labelled (Figure 2). This is so in follicles of every size up to an average diameter of 500 μ. In the following stages labelled nuclei are not observed. Labelling affects a greater percentage of nuclei in specimens fixed at later intervals (Figure 3) and after 3 h from injection affects almost every nucleus.

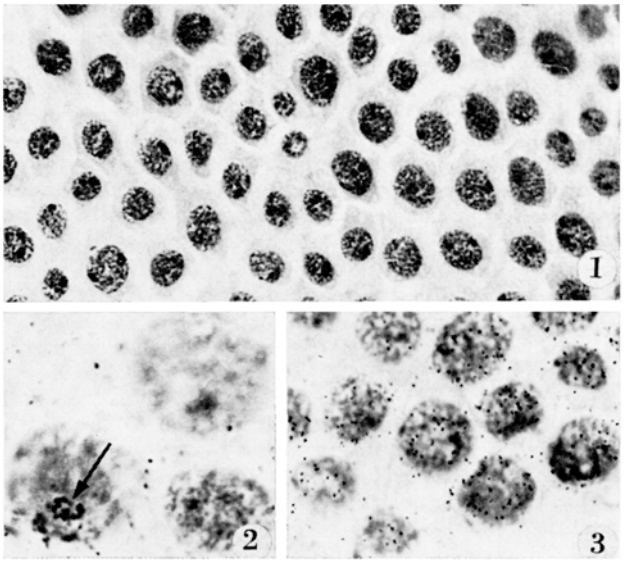

Fig. 1. Nuclei of variable size in follicular epithelium. Feulgen. × 400.

Fig. 2. 10 min after injection. The arrow indicates a labelled area around the nucleolus. Feulgen. × 1320.

Fig. 3. 45 min after injection. A high percentage of nuclei are labelled. Feulgen. × 760.

Conclusions. From cytophotometric measurements it appears that more or less continuous variations in DNA-amounts among different nuclei of one and the same follicle exist. This can be explained if one assumes the DNA synthetic period to be much longer than the inter-synthetic one. This hypothesis is supported by the high number of nuclei which, on autoradiographic analysis, show labelling even a short time after injection. The DNA synthetic period which is long compared with the non-synthetic interphase, might be caused by a remarkable degree of asynchrony in DNA-synthesis existing among the various chromatin elements in the same nucleus. This is also supported by the localized uptake of H^3-thymidine which is observed in some nuclei.

Riassunto. I nuclei delle cellule follicolari degli Afidi mostrano nell'ambito di uno stesso follicolo variazioni pressochè continue nel contenuto di DNA. Dopo brevi intervalli di tempo dall'iniezione di timidina tritiata, una elevata percentuale di questi nuclei risulta marcata. È stata fatta l'ipotesi che ogni ciclo di poliploidizzazione in questi nuclei si componga di un lungo periodo di sintesi di DNA seguito da uno breve di intersintesi. La lunghezza del periodo sintetico è dovuta presumibilmente all'asincronia nella sintesi di DNA che esiste tra i vari elementi cromatici in uno stesso nucleo.

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Influence of Halogenated Deoxyuridines on the Radiation Sensitivity of *Escherichia coli*

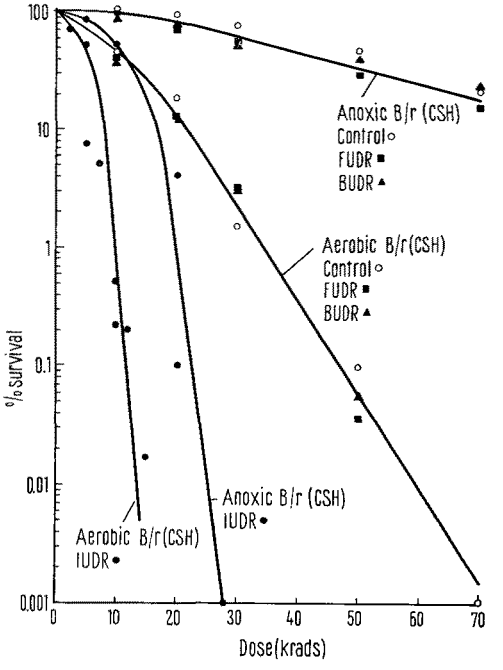
It has been shown that iodine containing compounds such as iodoacetamide[1], iodoacetic acid[2] and potassium iodide[3], when added to phosphate buffer suspensions of radioresistant bacteria can sensitize their inactivation by ionizing radiation. The mechanism of action of these compounds is believed due to short-lived radicals[2,4] formed by the reaction of the halogenated compounds with the radiolytic products of water, having the form[5] $RI + OH^{\cdot} \rightarrow ROH + I^{\cdot}$ with the iodine atom being responsible for enhanced bacterial killing. If this is the case, then other iodine containing organic compounds would be expected to act as radiation sensitizers when present in the medium during irradiation. The halogenated thymidine analogs are powerful mutagens when incorporated in cellular DNA, and also enhance the radiation sensitivity of a variety of cells when incorporated in their DNA[6]. We decided to examine the effect of the halogenated deoxyuridines upon bacterial radiosensitivity when merely present in the irradiation medium, rather than incorporated in the DNA, particularly 5'-iododeoxyuridine.

Materials and methods. 5'-Bromodeoxyuridine (BUDR), 5'-fluorodeoxyuridine (FUDR) and 5'-iododeoxyuridine (IUDR) were obtained from Calbiochem, Los Angeles, California, and freshly prepared as $10^{-3}M$ solutions in saline-phosphate buffer (pH 6.8). This concentration was shown to have no toxic effect on either unirradiated or irradiated bacteria. Iodoacetamide (IA) and potassium iodide (KI) used in some experiments for comparison with IUDR were reagent grade chemicals. The radiation resistant *Escherichia coli* B/r (CSH), having a D_0 or dose to inactivate 63% of the population (anoxic) of 23 krads, was grown from a loop in nutrient broth (Difco) with aeration at 37 °C to the middle or end of log phase (4 h) yielding an approximate titer of 5×10^8 cells/ml. The cells were refrigerated overnight, spun down, washed, resuspended in

1 C. J. Dean and P. Alexander, Nature *196*, 1324 (1962).
2 J. S. Lee, W. A. Anderson and P. R. Elliker, Radiat. Res. *19*, 593 (1963).
3 C. J. Dean and P. Alexander, Prog. Biochem. Pharmac. *1*, 46 (1965).
4 D. L. Dewey and B. D. Michael, Biochem. biophys. Res. Commun. *21*, 392 (1965).
5 L. Mullenger, B. B. Singh, M. G. Ormerod and C. J. Dean, Nature *216*, 372 (1967).
6 W. Szybalski, in *Research in Radiotherapy* (Publ. 888, Natn. Acad. of Sciences, National Research Council, Washington, D.C. 1961).

saline phosphate buffer (pH 6.8) and starved at 37 °C with aeration before use. The appropriate volume of sensitizer solution or buffer for the controls was added to a total volume of 2 ml and the mixtures held at 0 °C for 30 min prior to irradiation in screw cap glass vials. Pre-irradiation bubbling with either oxygen or nitrogen was carried out during this holding period. The vials were then exposed at icebath temperature to 280 KVP X-rays from a dual beam Picker Vanguard Unit, operating at 280 KV and 20 mA, at a dose rate of 1.24 krads/min, with continuous gas bubbling. Samples were withdrawn after graded X-ray doses, and after appropriate dilution the number of surviving bacteria was determined by plating on nutrient agar, incubating at 37 °C overnight, and counting the number of visible colonies produced.

Results and discussion. Typical survival curves obtained for anoxic and aerobic *E. coli* B/r (CSH) in the presence and absence of drug are shown in the Figure. These experiments with the halogenated deoxyuridines showed that IUDR as expected acted as a powerful radiosensitizer under both aerobic and anoxic radiation conditions with *E. coli* B/r (CSH), while FUDR and BUDR had no effect on bacterial radiosensitivity. While the bacterial sensitivity was greater under sensitized aerobic conditions the degree of sensitization by IUDR was greater under anoxic than aerobic conditions. The additional contribution to aerobic sensitivity was believed due to long-lived toxic products of radiolysis. This was shown in the following manner. When IUDR at $10^{-3}M$ was irradiated alone and added to unirradiated *E. coli* B/r (CSH) after 10 sec, cell killing was observed only if the irradiation were carried out aerobically. No cell killing was seen by irradiated solutions of BUDR or FUDR with *E. coli* B/r. However, when KI at $10^{-2}M$ was irradiated with 10 krads and added after 10 sec to unirradiated *E. coli* B/r (CSH), significant cell killing was observed, independent of whether the KI was irradiated aerobically or anoxically (Table). That this effect is primarily due to the formation of iodine, was demonstrated by irradiating solutions of IA, IUDR or KI, and then adding starch indicator after 10 sec – a deep blue color was observed in IA and IUDR solutions if irradiated aerobically but not anoxically, while KI gave a blue starch color whether irradiated anoxically or aerobically. This suggests that in the presence of oxygen, irradiated solutions of IUDR, IA or KI yield iodine which accounts for the additional enhanced aerobic radiosensitization shown in the Figure compared with the anoxic curve. Since the degree of sensitization is greater under anoxic than aerobic conditions, I_2 is less effective in killing cells than 2 I·. Under anoxic conditions, iodine is not formed from IUDR or IA hence the pronounced anoxic radiosensitization by IUDR (Figure) is due to the short-lived iodine atom product. Radiosensitization by IUDR therefore has 2 components, a long-lived aerobic poison product part (I_2) and a short-lived anoxic product (I·). DEWEY and MICHAEL[4] attribute the entire radiosensitizing effect by IA at $10^{-3}M$ with *Serratia marcescens* under aerobic conditions to the short-lived (< 1 sec) irradiation product of IA. We find however that by using higher concentrations ($10^{-2}M$) and larger doses of radiation (30 krad) we can also detect the long-lived toxic irradiation product (I_2) under aerobic conditions from IUDR or IA, but which is not detected if irradiation is under nitrogen. BUDR and FUDR when present in the irradiation medium are not radiosensitizers for *E. coli* in these experiments under aerobic or anoxic conditions indicating that only the iodine atom is involved in radiosensitization by halogen containing compounds[7].



Dose survival curve for log phase *E. coli* B/r (CSH) irradiated in saline-phosphate buffer at 0 °C with 280 KV X-rays in the presence (filled symbols) or absence (open symbols) of $10^{-3}M$ BUDR, FUDR or IUDR, under anoxic and aerobic conditions.

Effect of pre-irradiation of drug followed by addition of unirradiated *E. coli* B/r CSH

Drug/ concentration	Time elapsed before addition of bacteria	% survival Anoxic 10 krad	Anoxic 30 krad	Aerobic 10 krad	Aerobic 30 krad
KI/$10^{-2}M$	10 sec	24	0.14	24	0.10
IUDR/$10^{-3}M$	10 sec	100	100	38	2
IA/$10^{-2}M$	10 sec	94	90	0.005	$\sim 10^{-5}$
IA/$10^{-2}M$	30 min	~100	~100	10	–

Zusammenfassung. Halogenierte Deoxyuridine IUDR, BUDR und FUDR wurden im Medium als bakterielle Sensibilisatoren für Röntgenstrahlen untersucht. Es wird gezeigt, dass nur IUDR als Sensibilisator funktionieren kann, scheinbar sowohl durch die Bildung des langlebigen giftigen molekularen Jods als auch durch die Bildung des kurzlebigen giftigen Jod-Atoms.

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Division of Physical Biology, Sloan-Kettering Institute for Cancer Research, Walker Laboratory, Rye (New York, USA), 27 May 1968.

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