secondary effect of the high estrogen concentration observed in these animals, as suggested by King *et al.* (*14*). Alternatively, the increased cytoplasmic receptors may mean that there is a block in the translocation of estrogen receptors from cytoplasmic to nuclear sites with a subsequent buildup of receptors in the cytoplasm (*15*). The preliminary finding of significantly decreased nuclear binding (50 percent) in phenobarbital-treated animals supports the latter possibility.

Other long-term effects of phenobarbital exposure in utero have been suggested by several investigators (*16–22*). Middaugh *et al.* (*19*) found that the development of several neurologic reflexes was retarded in mice, and an increased incidence of congenital malformations was reported in humans (*21, 22*). Recently, a defect in sexual mating behaviors was found in rats exposed to this drug after birth (*6*).

The clinical significance of our results is unknown, but the striking and permanent effects of phenobarbital on reproductive function in animals suggest that this drug should be used with great care in pregnant humans. Many of the other psychotropic agents taken by women during pregnancy may also have delayed effects on the biologic functions of their offspring.

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DNA Polymerases in Parasitic Protozoans Differ from Host Enzymes

Abstract. *Analysis of extracts of the bloodstream forms of* Trypanosoma brucei *showed that both DNA polymerase-α and DNA polymerase-β activities were present. The detection of DNA polymerase-β in* T. brucei *demonstrates the presence of this enzyme in unicellular organisms. DNA polymerase-β is present also in* Leishmania mexicana. *The DNA polymerases in* T. brucei *are immunologically distinct from the host enzymes. The structural differences between the parasite and the host enzymes could be exploited for the development of agents to combat parasitic diseases.*

The existence of DNA polymerase-β (*1*) in animal, plants, and bacteria has been surveyed (*2*). DNA polymerase-β is found only in multicellular animal cells and is not present in free-living protozoans (*3, 4*), yeast (*5*), and slime molds (*6*), and it has not been detected in plants or in bacteria.

Relatively little information exists concerning the molecular biology and biochemistry of parasitic protozoa, which are generally found in a protected environment within the animal host or insect vector and contain the causative agents of diseases in both humans and domestic animals. *Trypanosoma brucei*, for example, is a flagellate protozoan that causes trypanosomiasis in livestock and is closely related to the trypanosomes responsible for human sleeping sickness.

One approach to combating parasitic diseases is to augment the host's immunological defense mechanisms. Another is to administer drugs targeted against specific enzymes and proteins of the parasites (*7*). In trypanosomiasis, large quantities of parasites can be obtained for exploratory biochemical studies by infecting mice or rats with *T. brucei*. We therefore studied enzymes involved in nucleic acid metabolism in *T. brucei* in an attempt to detect differences between the protozoan and mammalian enzymes. We now report the presence of both DNA polymerase-α and DNA polymerase-β in extracts of *T. brucei* (bloodstream form) and show that the enzymes in the parasite are distinct from the host enzymes.

Soluble extracts of *T. brucei* (*8*) were prepared, separated on sucrose gradients, and analyzed for DNA polymerase activities (*9*). The sucrose gradient profile of DNA polymerase activity in *T. brucei* is presented in Fig. 1. Both DNA polymerase-α and DNA polymerase-β are present, and the sedimentation characteristics of each are similar to that for the corresponding mammalian enzyme (*2*).

The detection of DNA polymerase-β activity in *T. brucei* is unusual, and it is necessary to demonstrate that the enzymes extracted from the purified *T. brucei* are not residual host enzymes, that is, rat DNA polymerase-α and rat DNA polymerase-β. This demonstration can be made with antiserums to rat DNA polymerases. The antibody for DNA polymerase-α is a rabbit immunoglobulin G fraction with broad specificity (*10*). This antiserum was prepared against a partially purified polymerase-α from calf thymus terminal deoxynucleotidyltransmerase-α from other mammalian sources as well as mammalian DNA polymerase-β. This antiserum is inactive against DNA polymerase-α from sea urchin, calf thymus terminal deoxynucleotidyltransferase, DNA polymerase-γ from rat liver, and *Escherichia coli* DNA polymerase I. The antiserum to DNA polymerase-β used in this study was obtained by immunization of a rabbit with homogeneous DNA polymerase-β from calf thymus (*11*). The antiserum to DNA polymerase-β inhibits DNA polymerase-β from all mammalian sources tested. However, the titer is lower for DNA

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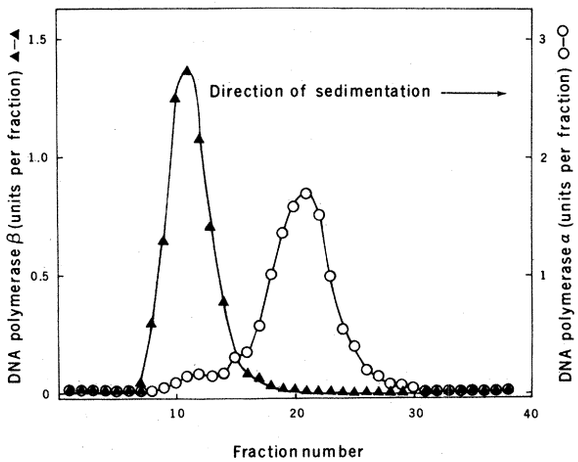

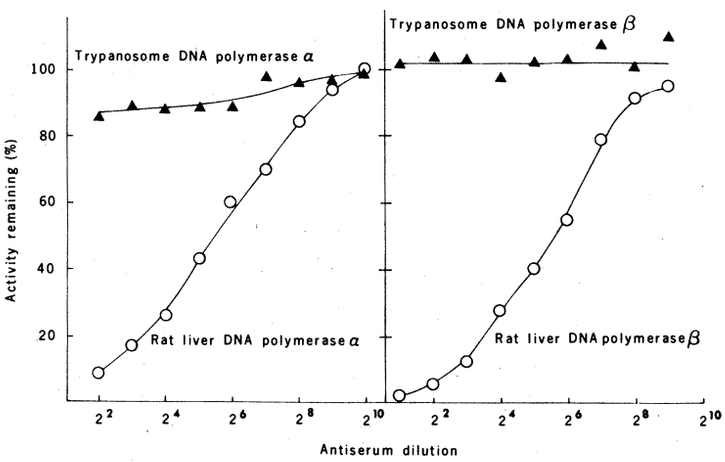

Fig. 1 (left). Sucrose gradient profile of DNA polymerases from *Trypanosoma brucei*. A soluble extract of *T. brucei* was analyzed for DNA polymerases before and after sucrose gradient fractionation (*2*). The amounts of enzymes present in *T. brucei* were 19 units of DNA polymerase-α and 6 units of DNA polymerase-β per 10^9 organisms. One unit of enzyme represents the polymerization of 1 nmole of ^{3}H-labeled deoxythymidine triphosphate into acid-insoluble material per hour in an activated DNA-primed reaction. The recovery of DNA polymerase-α activity from the sucrose gradient was 67 percent, and the recovery of DNA polymerase-β activity was 114 percent. Fig. 2 (right). Effects of antiserums to mammalian DNA polymerases on *T. brucei* DNA polymerases. The undiluted antiserum to DNA polymerase-α was an immunoglobulin G fraction containing 30 mg of protein per milliliter. The undiluted antiserum to DNA polymerase-β was obtained from rabbits. The antiserums were diluted with bovine serum albumin (1 mg/ml) in phosphate buffered saline. Enzyme purified on a sucrose gradient was first incubated with diluted antiserum for 16 hours at 4°C. Enzyme activities remaining were detected as described (*2*). The amounts of enzymes used were (in units) 0.06, 0.04, 0.17, and 0.14 for rat liver DNA polymerase-α, *T. brucei* DNA polymerse-α, rat liver DNA polymerase-β, and *T. brucei* DNA polymerase-β, respectively.

polymerase-β from nonmammalian vertebrates than for mammalian vertebrates. The antiserum to DNA polymerase-β has no effect on calf thymus terminal deoxynucleotidyltransferase, DNA polymerase-α, or DNA polymerase-γ.

The neutralization effect of the two antiserums on the *T. brucei* DNA polymerase-α and -β, and on control mammalian DNA polymerase-α and -β isolated from the liver of a 9-day-old rat are shown in Fig. 2. A slight inhibition of *T. brucei* DNA polymerase-α is seen at high concentrations of antiserum to DNA polymerase-α. This can be explained by the broad specificity of the antiserum to the DNA polymerase-α. Nevertheless, the data in Fig. 2 demonstrate that the *T. brucei* DNA polymerase-α differs from the rat liver DNA polymerase-α. A more dramatic effect was seen in the comparison of the effects of antiserum to mammalian DNA polymerase-β on the *T. brucei* and rat DNA polymerase-β. No inhibition of *T. brucei* enzyme was observed. Since the antiserum also inhibits DNA polymerase-β from nonmammalian sources (fish and chicken), the lack of inhibition of DNA polymerase-β from *T. brucei* indicates that the antigenic structure of the enzyme in this organism is different from that of the host enzyme.

It was previously reported (*12*) that *T. brucei* contains DNA polymerase-α activity that is not inhibited by an antiserum to HeLa cell DNA polymerase-α; however, no DNA polymerase-β activity was detected in that investigation. In our study substantial *T. brucei* DNA polymerase-β activity was demonstrable under the assay conditions used.

The presence of DNA polymerase-β in parasitic protozoa was further substantiated by analysis of amastigote and promastigote forms of *Leishmania mexicana* (*13*). Both forms of *L. mexicana* have a low-molecular-weight. *N*-ethylmaleimide-resistant DNA polymerase. The presence of DNA polymerase-β in *L. mexicana* promastigotes, which normally grow and divide within the gut of the insect vector, is of interest since the insect host does not have a typical DNA polymerase-β. The only multicellular animals in which DNA polymerase-β has not been detected are the Insecta, phylum Arthropoda. A low-molecular-weight DNA polymerase (< 50,000 daltons) is present in insect extracts, but this activity is *N*-ethylmaleimide-sensitive (*2*) and is therefore distinct from the activity demonstrated in the parasite.

It would be of interest to find out whether DNA polymerase-β is present in all the parasitic protozoa that require both insect and mammalian hosts to complete their life cycles. While the *T. brucei* DNA polymerases have certain characteristics in common with their mammalian counterparts (such as comparable in vitro assay conditions, molecular weight, *N*-ethylmaleimide sensitivity), the lack of immunological cross-reactivity between the parasite and host enzymes suggests real differences in protein structure. The structural differences between the parasite and the host enzymes provide a conceptual basis for designing therapeutic agents targeted against the parasite's enzymes (*7*).

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