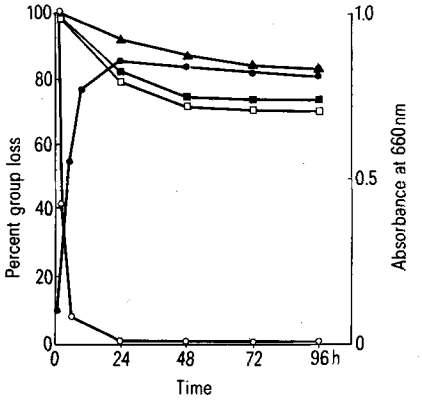

Growth of strain No. 21 *C. perfringens* and degradation of ^{3}H TC; ●——● absorbance of culture at 660 nm, ○——○ deconjugation of TC, ■——■ loss 3α-OH groups, □——□ apparent loss of 7α-OH groups and △——△ loss of tritium in crude spent bacterial medium.

However, about 36% of the tritium was lost on formation of 7α-, 12α-dihydroxy-3-keto-5β-cholanoate in vitro or in whole-cell *C. perfringens* cultures (table 2). The tritium loss (previously noted[14]) could be followed during the growth of *C. perfringens* cultures in TC-containing medium (figure). The appearance of this compound was verified by TLC. (Artifactual formation of methyl esters previously observed[14] was avoided by extraction at pH 3 instead of pH 1). Additionally, the loss of 3α-OH groups and apparent loss of 7α-OH groups (associated with the relative unreactivity of *E. coli* 7α-HSDH against this oxidation product[3]) closely paralleled the loss in label. Although the yield of 7α-, 12α-dihydroxy-3-keto-5β-cholanoate differed considerably from one strain to another, the percentage loss of tritium calculated from the ^{14}C/^{3}H ratio remained constant (table 2). Small losses of tritium were encountered in the remaining cholate in whole-cell cultures; none was measurable in vitro.

The discrepancy between the tritium lost from cholate by *C. perfringens* and that lost by *P. testosteroni* or *E. lentum* 3α-HSDH was rationalized by a stripping of protons from other sites on the steroid (possibly α- to C_3 position) on contact with the *C. perfringens* enzyme. These results support the conclusions of Panveliwalla et al.[11] in not recommending generally tritiated bile salt for human kinetic studies. Because of greatly differing *C. perfringens* populations in the human intestine[16], tritium loss, by this mechanism alone, could introduce a sizable and variable error in pool size estimation.

1 This work is supported by the National Cancer Institute.
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Absence of low molecular weight DNA polymerase activity from the nuclei of *Amoeba discoides*

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Summary. Amoeba discoides nuclear protein partially purified by passage through Sephadex G-200 showed 3 high-mol.-wt DNA polymerase activities which eluted in and just following the void volume. No low-mol.-wt (45,000 daltons) DNA polymerase β activity was detected. Nuclear protein layered on 5-20% sucrose gradients also showed an absence of low-mol.-wt DNA polymerase β. The void volume enzyme showed deoxyribonuclease activity, but no low-mol.-wt nuclease activity was detected.

DNA polymerase activity is found in many cellular structures[1]. A high-mol.-wt DNA polymerase found in the cytoplasm, DNA polymerase α[2] is the predominant activity found in growing cells[3-5], while DNA polymerase β is a well-characterized low-mol.-wt activity in the nuclei of many higher organisms. We wish to report the absence of a low-mol.-wt DNA polymerase activity from the nucleus of the large mononucleate Protozoan, *Amoeba discoides*.

Materials and methods. A. discoides ($_{T1}D_{13}$) were grown in mass cultures and nuclei were obtained as described previously[6]. Tritium-labelled DNA was obtained from *Tetrahymena pyriformis*[7] grown in proteose-peptone containing 2 µCi/ml ^{3}H-thymidine. Gel filtration with Sephadex G-200 and running buffer (20 mM Tris-HCl, pH 8.1, 1 mM EDTA, 1 mM β-mercaptoethanol, 0.02% sodium azide), and centrifugation on linear sucrose density gradients (5-20% sucrose in 50 mM Tris-HCl, pH 7.8, 1 mM EDTA, 1 mM β-mercaptoethanol, 50 mM KCl, 0.02% sodium azide) were carried out. The DNA polymerase assay composition was usually 50 mM Tris-HCl, pH 8.1, 10 mM $MgCl_2$, 30 mM KCl, 1 mM β-mercaptoethanol, 0.8 mM EDTA, 0.1 mM each of dATP, dGTP, dCTP, 0.1

mM ^{3}H-TTP and 0.016% sodium azide. The template was either 'activated' calf thymus DNA[8] or heat-denatured calf thymus DNA, and the assay time, 1 h at 37 °C. The assay conditions for deoxyribonuclease activity were the same as for the DNA polymerase activity, except for the absence of calf thymus DNA and deoxyribonucleotides. The nuclease activity substrate was ^{3}H-DNA from *Tetrahymena pyriformis*.

Results. Nuclei from *A. discoides* suspended in running buffer and disrupted by sonication were passed through Sephadex G-200. 3 high-mol.-wt DNA polymerase activities were found using 'activated' calf thymus DNA as template, and 2 when using heat-denatured DNA (figure 1). No incorporation into acid-precipitable material was found in any other fractions, for instance, fractions 29-32 where DNA polymerase β (45,000 daltons) might be expected to elute.

Nuclear preparations were layered onto 5-20% sucrose density gradients, centrifuged at 105,000 × g for 18 h and fractions examined for DNA polymerase activity. Activity was found only in the regions of the gradient co-incident with the γ-globulin marker (160,000 daltons) and none was found in the region of the ovalbumin marker (45,000 daltons).

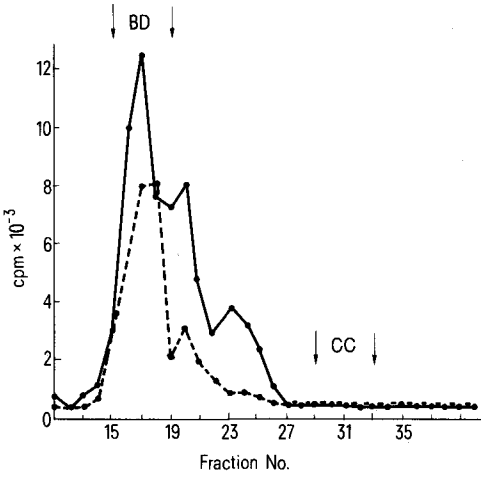

Fig. 1. DNA polymerase activity in *A. discoides* nuclear protein after passage through Sephadex G-200. Assay conditions as described in 'Materials and methods'. The fractions in which Blue Dextran (BD) and Cytochrome C (CC) eluted in an independent passage are indicated. ●——●——●, 'activated' DNA template; ●·····●·····●, heat-denatured DNA template.

The possibility that a low-mol.-wt nuclear deoxyribonuclease activity co-eluted with low-mol.-wt DNA polymerase activity and rendered its acid-precipitable DNA product too small for retention on glass fibre filters (GF/C) was examined, using ^{3}H-DNA from *Tetrahymena pyriformis* as substrate. A void volume deoxyribonuclease activity was shown by the rendering of the ^{3}H-DNA to an acid-soluble product (figure 2). This deoxyribonuclease activity co-eluted on Sephadex G-200 with the void volume DNA polymerase activity. No low-mol.-wt deoxyribonuclease activity was found.

Discussion. In a study of the phylogeny of DNA polymerase β, it has been shown that, with the possible exception of the Insecta, invertebrates possess DNA polymerases that are similar to those of vertebrates[9]. Among the Protozoa, no low-mol.-wt DNA polymerase β activity has been reported from a range of cells including *Dictyostelium discoideum*[10], *Euglena gracilis*[11], *Paramecium aurelia*[12], *Tetrahymena pyriformis*[13] and *Saccharomyces cerevisiae*[14]. Our failure to detect low-mol.-wt DNA polymerase activity in *A. discoides* is not inconsistent with the data available for other unicellular organisms. The 3-Os breakdown product of DNA polymerase B of *Euglena gracilis*[15] was shown to be sensitive to N-ethylmaleimide as the native enzyme, and therefore does not satisfy the criteria used to define DNA polymerase β activity.

Our studies have not as yet determined the nature of the nuclease activity which co-eluted with our partially-purified void volume nuclear polymerase activities. Nuclease activity is shown with DNA polymerase B of *E. gracilis* but not with DNA polymerase A[15]. The highly purified single polypeptide DNA polymerase of *T. pyriformis* has nuclease (and nucleoside diphosphokinase) activity[13], while enzyme II of *S. cerevisiae* can carry out a template-dependent deoxyribonucleoside triphosphate degradation reaction[16]. Polymerase activity associated with nuclease activity is not reported among higher eukaryotes which possess DNA polymerase β activity. It is possible that during the evolution of the Metazoa from the Protozoa, the aquisition of DNA polymerase β activity led to a loss of polymerase-associated nuclease activity, but the significance of this change is not known.

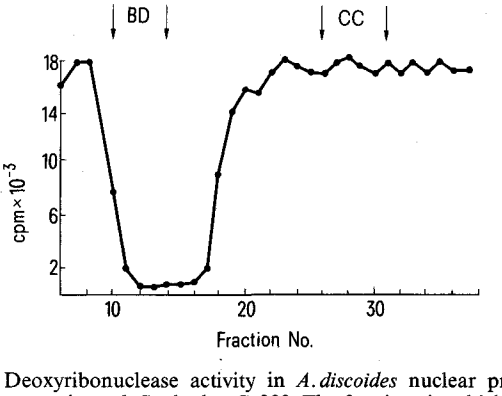

Fig. 2. Deoxyribonuclease activity in *A. discoides* nuclear protein after passage through Sephadex G-200. The fractions in which Blue Dextran (BD) and Cytochrome C (CC) eluted in an independent passage are indicated. Assay conditions as described in 'Materials and methods', using ^{3}H-DNA from *Tetrahymena pyriformis* as substrate.

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