DNA polymerases from other species. Table 1 indicates that the antibody is active at a 1 : 16 dilution against the 3.3*S* DNA polymerase purified from rabbit bone marrow. This is a rather striking result since the antibody was produced against calf thymus protein in a rabbit, that is, the animal produced an antibody that will react quite specifically with one of its own intracellular proteins. Other entries in Table 1 demonstrate that both forms of cellular DNA polymerase found in rat liver, mouse L cells, phytohemagglutinin-stimulated human lymphocytes (*5*), and rat liver mitochondrial DNA polymerase are inhibited by rabbit antibody to calf thymus 6*S* to 8*S* DNA polymerase. The last four entries confirm the specificity of this antibody in not inhibiting *E. coli* DNA polymerase I, and the converse, that rabbit antiserum to *E. coli* polymerase I serum does not inhibit calf thymus 6*S* to 8*S* DNA polymerase. Others (*6*) have demonstrated that *E. coli* polymerase II is not inhibited by antiserum to *E. coli* polymerase I, Table 1 shows that antibody to calf thymus 6*S* to 8*S* does not inhibit *E. coli* polymerase II. This confirms the specificity of the cross-reactivity of antibody to calf DNA polymerase with mammalian DNA polymerases.

The titrations of antibody to calf 6*S* to 8*S* polymerase have also been carried out with fixed amounts of antibody and variable amounts of antigen (6*S* to 8*S* calf thymus polymerase). Antigen-antibody precipitates were removed by centrifugation, and the supernatant fractions were assayed for DNA polymerase activity. The results confirmed the validity of fixed antigen-variable antibody titrations and also indicated that a precipitating antibody was present.

The final question to be answered is whether the antibody preparation contains two different populations of antibodies, one active against 6*S* to 8*S* polymerase and a second active against the 3.3*S* enzyme, or whether the same population of antibody molecules is active against both enzymes. To test these alternatives we mixed the antibody preparation with calf thymus 6*S* to 8*S* polymerase, and the mixture was centrifuged. The supernatant was then tested for activity against rabbit bone marrow and calf thymus 3.3*S* polymerase. The supernatants were depleted of antibody to 3.3*S* polymerase in proportion to the depletion of the antibody to calf 6*S* to 8*S* polymerase. We conclude that one antibody population is reacting with all forms of polymerase present.

Antiserums have been prepared against *E. coli* DNA polymerase I (*7*), T_2 DNA polymerase (*7*), Shope fibroma virus (*8*), and herpesvirus (*9*). In each instance the antiserum has been reported to be specific for its specific antigen (DNA polymerase) and no cross-reactions have been reported. The results that we have presented would not have been predicted on the basis of these earlier immunological studies. In retrospect it does seem reasonable that enzymes responsible for chromosome replication might be rather invariant in evolutionary time, particularly in determinant regions related to catalytic activity.

LUCY M. S. CHANG
F. J. BOLLUM
Departments of Biochemistry and *Oral Biology, University of Kentucky Medical Center, Lexington 40506*

References and Notes

1. M. Yoneda and F. J. Bollum, *J. Biol. Chem.* **240**, 3385 (1965).
2. L. M. S. Chang and F. J. Bollum, *ibid.* **246**, 909 (1971).
3. ———, *ibid.*, p. 5835; A. Weissbach, A. Schlabach, B. Fridlender, A. Bolden, *Nature* **231**, 167 (1971); E. F. Baril, O. E. Brown, M. D. Jenkins, J. Laszlo, *Biochemistry* **10**, 1981 (1971); J. T. Bellair, *Biochem. Biophys. Acta* **161**, 119 (1968); N. B. Furlong, *Texas Rep. Biol. Med.* **29**, 75 (1971); R. Howk and and T. Y. Wang, *Arch. Biochem. Biophys.* **133**, 238 (1969).
4. R. R. Meyer and M. V. Simpson, *Proc. Nat. Acad. Sci. U.S.* **61**, 130 (1968).
5. L. M. S. Chang and F. S. Bollum, unpublished results.
6. R. E. Moses and C. C. Richardson, *Biochem. Biophys. Res. Commun.* **41**, 1557 (1970); T. Kornberg and M. L. Gefter, *Proc. Nat. Acad. Sci. U.S.* **68**, 761 (1971).
7. H. V. Aposhian and A. Kornberg, *J. Biol. Chem.* **237**, 519 (1962).
8. L. M. S. Chang and M. E. Hodes, *Biochem. Biophys. Res. Commun.* **31**, 545 (1962).
9. H. M. Keir, H. Subak-Sharpe, W. I. H. Shedden, D. H. Watson, P. Wildy, *Virology* **30**, 154 (1966).
10. The antiserum was provided by Drs. L. Bertsch, and A. Kornberg. The *E. coli* polymerase I was obtained from Worthington Biochemical Corp. The *E. coli* polymerase II was a gift from Dr. C. C. Richardson (*6*); the DNA polymerase from rat liver mitochondria was fraction II (*4*).
11. Supported by grant CA 08487 from the National Cancer Institute. We are indebted to Dr. Thomas Roszman for many consultations on immunological problems. Dr. R. C. Gallo generously provided human PHA lymphocytes.

12 October 1971

Carcinoembryonic Antigen Present in Human Colonic Neoplasms Serially Propagated in Hamsters

Abstract. *Carcinoembryonic antigen, as measured by radioimmunoassay, is present in two different human colonic tumors that have been serially transplanted and maintained in the cheek pouches of unconditioned, adult golden hamsters. This finding shows that a human tumor-associated antigen can be produced in an animal host.*

Gold and Freedman have described (*1*) an antigenic material specific for embryonic digestive tissue and entodermally derived neoplasms of the gastrointestinal tract. This material, named carcinoembryonic antigen (CEA), has been used in the search for a practical means of cancer diagnosis and prognostication (*2*, *3*). One major limitation in the use of this antigen for sensitizing animals, in diagnostic tests, and in the study of its chemical nature and biogenesis has been the difficulty of procuring it from metastatic gastrointestinal cancers and the variability of its activity. The availability of almost unlimited supplies of two human, mucin-producing, colonic tumors (GW-39 and GW-77) growing in the cheek pouches and the hind leg musculature of unconditioned, adult golden hamsters (*4*, *5*) has prompted us to determine whether these implanted tumors might be a good source of consistently large quantities of CEA with relatively uniform activity. The presence of CEA in xenografted tumors would be an indication that such tumor-associated antigens are

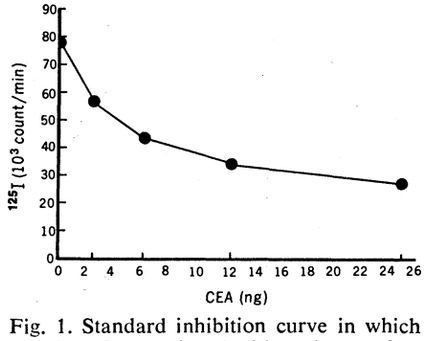

Fig. 1. Standard inhibition curve in which [^{125}I]CEA is complexed with antiserum free after incubation with known quantities of CEA.

indeed indigenous to the tumor cells and relatively independent of the host organism.

The GW-39 and GW-77 tumors growing in the cheek pouches of golden hamsters were excised, and 1 g of each was homogenized in 19 (GW-39) or 9 (GW-77) volumes of distilled water. The homogenate was centrifuged at 12,000*g* for 30 minutes. The supernatant was assayed for antigen content. Three other heterotransplantable tumors derived from human surgical tumor specimens, but which were found to be highly malignant in hamsters, were used as controls. These three tumors had species-specific properties more consistent with hamster than with human tissue (GW-176, originally a gastric carcinoma; GW-365, originally an adenocarcinoma of the lung; and GW-478, originally a gastric lymphoma) (*6*). The radioimmunoassay developed by Hansen *et al.* (*3, 7*) was used to detect CEA. Briefly summarized, known quantities (0 to 25 ng) of CEA diluted in 10 ml of 0.01*M* ammonium acetate (*p*H 6.8) were incubated with 0.1 ml of a 1 : 2500 dilution of goat antiserum to CEA (*8*) for 30 minutes at 45°C. A portion (2.4 ng) of [^{125}I]CEA, prepared and labeled according to the method of Thomson *et al.* (*9*), was then added to complex (with the antiserum in excess) after the first incubation with unlabeled CEA, and again incubated as before. The reaction was stopped by addition of 5 ml of zirconyl phosphate gel (*10*), which adsorbs the [^{125}I]CEA-antigen-antibody complex at *p*H 6.25, but not the free [^{125}I]CEA. The antigen-antibody complex was then sedimented by centrifugation, washed in 0.1*M* ammonium acetate buffer (*p*H 6.25), and centrifuged again. The pellet was used for counting radioactivity in a Packard gamma scintillation counter. The lower the counts, the greater is the amount of antiserum to CEA that is complexed by the original unlabeled CEA in the mixture, and is thus unavailable for precipitation with [^{125}I]CEA. Hence radioactivity (counts per minute) and quantity of CEA (nanograms) are inversely proportional. A standard curve was then established with known quantities of CEA (Fig. 1); the amount of antigen present in our tumor samples is deducible from the ^{125}I (counts per minute) values obtained in a repetition of the assay.

Table 1 provides the final average values of CEA per gram of each of the five tumors examined. The high amount of CEA in the GW-39 and GW-77 neoplasms not only agrees with other evidence that they retain properties characteritsic of human cells despite their long-term propagation in hamsters (*4, 11*), but supports their high degree of functional differentiation in this animal model. Our repitition of this assay for CEA in GW-39 and GW-77 tumors of other transplant generations has confirmed these results. Conversely, the lack of CEA in the other three neoplasms does not permit us to decide whether they are human, hamster, or human-hamster hybrid tumors.

Table 1. Average CEA content of five tumors of human origin continuously propagated in hamster cheek pouches.

Tumor	Passage No.	CEA content (μg/g)
GW-39	94	162
GW-77	102	41
GW-176	234	0
GW-365	155	0
GW-478	118	0

Our radioimmunoassay for CEA is not identical to that used by Thomson *et al.* (*9*), since we are only measuring an ion-sensitive site on CEA (*7*). This antigenic site has been found *not* to be restricted to gastrointestinal cancers (*3*). In colonic tumors, however, it can be considered as identical with Gold and Freedman's CEA. It thus appears that our GW-39 and GW-77 tumors contain CEA and an antigenic site on CEA similar to that found in many different human cancers (*3*). The capacity of these tumors to continue to produce such antigens when serially grafted in xenogeneic hosts constitutes strong evidence that these antigens are indeed specific to the tumors themselves and not products of any peculiar interaction between tumor and host. Accordingly, our results do not support the view of Apffel and Peters (*12*) that such tumor-specific antigens as CEA are "neither original constituents nor products of tumor cells," but glycoproteins produced by the liver (so-called "symbodies") in response to the tumor and coating the tumor cells. In order for us to accept this thesis, we would be obliged to prove the production of human glycoproteins in hamster hosts. The presence of a human tumor-associated antigen in our tumor xenografts introduces a new aspect to heterotransplantation of human tumors, and perhaps also a unique approach to studying the biogenesis of such human tumor-associated antigens in an animal model.

DAVID M. GOLDENBERG
Department of Pathology, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140
HANS J. HANSEN
Department of Biochemical Nutrition, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

References and Notes

1. P. Gold and S. O. Freedman, *J. Exp. Med.* **121**, 439 (1965); *ibid.* **122**, 467 (1965).
2. S. O. Freedman, P. Gold, J. Krupey, H. da C. Machado, *Can. Cancer Conf.* **8**, 407 (1969).
3. P. LoGerfo, J. Krupey, H. J. Hansen, *N. Engl. J. Med.* **285**, 138 (1971).
4. D. M. Goldenberg, *Arch. Geschwulstforsch.* **29**, 1 (1967).
5. ———, S. Witte, K. Elster, *Transplantation* **4**, 760 (1966); D. M. Goldenberg and E. R. Fisher, *Brit. J. Cancer* **24**, 610 (1970).
6. D. M. Goldenberg, *Int. Cancer Congr. 10th, Houston* (1970), abstr., p. 316; ——— and E. R. Fisher, *Proc. Amer. Ass. Cancer Res.* **10**, 30 (1969); D. M. Goldenberg, E. R. Fisher, J. C. Sieracki, R. Bhan, *ibid.* **11**, 30 (1970).
7. H. J. Hansen, K. P. Lance, J. Krupey, *Clin. Res.* **19**, 143 (1971).
8. H. J. Hansen, J. J. Hager, H. Cohen, J. Krupey, *ibid.*, p. 53.
9. D. M. P. Thomson, J. Krupey, S. O. Freedman, P. Gold, *Proc. Nat. Acad. Sci. U.S.* **64**, 161 (1969).
10. H. J. Hansen, O. N. Miller, C. H. Tan, *Amer. J. Clin. Nutr.* **19**, 10 (1966).
11. D. M. Goldenberg, R. D. Bhan, R. A. Pavia, *Proc. Soc. Exp. Biol. Med.* **135**, 657 (1970).
12. C. A. Apffel and J. H. Peters, *Progr. Exp. Tumor Res.* **12**, 1 (1969).

4 October 1971

Electric Enhancement of Bone Healing

Abstract. *A human congenital pseudarthrosis of the tibia, unresponsive to conventional treatment, was stimulated to healing by direct electric current. The method was modeled after prior experimental work in vivo in rabbits. X-ray photographs, histological techniques, and electron microscopy confirmed the presence of newly formed bone in the defect region.*

Recent experiments (*1–7*) dealing with electrical stimulation of bone tissue may be divided into two types. *Electrode-sensitive* experiments (*3, 8–10*), which tend to be equivocal, relate to remodeling effects at the electrode sites with accretion occurring at the negative electrode and resorption at the positive. Other investigators have performed the *current-sensitive* experiments (*1, 4, 7*) which evaluate the effects of current in promoting healing of lesions placed *between* the electrodes. The latter method prevents misinterpre-