addition of 8 drops of 50% ethanol to the planchets on the hot plate. The dCMP hydroxymethylase assay is linear with respect to both time and amount of extract used within the ranges of either studied in this paper.

Preparation of dialyzed extracts: A liter of cells of *E. coli* B was grown with vigorous aeration at 37°C in glycerol-casamino acids medium[7] to a concentration of 1.08×10^9 per ml. The contents of the vessel were chilled to 5°C, and within 2 hr aliquots of 120 ml were placed in 250-ml Erlenmeyer flasks. L-tryptophan was added to a concentration of 0.05 mM. The flasks were then placed in a 30°C waterbath for 2 min, after which vigorous aeration was begun through sintered glass tubes. After another 2 min, 9.3 phage per bacterium were added. After 25 min an aliquot of 100 ml was chilled by admixture with frozen medium, and was centrifuged at 8000 × *g* for 20 min. All subsequent steps were carried out between 0°C and 2°C. The pellet was resuspended in 4 ml of 0.1 *M* Tris chloride, pH 8.2, in 0.01 *M* Mg acetate. The suspension was frozen in liquid nitrogen and later forced through a Hughes press[18] at −20°C to break the cells. The thawed extract was then centrifuged at 100,000 × *g* for 1 hr. The supernatant solution was dialyzed twice for 5 hr in Dexstar tubing (7-mm diameter) against 50 volumes of a solution 0.025 *M* in respect to sodium phosphate, pH 7.0, and 0.005 *M* in respect to EDTA. The dialyzed extracts were stored at −20°C. No significant losses of enzyme activity occurred during the centrifugations or the dialysis, nor during subsequent storage for several months at −20°C, even though the extracts were frequently thawed to 0°C and refrozen.

Results.—Enzyme activity as a function of pH at 0°C and 30°C: Figure 1 shows the variation in activity of the T4*ts*$^+$ and G25W enzymes as a function of pH at 0°C, and of the T4*ts*$^+$, A41, G25W, and L13 enzymes at 30°C. It is clear that at 30°C the T4*ts*$^+$, A41, and L13 enzyme activities vary almost identically with pH; the

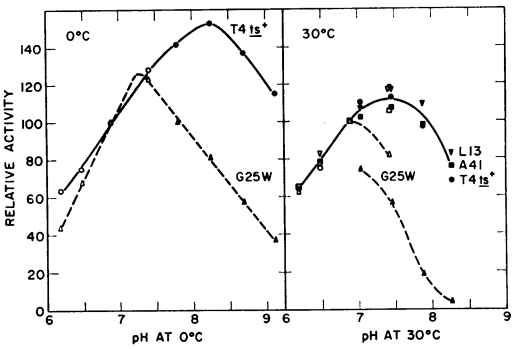

FIG. 1.—Variation with pH of the activity of dCMP hydroxymethylases assayed at 0° and 30°C. ○—● = T4*ts*+; △—▲ = G25W; □—■ = A41; ▽—▼ = L13. Hollow symbols represent sodium phosphate buffer; filled symbols represent Tris acetate buffer. Relative activities were determined for each enzyme by setting its rate in pH 6.9 phosphate buffer equal to 100. The absolute rates (in cpm per min) at this pH were, at 0°C: 20.7 and 8.4 for T4*ts*+ and G25W, respectively; at 30°C they were 230, 322, 105, and 24.2 for T4*ts*+, A41, L13, and G25W, respectively. Each plotted point represents a single rate derived from four aliquots, taken at intervals, from a single assay vessel containing 5 times the usual amount of reagents (*Methods*); these intervals were 8 min at 30°C and 90 min at 0°C. Each aliquot represented 10 μl of dialyzed extract.

maximum activity occurred in each case at about pH 7.5. The G25W enzyme, on the other hand, has a pH optimum of 6.9 at 30°C. In addition, with the G25W enzyme a distinct discontinuity appears between phosphate and Tris buffers, the enzyme being less active in Tris at a given pH. At 0°C, both pH optima are higher, being 7.3 and 8.25 for G25W and T4*ts*+, respectively.

Ratio of enzyme activity at 30°C to that at 0°C: The G25W and T4*ts*+ enzymes may be distinguished further by the ratio of activity at 30°C to that at 0°C. From the plotted data of Figure 1 and the absolute activities given in the legend, it was calculated that the ratios at pH 6.2, 6.5, and 6.9 are 3.6, 3.3, and 2.9, respectively, for G25W; and 11.2, 11.1, and 11.3, respectively, for T4*ts*+. Other experiments[19] have indicated that both the A41 and L13 enzymes are like the wild type in this respect.

Inactivation of enzymes as a function of temperature and dCMP concentration: In Table 1 are compared the activities of enzymes that have been heated at various temperatures for 1.5 min in the absence of substrate, dCMP (rows *A–D*). Also compared are the effects of substrate on the inactivation at 40°C (rows *D–G*). It is seen that the T4*ts*+, A41, and L13 enzymes are not seriously inactivated below 25°C, whereas the G25W enzyme loses considerable activity when kept at 20°C even for 1.5 min. In fact, as seen in Table 2 (row *C*), the G25W enzyme is unstable even at 0°C at pH 8.25 in the absence of dCMP. It is of interest that at 40°C a much lower concentration of dCMP is required by the T4*ts*+, A41, or L13 enzymes than is needed by the G25W enzyme to provide a comparable protection against inactivation (Table 1, rows *E* and *G*).

In other experiments[19] in which residual enzyme activity at 0°C was measured as a function of time of exposure at 40°C in the absence of dCMP, the rapid initial rate of inactivation appeared to slow drastically, except with L13, after about 95 per cent of the activity had been lost. However, the actual values of enzyme activity after 1 min of heating probably approach zero; the small apparent ac-

TABLE 1

INACTIVATION OF VARIOUS dCMP HYDROXYMETHYLASES AT SEVERAL TEMPERATURES AND dCMP CONCENTRATIONS

Treatment of Enzyme Prior to Assay: Temperature of exposure for 1.5 min	μmoles dCMP present	Residual Activity (as per cent of activity of untreated enzyme): T4*ts*+	A41	G25W	L13
A. 20°C	None	86	97	34	93
B. 25°C	None	83	86	14	89
C. 30°C	None	36	44	9.1	50
D. 40°C	None	4.3	5.9	6.2	0.6
E. 40°C	0.2	26	32	5.6	53
F. 40°C	0.6	71	77	8.5	88
G. 40°C	2.0	87	97	32	90

The following materials were mixed in a volume of 0.345 ml: 25 μmoles Tris acetate, (pH 8.25 at 0°C, pH 7.3 at 40°C), 25 μmoles 2-mercaptoethanol, 1 μmole EDTA, and dCMP as indicated. Tubes, each containing this mixture, were first warmed for 3 min in baths at the appropriate temperatures. Then 10 μl of dialyzed extract were added, and after 1.5 min the tubes were placed in ice water. The total amount of dCMP present was brought immediately to 2 μmoles and the volume was adjusted with water to 0.405 ml. The assay was started at 0°C within 5 min by addition of 95 μl of a solution containing 0.45 μmole tetrahydrofolate, 1 μmole C^{14}-HCHO, and 25 μmoles 2-mercaptoethanol. The reaction was stopped after 5 hr as described in the text. The values (in cpm) for the unheated control activities were 11,700, 14,900, 2090, and 5670 for T4*ts*+, A41, G25W, and L13, respectively.

tivities seen (e.g., in row *D*, Table 1) can be accounted for by reactivation (below) of each enzyme except the L13[20] during the assay.

Reactivation of enzymes heated at 40°C: In Table 2 are shown the effects of preincubation of the native and inactivated enzymes under several conditions. Significant reactivation occurs at 0°C and pH 8.25 with all the enzymes except L13, for which no reactivating conditions were found. Comparison of the data of rows *E* and *F* shows that the presence of dCMP is helpful but not obligatory for reactivation at 0°C, pH 8.25. Approximate initial rates of reactivation can be calculated by subtracting the percentages in row *D* from those in row *E* and dividing by 5 hr. Expressed in this arbitrary unit, reactivation at 0°C and pH 8.25 (the conditions of assay here and also for Table 1) occurs at the rates of 1.2, 1.5, and 0.7, respectively, for T4*ts*+, A41, and G25W. As noted above, these rates satisfactorily account for the "apparent" residual activity seen after long exposure at 40°C and for that seen with heated but unpreincubated enzyme (row *D*, Table 2).

Reactivation at 30°C, pH 7.05, is much faster; the respective rates, calculated from the data of rows *I* and *J*, are at least 53, 56, and 51. Note that the preincubations at 30°C were at a lower pH than those at 0°C, yet the lower pH *per se* increases the reactivation rate at 0°C by only two- to three-fold;[19] this is indicated also by comparing the data of rows *D* and *I*. Note also that the native G25W enzyme is unstable at 0°C, pH 8.25, in the absence of dCMP (row *C*).

Tests for protection of enzyme activity by other compounds: Several compounds related to dCMP were tested for their ability to protect the A41 hydroxymethylase (presumed to be wild type) from inactivation at 40°C. Neither protection nor inhibition of the unheated enzyme was exhibited by d-cytidine (2 μmoles), cytosine (2 μmoles), or CMP (0.84 μmole). However, dHMP (0.78 μmole), the product of the enzyme reaction, protected equally as well as did dCMP at the same concentration. The activity of the unheated enzyme in the presence of both dHMP and dCMP in equimolar concentrations was about half that seen in the presence of dCMP alone. This inhibition of the reaction by dHMP has been reported by Pizer and Cohen.[21]

Experiments with other ts mutants in gene 42: Four other mutants, *ts* L40, *ts* L66, *ts* G13, and *ts* G23, have sites of mutation that are also located in gene 42.

TABLE 2

REACTIVATION OF dCMP HYDROXYMETHYLASES PREVIOUSLY HEATED AT 40°C IN THE ABSENCE OF dCMP

Treatment of enzyme	Activity (as per cent of the activity of native enzymes) T4*ts*+	A41	G25W	L13
Assayed at 0°C, pH 8.25				
A. Native*	100	100	100	100
B. Native + 5 hr preincubation at 0°C with dCMP	111	102	110	100
C. Native + 5 hr preincubation at 0°C minus dCMP	108	101	18	98
D. Heated	3.5	4.5	4.2	<1
E. Heated + 5 hr preincubation at 0°C with dCMP	9.7	12	7.6	0.7
F. Heated + 5 hr preincubation at 0°C minus dCMP	7.4	8.9	5.9	0.5
Assayed at 0°C, pH 7.05				
G. Native*	100	100	100	100
H. Native + 1 hr preincubation at 30°C with dCMP	107	104	98	103
I. Heated	11	11	9.0	0.5
J. Heated + 1 hr preincubation at 30°C with dCMP	64	67	60	0.5

* Absolute activities (in cpm) for T4*ts*+, A41, G25W, and L13 in row *A* were 12,100, 16,900, 1880, and 7140, respectively; in row *G* they were 5000, 7200, 1880, and 2420, respectively.

The following materials were mixed in a volume of 0.345 ml: 25 μmoles Tris acetate (pH 7.3 at 40°C, pH 8.25 at 0°C), 25 μmoles 2-mercaptoethanol, and 1 μmole EDTA. For rows *D*, *E*, *F*, *I*, and *J*, tubes containing this mixture were placed in a waterbath at 40°C for 5 min and then 10 μl of dialyzed extract were added. Three min later the tubes were placed in ice water. For rows *A*, *B*, *C*, *G*, and *H* this incubation at 40°C was omitted. Within 10 min 80 μl of water were added containing (for the tubes of all rows except *C* and *F*) 2 μmoles of dCMP, and (for the tubes of rows *G–J*) 15 μmoles Na phosphate, pH 6.5; this phosphate lowered the pH to 7.05 at 0°C (or to 6.85 at 30°C). For rows *B*, *C*, *E*, and *F* the tubes were then left on ice for a 5-hr period ("preincubation") prior to assay. For rows *H* and *J* the tubes were placed at 30°C for 1 hr and then chilled to 0°C. For rows *A*, *D*, *G*, and *I*, addition of extract was scheduled to coincide closely with the termination of the preincubation for the other rows. Then to all tubes 80 μl of water were added, containing (for the tubes of rows *C* and *F*) 2 μmoles dCMP. The 5-hr assay at 0°C was begun immediately, exactly as in Table 1.

Mapping evidence[22] indicates that all the L mutations are located at one site and that all the G mutations are at a distinctly different site. Preliminary experiments with the dCMP hydroxymethylases produced by these mutants indicate a corresponding grouping of properties, i.e., the two L enzymes have activity and stability in the presence of dCMP at 40°C comparable to those of the L13 enzyme, whereas the two G enzymes are about as unstable as the G25W enzyme.

Discussion.—The L13 enzyme is similar to the wild type in all respects studied except that it cannot be reactivated after heating at 40°C. That the G25W enzyme is different from the wild type is manifested by a number of properties. Some of these differences appear to be attributable to a poor affinity of the enzyme for its substrate, dCMP. A direct determination of the Michaelis constant for dCMP would be of limited value with these relatively unpurified extracts, and difficult to perform in the case of the G25W enzyme, which is so labile in the absence of dCMP. Nonetheless, since dCMP is specifically required to protect the wild-type enzyme at 40°C, presumably by binding at the active site, the protective ability of dCMP can be considered a rough measure of the K_m for dCMP. Thus, the fact that the G25W enzyme requires 10 times as much dCMP as does the wild type for comparable protection (Table 1) indicates that the K_m for dCMP of the G25W enzyme is about 10 times that of the wild-type enzyme, at 40°C, pH 7.3. The lability of the G25W enzyme is clearly not due solely to its low binding affinity for dCMP, for even when no dCMP is present to "protect" either it or the other enzymes, only the G25W enzyme is inactivated below 25°C (Table 1).

In infections carried out at 42.5°C with the three L mutants, no DNA was made and no dCMP hydroxymethylase was detectable. With the three G mutants at 42.5°C very little DNA synthesis occurred, yet just as much dCMP hydroxymethylase

activity was detected in the high-temperature infections with the G mutants as in infections carried out at 30°C.[19] Thus, it appears likely that, *in vivo*, the L mutants fail to make DNA at 42.5°C because active enzyme either is not made or is inactivated rapidly and irreversibly, whereas the G mutants fail to make much DNA at 42.5°C simply because the enzyme *functions* poorly. Neither DNA nor hydroxymethylase production by T4*ts*+ was impaired at 42.5°C.

This demonstration of two different types of alteration in the properties of the dCMP hydroxymethylases induced by two phage mutants, which map genetically at two different loci within the same gene, constitutes the strongest evidence to date that the structural gene for this enzyme resides in the phage rather than in the bacterial host. This question has been discussed by others.[23, 24] Two further lines of supporting evidence have appeared recently. Mathews and Cohen[25] have shown that the affinities of dUMP and FdUMP for the thymidylate synthetase induced by T2 differ from those for the corresponding T6 enzyme. Dirksen, Hutson, and Buchanan[26] have provided evidence that a number of *amber* mutants of T4 that induce no detectable dCMP hydroxymethylase activity in *E. coli* B do cause the formation, in permissive K12 hosts, of hydroxymethylases, the properties of which vary from mutant to mutant with respect to pH and heat inactivation.

Since the present *ts* mutants involve point mutations,[22] it can be argued, by analogy with the work of Ingram[27] and Baglioni[28] on abnormal hemoglobins and of Helinski and Yanofsky[29] on altered tryptophan synthetases from *E. coli*, that the properties of the G25W and the L13 hydroxymethylases reflect a single amino acid substitution, different for each. In view of the technical advantages in experiments with bacteriophage,[4] the present studies illustrate the potential inherent in such phage mutants for the eventual correlation of enzyme properties with protein structure and genetic fine-detail mapping.

Summary.—Among the temperature-sensitive (*ts*) mutants of bacteriophage T4 isolated by Edgar and co-workers[5] are two, *ts* G25W and *ts* L13, that contain sites of mutation located in the same gene as that of mutant *amber* N122. This *amber* mutant has been shown[2] to be unable to cause formation of dCMP hydroxymethylase upon infection of *E. coli* B. Several properties of the hydroxymethylases formed upon infection by the two *ts* mutants and by the wild-type phage have been compared. Relative to the wild-type enzyme, the G25W enzyme has a lower pH optimum, a much smaller temperature coefficient between 0°C and 30°C, is inactivated far more rapidly at 40°C in the presence of substrate, dCMP, and is quite unstable even at 0°C, pH 8.25, in the absence of dCMP. The L13 enzyme is similar to the wild-type enzyme in all respects studied except that it cannot be reactivated after inactivation in the absence of dCMP at 40°C. Two properties of the wild-type enzyme are described for the first time: (*a*) a marked protective effect of both substrate and product, dCMP and dHMP, respectively, against heat inactivation at 40°C; and (*b*) the ability of the enzyme to undergo extensive reactivation after exposure of the unprotected enzyme to heat at 40°C.

These results constitute strong evidence that the structural gene for dCMP hydroxymethylase, an "early enzyme" of phage infection, resides in the phage itself. The results also serve to illustrate the promise offered by phage systems, which are readily manipulated, to provide new correlations between genetic fine-detail mapping and enzyme properties and structure.

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[1] The following abbreviations are used: dCMP, deoxycytidylate; dHMP, 5-hydroxymethyldeoxycytidylate; Tris, tris (hydroxymethyl) aminomethane; EDTA, ethylenediamine tetraacetic acid, disodium salt; dUMP, deoxyuridylate; FdUMP, 5-fluorodeoxyuridylate.

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[9] This preheating step was prompted by the observation that when C^{14}-labeled HCHO was added to the rest of the reagents plus enzyme at 0°C (assay pH = 8.25, in 0.05 *M* Tris acetate), enzyme activity was undetectable for 15 min and then did not attain a maximum rate until 45 min later. Preheating the HCHO and tetrahydrofolate eliminates this lag and suggests that the lag is due to slow formation at 0°C of the active substrate, N^5,N^{10}-methylenetetrahydrofolate. The rate of this complex formation has been examined by others at higher temperatures.[10-12] Possibly the limiting reaction here is the dissociation of methylene glycol, the hydrate of formaldehyde, which is the predominant form of HCHO in aqueous solutions.[13]

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[16] This hydroxylamine solution was prepared fresh in cold water just before use, by dilution of 1 *M* solution that had been stored at 4°C for no longer than 3 months. Hydroxylamine, which removes the HCHO from the tetrahydrofolate by forming a volatile complex with HCHO,[12] decreased the radioactivity of the reagent blank 2 to 5 times relative to that seen in its absence. The addition of 200 μmoles of unlabeled HCHO to "dilute" the C^{14}-labeled HCHO[8] was discontinued because it was noted that this caused the pH to drop about 5 units by reaction with the Tris buffer. This increase in acidity was deleterious to proper adsorption of the dHMP to the Dowex-1-resin.

[17] To keep the fluid from running to the edge of the planchet, with its attendant counting errors, a water-repellent ring was drawn (about 3 mm wide) at the periphery of the planchet with a black marking pen such as Carter's "Marks-a-Lot." Pens of other colors were less satisfactory.

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*ELECTROCHEMICAL PRECIPITATION OF HUMAN BLOOD CELLS AND ITS POSSIBLE RELATION TO INTRAVASCULAR THROMBOSIS**

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Several recent experiments have suggested that surface chemical phenomena are involved in both the prevention and production of intravascular thrombosis.[1-4] These experiments indicated that polarization of the blood vessel wall resulted in the formation of an intravascular thrombus on the positively polarized wall. However, the precise mechanism is not known.

We have carried out simple experiments designed to measure the precipitation potential of the various cellular elements of blood on metal electrodes. It was found that human erythrocytes and leucocytes deposit semireversibly on both platinum and gold at a reproducible potential. This potential is the same for both metals, and at a pH of about 7.4 is approximately + 0.33 volt ± 0.02 volt with respect to the reversible hydrogen electrode.

The precipitation potential is demonstrably independent of concentration of red blood cells over a range of three decades (10^3–10^6 RBC/ml) but depends on the pH of solution, changing approximately 85 mv ± 15 mv per unit of pH change.

These findings show that the cellular elements of blood undergo some change in contact with a surface polarized to a potential more positive than a critical value. This would seem to be due to a reduction in the density of negative charge[5] on the blood cell wall with a consequent reduction in the stability conferred by mutual repulsion of the particles. We are not suggesting that a simple process of this type is necessarily all that is involved in such a complex process as the clotting of blood, but rather that since thrombosis may be induced in such a simple and controllable way, useful information may be gained from a study of this phenomenon.

By implication at least, this would suggest that the blood vessel wall exists in a state which ordinarily prevents cellular precipitation on the wall by the mechanism described here. An unproved corollary of this general observation would suggest that the protein elements in the plasma also have a charge similar to that of red cells. This possibility is now being investigated.

Materials and Methods.—Precipitation chamber: The precipitation chamber consisted of a Lucite box with optical glass sides. A thin platinum rod was inserted through the side of the box close to the anterior optical glass surface through which it was observed. This platinum rod served as the precipitation electrode. Two 5-mm holes were drilled into the top surface of the chamber in the upper cor-