

# MASTER

## DNA REPLICATION AND THE REPAIR OF DNA STRAND BREAKS IN NUCLEI OF PHYSARUM POLYCEPHALUM

### PROGRESS REPORT

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— Principal Investigator: E.N. Brewer, Ph.D.  
Co-Investigator: O.F. Nygaard, Ph.D.  
Research Associate: G. Kuncio, Ph.D.

Division of Radiation Biology  
Department of Radiology  
Case Western Reserve University  
Cleveland, Ohio 44106

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DNA REPLICATION AND THE REPAIR OF DNA STRAND  
BREAKS IN NUCLEI OF PHYSARUM POLYCEPHALUM

Abstract

Isolated nuclei and intact plasmodia of Physarum contain a heat-stable stimulator of nuclear DNA replication. This substance has been purified extensively and found to contain both protein and carbohydrate. The molecular weight, estimated by gel filtration, is ca. 30,000 d. The purified material does not exhibit DNA polymerase or DNase activity, and does not stimulate DNA polymerase activity per se. In the presence of the stimulatory factor, DNA chain elongation occurs at an elevated rate, and continues for a longer time than in its absence, but  $G_2$  nuclei are not stimulated to initiate DNA synthesis.

Double-strand breaks in nuclear DNA of irradiated plasmodia are repaired in vitro to a greater extent following nuclear isolation during  $G_2$ , and the DNA of unirradiated plasmodia is less susceptible to double-strand breakage during cell-free nuclear incubation, than is the DNA of S-phase nuclei. This correlation suggests a common basis for both observations, for example an increase in deoxyribonuclease activity or a decrease in DNA ligase activity during the S period. This, in turn, may account for the cell cycle-dependent sensitivity of this organism, in terms of mitotic delay, to ionizing radiation.

Exposure of plasmodia to 4-nitroquinoline-N-oxide results in substantial DNA single-strand breakage. Strand breaks are produced at all times of the division cycle within 1 hour of exposure to the carcinogen at  $10^{-4}$  M. However, breaks are repaired to a greater extent when exposure to the carcinogen occurs during the  $G_2$  period. These results are similar to those found for exposure of the organism to ionizing radiation.

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E.N. Brewer, Ph.D., Principal Investigator

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O.F. Nygaard, Ph.D., Co-investigator

Time devoted to project since the beginning of the current term of agreement: 5%  
Time expected to be devoted to project during the remainder of the current term: 5%

G. Kuncio, Research Associate

Time expected to be devoted to project during the remainder of the current term: 100%

DNA Replication and the Repair of DNA Strand Breaks  
in Nuclei of Physarum polycephalum

The processes of eukaryotic DNA replication and repair would be better understood if the molecular species which participate in these events could be identified. For this reason we have continued our investigations of the biochemical requirements for nuclear DNA replication and repair in isolated nuclei:

(A) The heat-stable factor capable of stimulating DNA replication in isolated nuclei of Physarum has been purified considerably. The factor appears to be a glycoprotein, possibly of nuclear origin. Both the rate and overall extent of DNA synthesis are increased in the presence of the stimulatory material.

(B) The lesser degree of repair of radiation-induced DNA double-strand breaks in nuclei isolated from S-phase plasmodia, as compared to G<sub>2</sub>-phase plasmodia, occurs also in unirradiated cultures: i.e., DNA of nuclei isolated from unirradiated S-phase cultures undergoes a significantly greater degree of strand breakage during in vitro incubation than does that of G<sub>2</sub>-phase nuclei.

The possible relationship between these two phenomena is currently under investigation. (C) We have initiated a study of DNA strand breakage and repair, in vivo, resulting from the effects of environmental carcinogens.

Preliminary results indicate that 4-nitroquinoline-N-oxide produces substantial numbers of DNA single strand breaks (or alkali-labile bonds) in Physarum. These breaks are repaired to a greater extent when exposure to this carcinogen occurs during the G<sub>2</sub> phase of the mitotic cycle.

These results are described in greater detail below.

(A) Stimulation of nuclear DNA replication by a glycoprotein material extracted from nuclei and plasmodia of Physarum.

Many of the proteins which are involved in the replication and repair of the prokaryotic genome have been identified (Alberts and Sternglanz,

Nature, 269, 655-661, 1977). It is likely that the DNA replication and repair processes in eukaryotic organisms are at least as complex, involving a host of gene products. However, with the possible exception of DNA polymerase  $\alpha$  (Chiu and Baril, J. Biol. Chem., 250, 7951-7957, 1975; Bertazzoni et al., Proc. Natl. Acad. Sci. U.S., 73, 785-789, 1976), the proteins, as well as substances other than DNA and protein, which play a role in these processes are unknown. Some progress has been made toward a solution to this problem, notably in G.C. Mueller's laboratory (Thompson and Mueller, Biochim. Biophys. Acta, 414, 231-241, 1975; Seki and Mueller, Biochim. Biophys. Acta, 435, 236-250, 1976). In addition, the recent success in obtaining limited DNA synthesis in isolated chromatin preparations (Su and DePamphilis, Proc. Natl. Acad. Sci. U.S., 70, 3466-3470, 1976; Knopf and Weissbach, Biochemistry, 16, 3190-3194, 1977; Edenberg, Waqar and Huberman, Nucl. Acids Res., 4, 3083-3095, 1977) and in soluble nuclear extracts (Yamashita, Arens, and Green, J. Biol. Chem., 252, 7940-7946, 1977; Kaplan, Kleinman, and Horwitz, Proc. Natl. Acad. Sci. U.S., 74, 4425-4429, 1977) suggests that nuclear subfractionation may be a valuable method for identifying both chromatin and non-chromatin nuclear constituents which may participate in the DNA replication and repair processes.

We have reported previously the extraction of a DNA polymerase activity from the nuclear fraction of Physarum homogenates (Brewer, Biochim. Biophys. Acta, 402, 363-371, 1975, COO-78-334), and of a heat-stable stimulator of DNA synthesis in well-washed nuclei (Annual Report, 1976-77, COO-2486-366). During the current contract period we have purified the latter substance extensively, and have found it to contain both protein and carbohydrate. For the sake of simplicity we shall refer to this material as a "glycoprotein", although we cannot rule out the possibility that the active principle is associated with only one of these two moieties.

The glycoprotein material is present at about the same level at all times of the mitotic cycle. The material does not trigger the initiation of DNA synthesis in  $G_2$ -phase nuclei, but increases the rate and overall extent of synthesis in S-phase nuclei. The size of DNA replication (Okazaki) intermediates is not greatly different in stimulated vs. unstimulated nuclei. Stimulatory activity is not associated with DNase or polymerase activity. The stimulatory activity present in plasmodial extracts is not affected significantly by treatment of intact plasmodia for 1 hr. with cycloheximide (10  $\mu$ g/ml). The active material has no effect on DNA polymerase activity assayed in the presence of an exogenous (salmon sperm) template.

~~These results are described in greater detail in the accompanying manuscript (Appendix A).~~

#### B. Rejoining of Gamma-Radiation-Induced DNA Double-Strand Breaks *in vitro*

The radiation sensitivity of Physarum, in terms of mitotic delay, is cell-cycle dependent (i.e., delay of mitosis is much greater when plasmodia are irradiated during early S phase, as compared with exposure during the  $G_2$  period (Nygaard, Brewer, Evans and Wolpaw, in Adv. Radiat. Res. Vol. 2, pp. 989-995, 1973, COO-78-221). This mitotic cycle dependence is closely associated with the ability of the organism to repair nuclear DNA strand breaks at different times in the division cycle: DNA repair is far more extensive during the  $G_2$  period than during S (Brewer and Nygaard, Nature New Biol., 229, 108-110, 1972, COO-78-267).

Radiation-induced DNA double-strand breaks are repaired in homogenates and isolated nuclei of Physarum. This cell-free repair activity shows the same mitotic cycle dependence as does strand break rejoining *in vivo* (Annual Report, 1976-1977, COO-2486-366). More recently, we have found that

the cell cycle-dependent repair of DNA double-strand breaks in vitro is paralleled by a difference in the susceptibility of nuclear DNA to the double-strand breakage which occurs during incubation of unirradiated nuclei: isolated nuclei (homogenates) obtained from early S cultures incur far more double-strand breaks during incubation in vitro than do nuclei obtained from  $G_2$  plasmodia (Appendix B).

The mechanism responsible for this phenomenon is, at present, unknown. It is possible that changes in the configuration of DNA or chromatin as the nuclei enter the S-phase render parental DNA strands more susceptible to endogenous endonuclease activity. Conversely, cell cycle-dependent changes in DNase or DNA ligase activities per se could account for the difference in DNA strand breakage (see Proposed Research).

Finally, owing to the present commercial availability of DNA markers suitable for use in both alkaline and neutral sucrose density gradients, we have been able to obtain a more reliable estimate of the molecular weights of single- and double-strand DNA molecules than was possible previously (McGrath and Williams, Biophys. J., 7, 309-317, 1967; Brewer, J. Mol. Biol., 68, 401-412, 1972, COO-78-260). The molecular weight of the double-strand DNA units of Physarum, as determined by neutral sucrose density gradient centrifugation, is approx.  $6.7 \times 10^7$  d, whereas the single-strand molecular weight obtained by alkaline sucrose density gradient centrifugation is approx.  $2.8 \times 10^7$  d. Hence, the single-strand molecular weight is approximately half the double-strand value, and the nuclear DNA of Physarum does not, therefore, appear to contain discontinuities as we suggested previously (op. cit.).

The results discussed in this section are presented in greater detail in the accompanying manuscript (Appendix B).

C. Production and Repair of DNA Strand Breaks by 4-Nitroquinoline-N-oxide.

We proposed previously to extend our studies of the biological effects of  $\gamma$ -radiation to include investigations of the production and repair of DNA strand breaks by various environmental pollutants. As a preliminary step in this study, we have tested the abilities of three well-known chemical carcinogens to produce DNA strand breaks in Physarum when present in the culture medium for prolonged periods (up to 18 h). Of the three chemicals tested, Adriamycin (5 $\mu$ g/ml) and nitrofurazone ( $10^{-3}$  M) produced no detectable DNA strand breaks as determined by alkaline sucrose density gradient centrifugation analysis. However, 4-NQO at  $10^{-5}$  M produced a significant number of DNA single-strand breaks. Strand breaks are produced within 1 hr of exposure to this compound at  $10^{-4}$  M (Fig. 1). Such breaks are repaired to greater extent when exposure occurs during the  $G_2$  period, as compared with the S period (Fig. 2). These results have encouraged us to pursue our investigation of the effects of environmental pollutants on nuclear DNA using 4-NQO as a model chemical carcinogen (see Proposed Research), as well as to determine the abilities of other chemical carcinogens and environmental agents to effect the production of DNA strand breaks in Physarum.

Figure 1. DNA strand breaks produced by exposure of intact plasmodia of Physarum to 4-nitro-quinoline-N-oxide. Plasmodia were labeled from the time of fusion with [ $^3$ H]thymidine ( $10\mu\text{Ci}/\text{ml}$ ), or [ $^4$ C]thymidine ( $0.4\mu\text{Ci}/\text{ml}$ ).  $^3$ H-labeled plasmodia in mid- $G_2$  (preceding the third synchronous mitosis after fusion of microplasmodia) were exposed to  $10^{-4}$  M 4-NQO for 1, 2, or 4 hrs.  $^{14}$ C-labeled plasmodia served as untreated controls. Portions (1/6 each) of the treated and untreated plasmodia were combined, nuclei were isolated, and alkaline sucrose density gradient centrifugation of the labeled DNA's carried out as described previously (Brewer, J. Mol. Biol., 68, 401-412, 1972, COO-78-260). Direction of sedimentation is from right to left.

Figure 2. Rejoining of DNA strand breaks produced by exposure to 4-NQO.  $[^3\text{H}]$ -labeled plasmodia were exposed to  $10^{-4}$  M 4-NQO for 1 hr. during either early S or mid- $G_2$ , then returned to fresh growth medium for a 2-hr recovery period. Other experimental details are as described for Fig. 1.

Figure 2a.

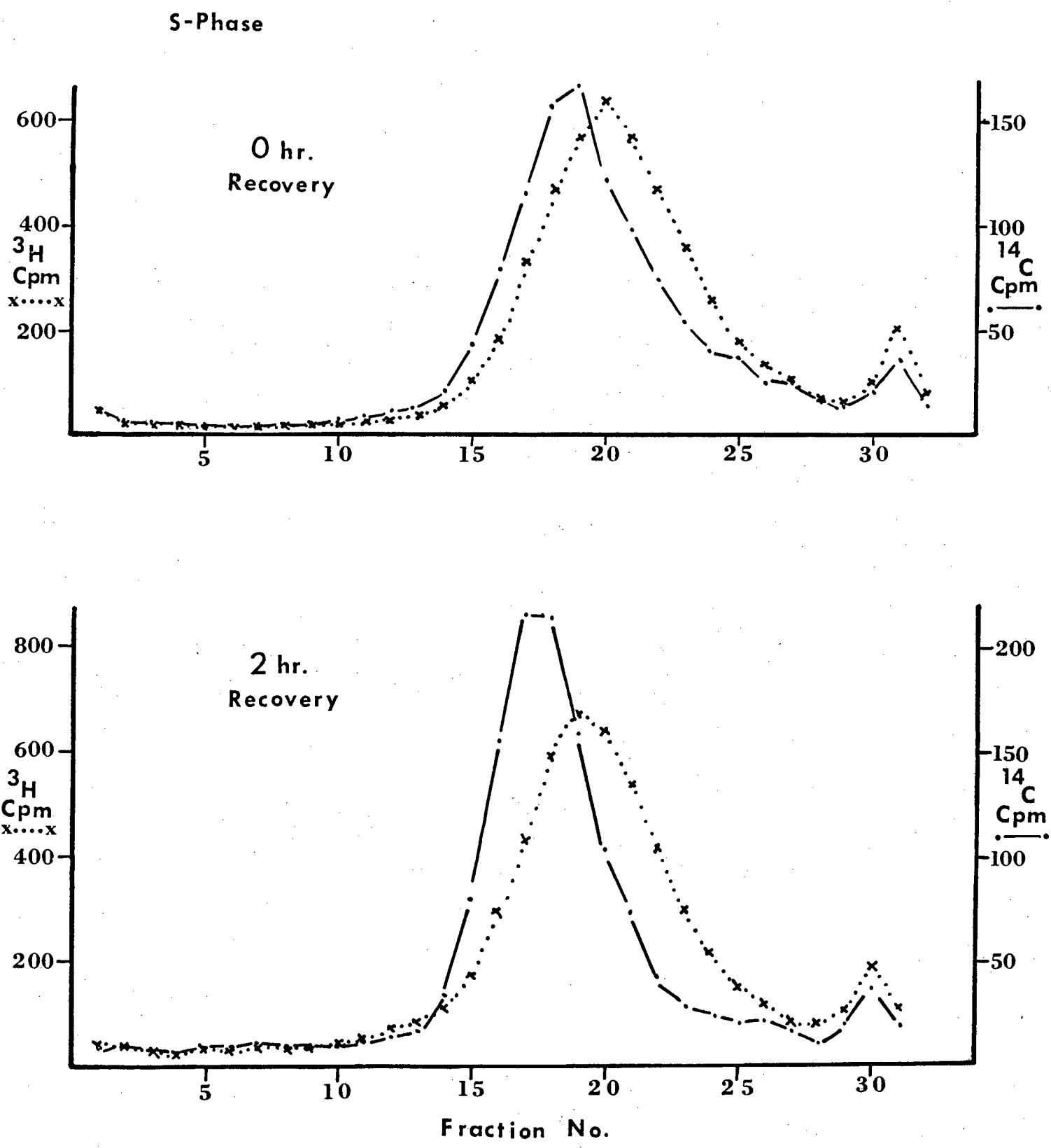


Figure 1.

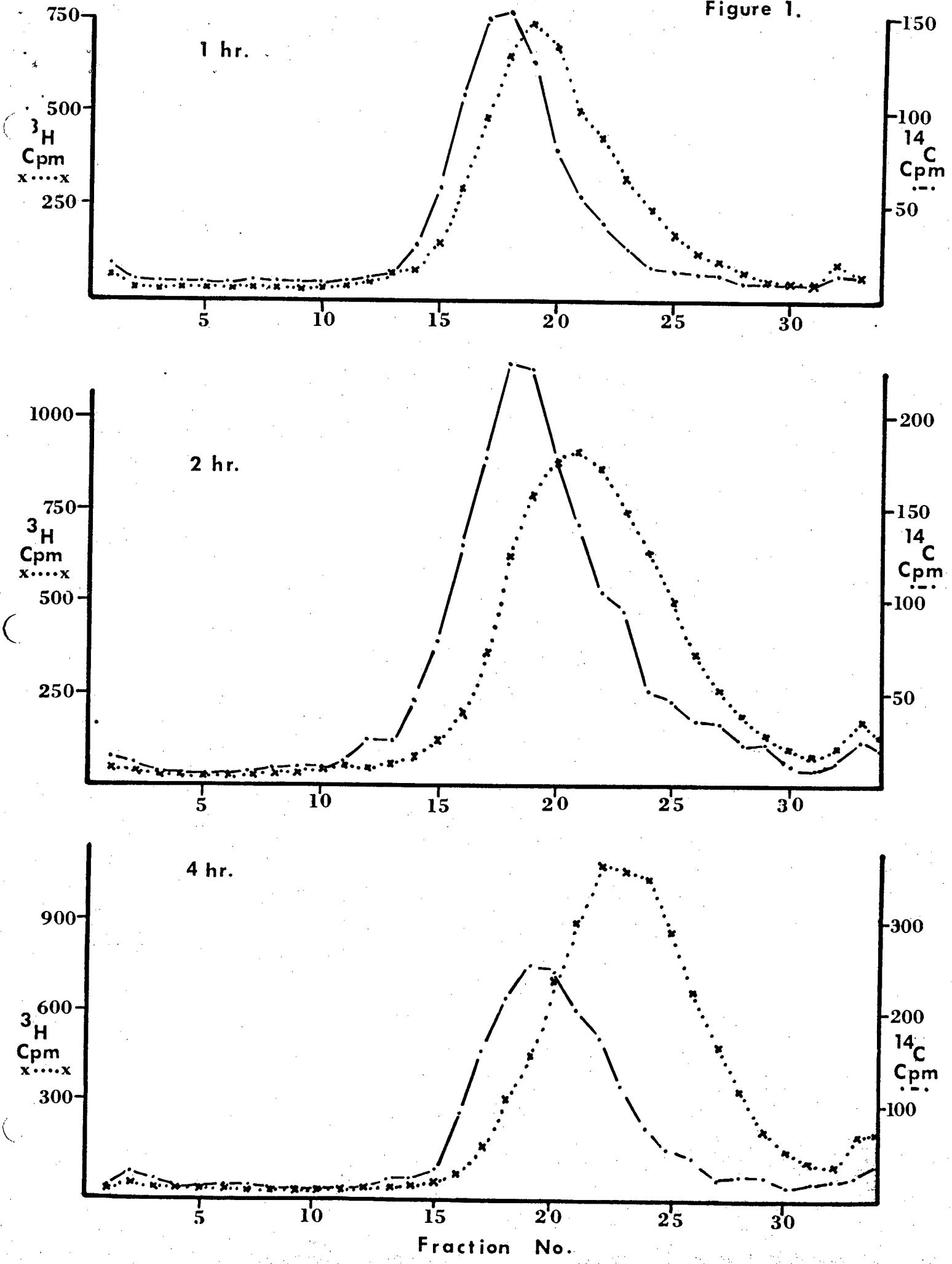


Figure 2b.

