the *vs* gene; while T4*tk3*, T4*tk25*, and T4*farP13* contain extensive deletions which include the *vs* gene (*7*).

A comparison of the genotypes of the *E. coli* strains indicates that the simultaneous presence of the *relA* and *valS*^{ts} alleles is crucial for the differential plating phenomenon. Introduction of the normal *valS* allele into strain CP 790302 via P1*vir* transduction (GM 23) restores its ability to plate normal phage. An isogenic (except for *relA*) pair of strains, CP 78 and CP 79, also contain the normal *valS* allele and do not exhibit differential plating of normal versus *vs* phage. This is also true for strains KL 16, K 10, JP 1116, and NP 37, which are *rel-1* (isogenic with *relA*) but harbor the wild-type *valS* allele. In addition, since JP 1116 and NP 37 produce thermolabile phenylalanyl-tRNA synthetases, temperature sensitivity of a different aminoacyl-tRNA synthetase is not sufficient for the phenomenon. Finally, while NP 29 and NP 910212 contain *valS*^{ts} alleles they plate all phage efficiently, presumably because they are *relA*$^{+}$.

Whether the inability to plate bacteriophage T4 on strain CP 790302 is dependent on the particular allelic state of the *valS* and *relA* genes remains to be determined. Strains NP 29 and NP 910212 most probably contain different allelic states of the *valS*^{ts} gene as they were isolated independently (*8*). On the other hand, the *valS*^{ts} allele in CP 790302 comes from NP 910212 by P1*vir* transduction (*9*). In contrast, the *relA* alleles in the strains in Table 1 are identical, having been derived from CP 79.

These data thus demonstrate that the simultaneous presence of certain mutations within the *relA* and *valS* genes render *E. coli* nonpermissive for T4 bacteriophage that contain a wild-type *vs* gene. The implication exists, therefore, that the *relA* gene product or subsequent metabolites, for example, ppGpp, may play a role during the development of bacteriophage T4 in *E. coli*. Since the *relA* locus influences a large number of biosynthetic and catabolic systems in bacteria (*10*), and has also been shown to affect the accumulation of RNA in T4-infected *E. coli* (*11*), an explanation of the mechanism of the phenomenon is not possible at this time.

GEORGE L. MARCHIN
Division of Biology, Kansas State University, Manhattan 66506

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28 January 1980; revised 7 April 1980

Myocardial Infarct Imaging of Antibodies to Canine Cardiac Myosin with Indium-111-Diethylenetriamine Pentaacetic Acid

Abstract. *Antibodies, by virtue of marked selectivity and affinity, may lend themselves to identification of structures of unique antigenic specificity in vivo. In experimental myocardial infarction in dogs, F(ab')$_2$ fragments of antibodies to cardiac myosin that had been labeled with iodine-131 were shown to localize within the lesion. Because the energy characteristics of iodine isotopes are not ideal for imaging with a gamma camera, a new method for labeling antibody fragments with divalent or polyvalent radionuclides was developed. A bifunctional chelating agent, diethylenetriamine pentaacetic acid was covalently coupled, by an amide bond, to Fab fragments of antibodies to canine cardiac myosin. A stable chelate was then formed with indium-111, a nuclide that has appropriate half-life and energy characteristics for gamma imaging. Antibodies treated in this way retain their antigen-binding activity and are useful in locating myocardial infarcts in vivo.*

Irreversible ischemic tissue injury results in a loss of integrity of the cell's plasma membrane, as evidenced by leakage of intracellular enzymes from regions of myocardial infarction (*1*). This increase in membrane permeability should also permit the entry of extracellular molecules into the damaged cell. If the extracellular molecule is an antibody specific for an intracellular component, it should concentrate within the injured tissue. We have previously demonstrated that, following experimental myocardial infarction in dogs, ^{125}I-labeled F(ab')$_2$ fragments of antibodies specific for cardiac myosin localize and concentrate within the lesion (*2*). Neither ^{131}I nor ^{125}I are ideal gamma-imaging

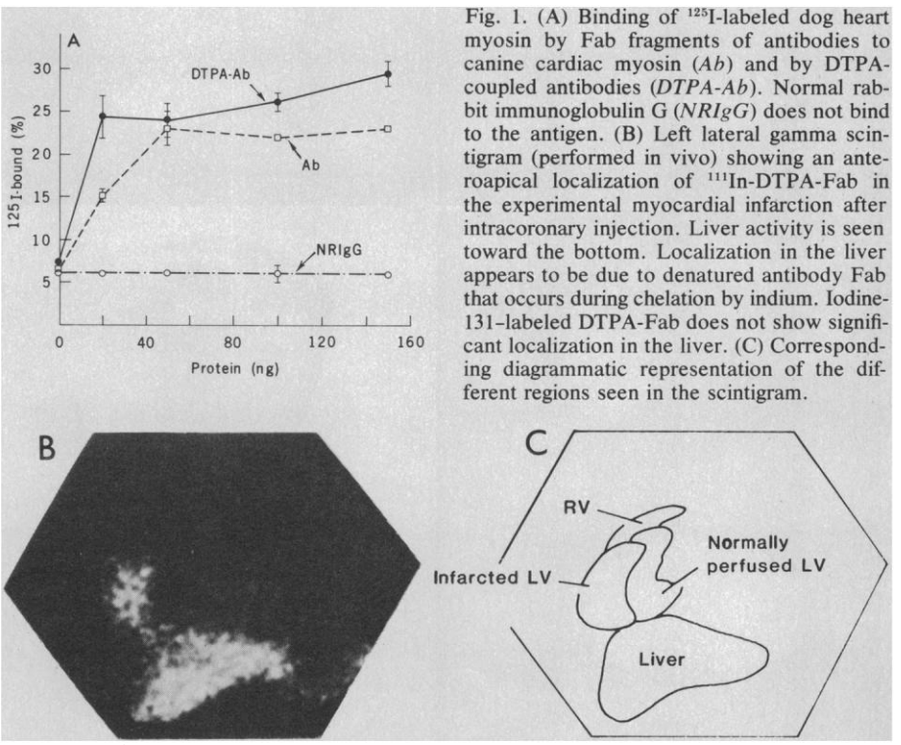

Fig. 1. (A) Binding of ^{125}I-labeled dog heart myosin by Fab fragments of antibodies to canine cardiac myosin (*Ab*) and by DTPA-coupled antibodies (*DTPA-Ab*). Normal rabbit immunoglobulin G (*NRIgG*) does not bind to the antigen. (B) Left lateral gamma scintigram (performed in vivo) showing an anteroapical localization of ^{111}In-DTPA-Fab in the experimental myocardial infarction after intracoronary injection. Liver activity is seen toward the bottom. Localization in the liver appears to be due to denatured antibody Fab that occurs during chelation by indium. Iodine-131-labeled DTPA-Fab does not show significant localization in the liver. (C) Corresponding diagrammatic representation of the different regions seen in the scintigram.

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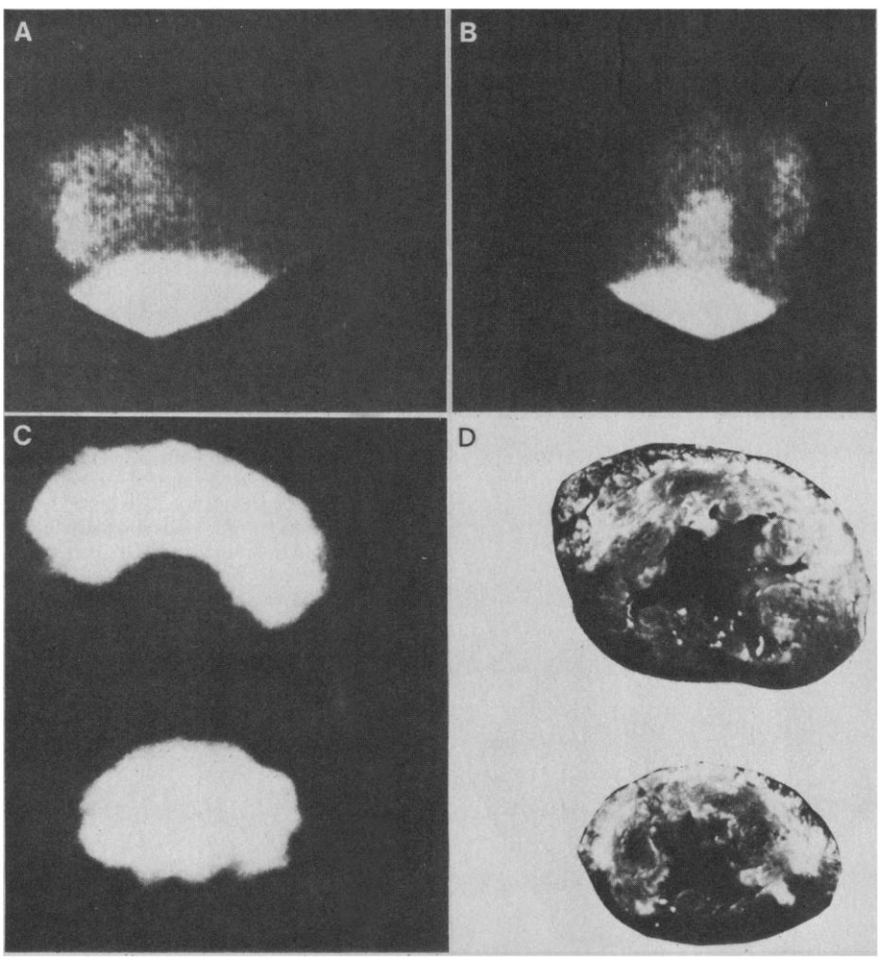
agents, however. The former isotope has too high an energy of emission, the latter too low. In addition, each has a long half-life, resulting in excessive radiation dosage if used in clinical studies. For these reasons we investigated other methods for labeling antibodies.

We now report the use of a bifunctional chelating agent, diethylenetriamine pentaacetic acid (DTPA), to label anti-myosin-specific Fab fragments with indium-111 and the use of this labeled antibody to image experimental myocardial infarctions in dogs. Indium-111 was used for the following reasons: (i) it has a short half-life of 67 hours, (ii) it has energy emission peaks at 173 and 247 keV that can be imaged by use of conventional collimators with an Anger camera, (iii) it has a highly efficient disintegration-to-photon production ratio of 100:183, and (iv) it has no beta emission (*3*).

Antibodies specific for canine cardiac myosin were purified by myosin-Sepharose affinity chromatography of rabbit immune serum (*2*). Purified antibody was treated with papain for 1.5 hours at 37°C, *p*H 7.0, at an enzyme to substrate ratio of 1:100 to produce Fab fragments (*4*). Fab and Fc fragments were separated by protein A–Sepharose chromatography, which binds the Fc fragments as well as undigested antibody (*5*). The Fab fragments of antibody were not bound to the column. The Fc and undigested antibody that remain on the column can be recovered with 3*M* guanidine hydrochloride. Antibody Fab fragments were concentrated by vacuum dialysis, then dialyzed against 0.1*M* $NaHCO_3$, *p*H 8.0. One milliliter of the concentrated solution of Fab (5 mg/ml) was then reacted with carboxycarbonic anhydride of DTPA (0.025 m*M*), prepared as described by Krejcarek and Tucker (*6*). The efficiency of covalent coupling of DTPA to Fab was determined by the chelating capacity of DTPA-Fab for ^{111}In at *p*H 3.5; the antibody activity of DTPA-Fab was determined by its capacity to bind the ^{125}I-labeled cardiac myosin (CCM) (*2*). Figure 1A shows that the covalent attachment of DTPA to Fab does not impair antigen binding. The capacity of unmodified Fab and DTPA-Fab to bind ^{125}I-CCM are indistinguishable.

The binding of ^{111}In to DTPA-Fab was performed at *p*H 3.5 in 0.1*M* glycine hydrochloride at room temperature for 15 minutes. Free and chelated ^{111}In were separated by Sephadex G-25 column (10 ml) chromatography, using 0.3*M* sodium phosphate, *p*H 7.0, 0.15*M* NaCl solution (0.3*M* phosphate-buffered saline) to elute the column. The radioactive peak in the void volume was dialyzed against 2 liters of 0.3*M* phosphate-buffered saline for 2 to 3 hours, then against lactate-Ringer solution for 6 to 12 hours. All samples of ^{111}In-DTPA-Fab prepared were used within 24 hours.

Myocardial infarction was produced in nine dogs by ligation of the left anterior descending coronary artery for 4 hours, after which the occlusive ligature was removed to permit reperfusion (*2*). Indium-111-DTPA-Fab was injected into the orifice of the main left coronary artery through a catheter placed under direct fluoroscopy, as previously described (*7*), or it was injected intravenously. One millicurie of ^{111}In-DTPA-Fab was used for the intracoronary injection and 1.5 to 2 mCi for the intravenous injection. Images were obtained with a portable Anger camera immediately after intracoronary injection and again 24 and 48 hours later. Figure 1B shows a left lateral gamma image of ^{111}In-DTPA-Fab localization in the anterolateral wall of the canine left ventricle, the site of the experimental myocardial infarction. A diagrammatic localization of the structures



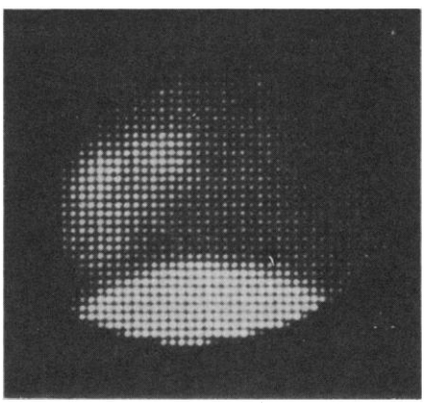


Fig. 2 (left). (A) Left lateral scintigram (obtained in vivo) showing anteroapical localization of ^{111}In-DTPA-Fab 24 hours after intravenous injection of the antibody; (B) anteroposterior scintigraphic view of the same dog showing anteroapical localization of the ^{111}In-DTPA-Fab; (C) the in vitro gamma scintigram of the two apical slices of the infarcted myocardium showing localization of ^{111}In-DTPA-Fab; and (D) the corresponding TTC-stained slices demonstrating normal myocardium as darkly stained areas and the infarcted myocardium as lighter colored or white areas. Fig. 3 (above). Left lateral scintigram showing anteroapical localization of ^{99m}Tc-DTPA-anti-myosin Fab 24 hours after intravenous injection of the compound. The scintigram was performed in vivo. Radioactivity in the lower region of the scintigram represents liver uptake.

visualized is shown in Fig. 1C, as determined by a thallium-201 scan. Three animals that received the ^{111}In-DTPA-Fab intravenously were imaged 24 hours later; the infarct was clearly revealed, as shown for one of the dogs in Fig. 2, A and B. In each of the animals studied, the infarcts were identified postmortem by histochemical staining with triphenyltetrazolium chloride (TTC) (*8*). The stained region corresponded to the region of radioactive antibody concentration. A representative comparison is shown in Fig. 2, C and D. In three control animals, subjected to sham operation but not coronary ligation, there was no localization of labeled antibody either in vivo or in vitro.

In preliminary experiments, gamma camera images have also been obtained with technetium-99m–labeled antimyosin-Fab and positron camera images with gallium-68–labeled antimyosin Fab. Figure 3 shows a left lateral gamma image of ^{99m}Tc-DTPA-Fab localization in the anterolateral wall of the canine left ventricle, the site of experimental myocardial infarction after intravenous injection.

These experiments not only demonstrate the potential utility of specific antibody fragments in the localization of lesions characterized by unique antigenic identity in vivo, but also provide an approach to the labeling of such specific antibodies with various radionuclides that possess optimal imaging properties.

BAN AN KHAW, JOHN T. FALLON
H. WILLIAM STRAUSS, EDGAR HABER
Department of Medicine,
Massachusetts General Hospital,
Harvard Medical School,
Boston 02114

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22 October 1979; revised 19 December 1979

Mitochondrial DNA Is a Major Cellular Target for a Dihydrodiol-Epoxide Derivative of Benzo[*a*]pyrene

Abstract. *When mammalian cell cultures are exposed for 2 hours to (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo*[a]*pyrene, a mutagenic and carcinogenic derivative of benzo*[a]*pyrene, the extent of covalent modification of mitochondrial DNA is 40 to 90 times greater than that of nuclear DNA. Evidence is presented that this reflects the lipophilic character of the derivative and the very high ratio of lipid to DNA in mitochondria. These results suggest that mitochondrial DNA may be an important cellular target of chemical carcinogens.*

Recent studies have provided evidence that a dihydrodiol-epoxide, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BDPE), is the most potent carcinogenic and mutagenic metabolite formed in mammalian cells and tissues exposed to the environmental pollutant benzo[*a*]pyrene (*1*). The BPDE binds covalently to nuclear DNA (nDNA) and cellular RNA in vivo and also forms covalent adducts with a variety of nucleic acids in vitro (*1*). The major adduct consists of a guanine residue linked via its 2-amino group to the 10 position of benzo[*a*]pyrene (*1*). The covalent binding of BPDE or other ultimate carcinogens with nDNA is often emphasized as the initial and crucial event in the malignant transformation of cells by chemical carcinogens. We now report that in mammalian cell cultures exposed to BPDE, mitochondrial DNA (mtDNA) is modified to a much greater extent than nDNA. Our results raise the possibility that carcinogen modification of mtDNA may play an important role in the carcinogenic process.

When three different cell types were exposed to [^{3}H]BPDE for 2 hours, the extent of modification of mtDNA was much higher than that of nDNA (Table 1). The values of 3 to 12 BPDE residues per 10^6 DNA base residues that we ob-

Table 1. Extent of modification of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) when cell cultures are exposed to [^{3}H]BPDE. The 10T^1/$_2$ mouse embryo cell line (*12*) and a chloramphenicol-resistant subline of mouse L cells (*12*) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 percent fetal bovine serum (FBS) in 10-cm tissue culture plates (Nunclon) at 37°C in an atmosphere of 5 percent CO_2 and air. The rat liver epithelial cell line K-16 (*12*) was grown under the same conditions but in F12 medium with 10 percent FBS. The cellular DNA was uniformly labeled by growing the cells continuously in the presence of [^{14}C]thymidine (0.02 μCi/ml; 41 mCi/mmole) for five to six passages. Confluent cultures were washed with phosphate-buffered saline (PBS) and incubated at 37°C for 2 hours with 3 ml of serum-free medium per plate containing [^{3}H]BPDE (1.8 μg/ml; 411 mCi/mmole). Cells from 20 to 30 plates were then washed with PBS, dissociated with 0.1 percent trypsin-Versene and sedimented at 2500 rev/min. The cells were then resuspended in 5 ml of 0.25*M* sucrose containing 10 m*M* EDTA, bovine serum albumin (0.5 mg/ml), and 20 m*M* Hepes buffer, and homogenized in a glass homogenizer. The homogenate was centrifuged at 2500 rev/min for 5 minutes; the pellet was suspended in 3 ml of sucrose-buffer solution and recentrifuged to yield the nuclear pellet. Both supernatant fractions were combined and recentrifuged several times at 2500 rev/min until no visible pellet was seen. The final supernatant fraction was then centrifuged at 10,000 rev/min for 20 minutes to yield a mitochondrial pellet. To extract DNA, the nuclear and mitochondrial pellets were suspended for 1 hour at 37°C in a buffer solution containing 20 m*M* NaCl, 1 m*M* EDTA, 20 m*M* tris-HCl (*p*H 7.8), 0.1 percent sodium dodecyl sulfate, and proteinase K (200 μg/ml). The nDNA was then extracted with hot phenol and incubated for 1 hour with RNAase and then for 1 hour with proteinase K. The hot phenol extraction was repeated, and the nDNA was collected (*13*). To remove noncovalently bound radioactivity, the nDNA was solubilized in buffer containing 20 m*M* NaCl, 1 m*M* EDTA, 20 m*M* tris-HCl (*p*H 7.8), then sonicated, extracted several times with ethyl acetate until it reached a constant specific activity, precipitated with ethanol, and assayed for ^{14}C and ^{3}H content and absorbance at 260 nm. The resulting values were used to calculate extent of modification by [^{3}H]BPDE. The mtDNA was extracted several times with chloroform-isoamyl alcohol (24:1). The aqueous phase was then centrifuged in a CsCl-ethidium bromide gradient at 40,000 rev/min for 40 hours. A single peak of DNA banding at a density of 1.59 to 1.60 g/cm^3, corresponding to the known density of mammalian closed circular supercoiled mtDNA (*14*), was obtained. Fractions constituting this peak were combined, the ethidium bromide was extracted with isopropyl alcohol saturated with H_2O and CsCl, and any residual noncovalently bound [^{3}H]BPDE was removed by repeated extractions with ethyl acetate until the mtDNA had a constant specific activity. The mtDNA was then dialyzed overnight against buffer containing 2 m*M* NaCl, 0.1 m*M* EDTA, 2 m*M* tris-HCl (*p*H 7.8) and lyophilized. The extent to which mtDNA was modified by [^{3}H]BPDE was also calculated from the ratio of ^{3}H to ^{13}C.

Cell culture	Residues, [^{3}H]BPDE bound per 10^6 bases		Ratio of mtDNA to nDNA
	nDNA (No.)	mtDNA (No.)	
10T^1/$_2$	12	766	84
L cells	3	126	42
K-16	10	900	90

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