# The Binding of Benz [*a*] anthracene to Replicating and NonReplicating DNA in Cell Culture (35131)

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(Introduced by John H. Weisburger)
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Previous studies (1) have demonstrated that binding of 7, 12-dimethylbenz [*a*] anthracene (DMBA) to DNA does not require DNA synthesis. DNA which had replicated in the presence of DMBA was separated from nonreplicating DNA by the addition of 5-bromodeoxyuridine (BUDR) to cultures of fetal mouse skin and subsequent isopycnic density sedimentation of extracted DNA. More binding was found to DNA which had not replicated while DMBA was present than to that which had. That this was the case for the strong carcinogen DMBA did not necessarily imply that all the polycyclic hydrocarbons acted this way with regards to binding during the cell cycle. In fact, Alfred and DiPaolo (2) had reported that the weakly carcinogenic hydrocarbon benz [*a*] anthracene (BA) (3–5) reached a maximum binding to hamster embryo cell DNA during the first 4 hr of the release of the cells from a thymidine block. This was the same time that maximum DNA synthesis had occurred in their culture system. DMBA in their system did not reach a maximum binding until 24 hr, a time when more than one complete cycle had occurred.

Furthermore, weakly carcinogenic hydrocarbons had previously been shown to be very poor binders to DNA when painted on mouse skin. (6) These studies suggested the possibility that weak carcinogens could only bind to DNA that was being replicated. If this were so, it might indicate that binding of carcinogens to DNA during replication was not as important to carcinogenesis as binding in some other part of the cell cycle, if indeed binding was important. For this reason BA was tested in the cell cultures system which utilized BUDR to separate the replicating from nonreplicating DNA.

*Materials and Methods.* The cell culture technique, basic media and DNA isolation were performed as previously reported (1). The preparation of the hydrocarbon-containing medium and the scintillation counting procedure are described below.

*BA Medium.* $^3$H-BA generally labeled was obtained from Amersham-Searle at a specific activity of 750 mCi/mmole. Approximately 5000 $\mu$Ci (1500 $\mu$g) in 5 ml of benzene was exposed to a stream of nitrogen until the benzene had evaporated. The $^3$H-BA was redissolved in 100 ml of acetone in semidarkness and slowly added to 15 ml of fetal calf serum being slowly stirred in an ice bath. This was allowed to mix for 30 min in the dark and an additional 100 $\mu$l of acetone was used to rinse the $^3$H-BA ampoule and then added slowly to the serum. The solution was mixed for 4 hr in the dark in an ice bath and then added to 85 ml of thymine-free medium 199 and 1 ml of antibiotic mixture giving a final radioactivity of 50 $\mu$Ci of $^3$H-BA per /ml $^3$H-BA/ml (15 $\mu$g/ml). This stock solution was stored in the dark at 4°. For the experimental medium a 1:20 dilution of the stock mixture was used with either BUDR or thymine-containing medium making a final radioactivity of 2.5 $\mu$Ci/ml (0.75/ $\mu$g/ml).

*Collection of fractions and scintillation counting.* Following ultracentrifugation of the isolated DNA at 45,000 rpm for 66 hr, 0.4-ml fractions were collected. The absorption of each fraction was read at 260 m$\mu$ in a Gilford Model 240 spectrophotometer. To a 150-$\mu$l sample of each fraction was added 200 $\mu$l of carrier DNA solution (2 mg/ml). The mixture was precipitated by cold 20% TCA

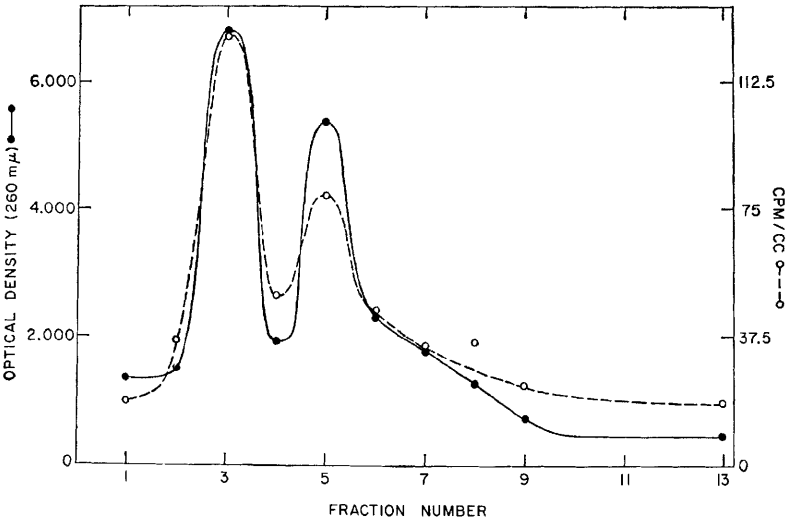


FIG. 1. CsCl density-gradient profile of DNA from cells incubated simultaneously with $6 \times 10^{-6}$ *M* BUDR and 2.5 μCi/ml of $^3$H-BA (0.75 μg/ml) for 20 hr.

followed by two washings with cold 3% TCA and two methanol washes. The precipitate was dissolved in 0.5 ml of NCS reagent (Amersham-Searle) and 20 ml of Spectrafluor PPO-POPOP scintillation fluid (Amersham-Searle). The radioactivity was counted for 100 min for each sample in a Beckman LS 250 scintillation counter using the wide $^3$H window.

*Results.* Figure 1 shows the results of a simultaneous incubation of $^3$H-BA and BUDR for 20 hr prior to DNA extraction and ultracentrifugation. Figure 2 represents the binding resulting from incubation with $^3$H-BA in thymine-containing medium for 20 hr. The peak to the left of Fig. 1 represents DNA which was newly synthesized and incorporated BUDR during the 20-hr exposure to the hydrocarbon. The upper peak contains the DNA not replicated during that time period. It is apparent that binding occurs in both peaks and synthesis is not a requirement. There is a greater degree of binding in the newly replicated peak. Because the de-

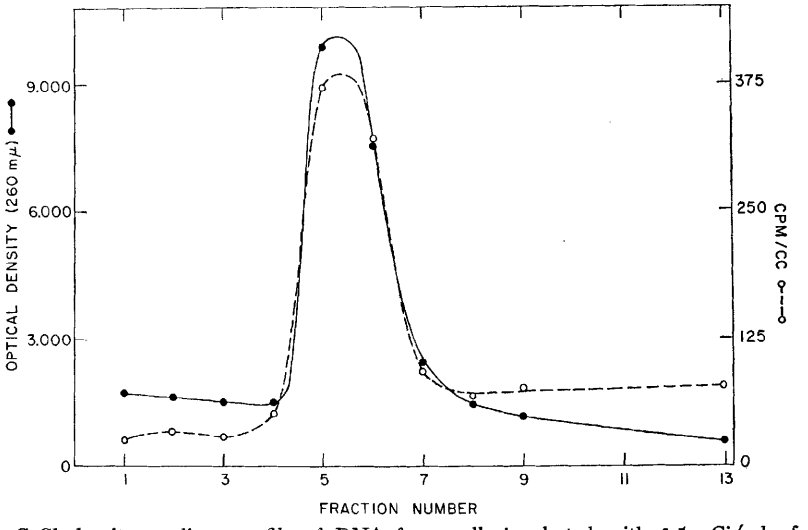


FIG. 2. CsCl density-gradient profile of DNA from cells incubated with 2.5 μCi/ml of $^3$H-BA (0.75 μg/ml) in thymine-containing medium for 20 hr.

TABLE I. Binding of BA-$^3$H and DMBA-$^3$H to DNA of Cells in Tissue Culture.

| Study | Cell type | Hydrocarbon conc in medium: DMBA-$^3$H ($\mu$g/ml) | Hydrocarbon conc in medium: BA-$^3$H ($\mu$g/ml) | Time of exposure (hr) | DNA binding (molecules hydrocarbon $^3$H/$10^6$ nucleotides) |
|---|---|---|---|---|---|
| Yuspa *et al.* (1) | Fetal mouse skin | 0.065 | | 15 | 17.4 |
| | Fetal mouse skin | | 0.75[a] | 20 | 0.35 |
| Diamond *et al.* (7) | Mouse embryo | 0.05 | | 18 | 14.0 |
| | Hamster embryo | 0.012 | | 24 | 3.9 |
| Alfred and DiPaolo (2) | Hamster embryo | 0.115 | | 24 | 3.5 |
| | Hamster embryo | | 0.103 | 24 | 1.0 |

[a] Binding levels calculated from data shown in Fig. 2.

gree of binding is so small in both peaks, however, it is not possible to say that the differences between the peaks is truly significant. Figure 2 demonstrates that total binding of BA to DNA of cells in all phases of the cell cycle is still of a small degree at 20 hr.

*Discussion.* As previously shown for other weak carcinogens (6), benzanthracene binds poorly to DNA of mouse cells. The results show that this poor binding is not due to a requirement to bind to DNA which is undergoing replication. Although the binding is somewhat higher to this newly synthesized peak, it is still extremely poor compared to that shown for the strong carcinogen DMBA (1) even though the latter was used at a much lower concentration (Table I).

The marked difference between binding levels of BA and DMBA which we observed was not seen by Alfred and DiPaolo (2) (Table I). In their study, the maximum level of binding of BA to hamster embryo cells occurred after 4-hr incubation; this level was higher than the maximum level of binding reached with DMBA at 24-hr incubation. In experiments by Diamond *et al.* (7) (Table I), the level of binding of DMBA to DNA of mouse cells was similar to that in our experiments. They also studied binding of this carcinogen to hamster cells, but at one-tenth the concentration used by Alfred and DiPaolo. The reason for the higher level of binding by BA and lower level by DMBA seen by Alfred and DiPaolo relative to our findings could result from differences in experimental systems. They blocked the cell cycle with thymidine and then released the block before incubating with hydrocarbon, and also used another species and cell type.

*Summary.* Replicating and nonreplicating DNA were isolated in an isopycnic density gradient from cultured fetal mouse skin cells after incubation with 5-bromodeoxyuridine for 20 hr. Benz[a]anthracene, when incubated in the cultures simultaneously with bromodeoxyuridine, binds to both replicating and nonreplicating DNA. The binding of benz[a]anthracene to mouse DNA is considerably less than that previously reported for 7,12-dimethylbenz[a]anthracene.

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