The Distribution and Fate of Bromodeoxyuridine and Bromodeoxycytidine in the Mouse and Rat*

JOSEPH P. KRIS AND LÁSZLÓ RÉVÉSZ†

WITH THE TECHNICAL ASSISTANCE OF LUCIE TUNG AND SUSAN EGLOFF

(Departments of Medicine and Radiology, Stanford University School of Medicine, Stanford, California)

SUMMARY

Bromodeoxyuridine (BUdR) was rapidly and extensively degraded in vivo in the rat, with the concomitant formation of bromouracil and bromide ion. The liver is a major site of dehalogenation. A portion of the administered compound escaped degradation and was incorporated into the DNA of various tissues in a pattern similar to that of thymidine incorporation.

In the rat, bromodeoxycytidine (BChR) was more slowly degraded than BUdR. Neither bromocytosine nor bromouracil could be detected as degradation products. Following administration of BChR-Br82, the distribution of Br82 activity in the DNA of various organs was different from that which followed injection of BUdR-Br82. Some evidence was presented which suggests that such a difference may be due to deoxycytidylate deaminase activity in particular tissues, such as the bone marrow.

Following incorporation of Br82 by Ehrlich ascites cells after intraperitoneal administration of BUdR-Br82 or BCdR-Br82 to mice, the radioactive label was conserved during neoplastic cellular multiplication for at least four successive generations.

Ehrlich ascites cells, exposed in vivo to repeated intraperitoneal injections of BUdR and subsequently irradiated with 550 r, showed a greater inhibition of their post-treatment growth curves in vivo than did irradiated cells not exposed to BUdR.

The evidence that incorporation of the halogenated thymidine analog, 5-bromodeoxyuridine (BUdR), into the deoxyribonucleic acid (DNA) of mammalian cells cultured in vitro may result in an increased susceptibility of the cells to the lethal effects of x-radiation has recently been reviewed by Djordjevic and Szybalski (8). These authors pointed out that radiosensitization is most readily elicited when BUdR has replaced an appreciable quantity of the thymidine in both strands of DNA. Quantitative incorporation of BUdR into DNA of cells in vitro is markedly affected by the concentration of BUdR in the medium and by the duration of incubation (19). BUdR-monophosphate (BUdRP) may also be recovered from the DNA of neoplastic cells after they have been incubated with bromodeoxycytidine (BChR) (7). The possible clinical usefulness of BUdR or BChR in inducing radiosensitization of tumor tissue will depend not only upon local factors governing their incorporation into DNA, but also on the fate of these compounds in vivo. With the use of BUdR-Br82 and BChR-Br82, a study was undertaken to investigate the extent to which these compounds are incorporated into the DNA of various tissues of the rat and mouse in vivo after parenteral administration. As contrasted to the use of H1 or C14 as the isotopic label, the use of Br82 conveniently permits both the detection of minute quantities of bromine-containing compounds in DNA after administration of tracer quantities of BUdR and BChR and an estimation of the rate and extent of their dehalogenation. Observations were also made on the conservation of the incorporated compounds within cells during multiplication and on the proliferation of BUdR-treated cells in vivo after x-radiation.

* This investigation was supported by grant CY-4096 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland.
† On leave from the Institute for Tumor Biology, Karolinska Institutet, Stockholm, Sweden.

Received for publication October 9, 1961.
MATERIALS AND METHODS

Animals.—C57BL/Ka and C3H/Ka male mice, weighing 20–30 gm., and male Long-Evans rats, weighing approximately 200 gm., were used. The animals were maintained on Purina chow diet and water ad libitum.

Tumors.—An Ehrlich ascites tumor line, referred to as ELD, was used. A number of cellular growth properties of this tumor are summarized in a recent publication (28). In this laboratory the tumor cells were carried in C57BL male mice by weekly serial transfer of 0.1 ml. ascitic fluid, diluted with sterile 0.9 per cent saline in a proportion of 1:9. The cell concentration was determined in an aliquot of the ascitic fluid in a hemocytometer after dilution with Turk’s solution. All intraperitoneal injections were made by a 25-gauge needle through the musculature of the lumbar region in order to avoid leakage of peritoneal fluid to the outside. The multiplication of the intraperitoneally inoculated tumor cells was determined by a quantitative rinsing procedure (18).

Radioactive compounds.—Synthesis of BUdR-Br\textsuperscript{82} was performed from deoxyuridine, according to Kriss and Révész (19). BCdR-Br\textsuperscript{82} was synthesized in a manner identical to that of BUdR-Br\textsuperscript{82}, except that deoxycytidine instead of deoxyuridine was used as a starting material. The mobility of these radiobromine compounds is identical to that of standard BCdR. The specific activities of the radiobromine compounds are given in the section describing the experiments. In some experiments, C\textsuperscript{14}-labeled thymidine\textsuperscript{3} and H\textsuperscript{3}-labeled deoxycytidine-5-monophosphate\textsuperscript{4} were also used.

Blood clearance.—A polyethylene cannula filled with heparin solution (1000 USP units/ml) was inserted and secured into one carotid artery of a rat anesthetized by nembutal (45 mg/kg body weight). The cannula was then clamped, and 200 USP units of heparin were administered intravenously. A physiological saline solution of the rat and the cannula was momentarily unclamped, and a few drops of blood were collected onto wax paper. An aliquot, 10 or 20 λ of blood, was added to 1 ml. of

\textsuperscript{1} Obtained from the Institute for Tumor Biology, Karolinska Institutet, Stockholm.

\textsuperscript{2} California Corp. for Biochemical Research, Los Angeles, Calif.

\textsuperscript{3} New England Nuclear Corp., Boston, Mass., 1 mc/mmole.

\textsuperscript{4} Schwarz Laboratories, Inc., Mt. Vernon, N.Y.; sp. act., 100 mc/mmole.

\textsuperscript{5} For details, see (19).

\textsuperscript{6} The radioactivity was expressed as per cent of the administered activity per mg. DNA in the sample. BUdR-Br\textsuperscript{82}, 0.5–5 μc., was administered to mice intravenously, intraperitoneally, or subcutaneously. At stated intervals after the injection, the animals were weighed and sacrificed. The following organs were excised in toto: skin, spleen, liver, kidneys, testes, stomach, intestine, and colon. The three latter were evacuated of their contents by being rinsed with water. Each of the organs and also both legs were weighed and placed in separate test tubes, filled with water to a volume of 5 ml., and their activity was counted in a well-type scintillation counter. A tube containing a Br\textsuperscript{82} standard in 5 ml. water was also counted. The skin was counted in two portions. The carcass remaining after removal of the organs was placed in a 50-ml beaker, water was added to a total volume of 25 ml., and the radioactivity was measured over the crystal of the scintillation counter. A 50-ml beaker containing a Br\textsuperscript{82} standard in 25 ml. water was counted under similar conditions. The radioactivity in the organs was expressed as per cent of administered activity per gram wet tissue.

Analytic methods.—The methods used for paper chromatography and the measurement of radioactivity and DNA content of tissues were reported in a previous study (19).

Fractionation of PCA-soluble, Br\textsuperscript{82}-containing compounds was accomplished by passing the solution through a column containing 20- to 50-mesh, Dowex-1-Cl, anion exchange resin, which allows passage of BUdR and BCdR to the eluate but retains bromide. With nonradioactive BUdR, BCdR, and bromide-Br\textsuperscript{82} used as test substances, it was found that washing the resin columns sequentially with 2 volumes each of water, N/100 HCl, and N/10 HCl resulted in essentially complete recovery of BUdR and BCdR in the pooled eluate as determined by spectrophotometric meas-
measurements. Such treatment also elutes bromouracil and bromocytosine from the resin column, while there is complete retention of Br$^{82}$-bromide on the resin.

Descending paper chromatography of synthesized compounds, plasma samples, and PCA-soluble fractions of plasma was carried out with solvent systems of butanol:water (86:14) with 5 per cent ammonia and 65 per cent v/v aqueous isopropanol:2.0 N HCl.

The measurement of the activity of Br$^{82}$, C$^{14}$, and H$^{3}$ in the same sample was carried out in a liquid phosphor counter as follows: The samples to be counted, together with Br$^{82}$, C$^{14}$, and H$^{3}$ standards containing amounts of perchloric acid and phosphor equivalent to those in the samples, were first counted at a low-voltage tap setting (tap 2). At this voltage tap, the lower discriminator can be set so that C$^{14}$ and H$^{3}$ activity are not detected, and only Br$^{82}$ ($\beta$ energy = 0.44 Mev) is counted. The samples and standards are then counted at an intermediate voltage (tap 6) and a high voltage (tap 10). The Br$^{82}$ contribution for each sample at taps 6 and 10 can be calculated from the values for the Br$^{82}$ standard at the three tap settings. Subtraction of the Br$^{82}$ contribution at taps 6 and 10 from the total count at these settings gives the activity due to C$^{14}$ and H$^{3}$. C$^{14}$ activity ($\beta$ energy = 0.155 Mev) is recorded maximally at tap 6, whereas H$^{3}$ activity ($\beta$ energy = 0.018 Mev) is more efficiently detected at tap 10. From the values of the C$^{14}$ and H$^{3}$ standards at taps 6 and 10, and the total sample counts at these taps, double equations can be solved for C$^{14}$ and H$^{3}$ activity, the final expressions being

$$\frac{C^6}{1 - ab} = T^6 - bT^{10} \quad \text{and} \quad \frac{H^6}{1 - ab} = T^6 - aT^{10}.$$  

Where: $T^6$ and $T^{10}$ = total sample counts at taps 6 and 10, respectively, less Br$^{82}$ contribution

$C^6$ and $C^{10}$ = C$^{14}$ contribution at taps 6 and 10, respectively, less Br$^{82}$ contribution

$H^6$ and $H^{10}$ = H$^{3}$ contribution at taps 6 and 10, respectively, less Br$^{82}$ contribution

a = $\frac{C^{10}}{C^6}$, by standard C$^{14}$

b = $\frac{H^6}{H^{10}}$, by standard H$^{3}$.

X-radiation.—Aliquots of ice-cooled suspensions of ELD tumor cells were irradiated in air in a flat-bottomed plastic irradiation chamber with a diameter of 30 mm. and a height of 40 mm. X-rays were generated at 250 kv. and 15 ma. (HVL = 0.84 mm. Cu). The dose rate, without added filter, at 50 cm. target-based distance, was 200 r.p.m.

RESULTS

Clearance of Br$^{82}$-labeled BUdR and BCdR from the blood.—The clearance of BUdR from the blood of rats was studied in two experiments. In each experiment, thymidine (TdR) was administered simultaneously as a standard for comparison. In the first experiment each of two rats was given injections intravenously of equimolar quantities (22 $\mu$mole) of BUdR-Br$^{82}$ and TdR-C$^{14}$, 2 $\mu$c, and 1.4 $\mu$c, respectively. Serial blood samples were obtained via a carotid cannula during 60 minutes. The Br$^{82}$ and C$^{14}$ radioactivities in the PCA-soluble fraction of 20 $\lambda$ aliquots of each blood sample were determined. From these values, and from the value for blood volume of the animals, as estimated from the respective body weight figures, the total amount of radioactivity in the circulation was calculated and expressed as per cent of the administered radioactivity. No correction was made for the blood lost due to sampling. The results are shown in Chart 1. It will be noted that the data obtained from the two animals are in good agreement. The clearances of Br$^{82}$ and C$^{14}$ differed markedly in both rats. The C$^{14}$ content of the blood decreased quickly, and less than 2 per cent remained at the conclusion of the experiment after 1 hour. On the other hand, within the first 5 minutes after injection, the Br$^{82}$ activity of the blood fell to about 19 per cent. Thereafter it rose a few per cent and subsequently maintained a level of about 20 per cent until the end of the experiment.

An explanation for the disparate curves for Br$^{82}$ activity on the one hand and C$^{14}$ activity on the other was provided by observations which were made in the second experiment. Equimolar quantities (22 $\mu$mole) of BUdR-Br$^{82}$ and TdR-C$^{14}$
(50 μc. and 1.4 μc., respectively) were injected intravenously in a mixture, and 20 blood samples were taken at intervals for a 60-minute period. A 0.5-ml. aliquot of the PCA-soluble fraction of each sample was counted for its Br\(^{82}\) and C\(^{14}\) activity in the liquid scintillation counter. The remainder of the PCA-soluble fraction was counted for its Br\(^{82}\) activity in a well-type scintillation counter and then passed through an anion exchange resin column. The column was eluted sequentially with 2 volumes each of water, N/10 HCl, and N/10 HCl, and the radioactivity of the pooled eluate of each sample was determined.

Chart 2 shows the Br\(^{82}\) activity in the eluates of the samples, expressed as per cent of the activity of the PCA-soluble blood fraction before resin treatment. It will be noted that the eluate contained about 80 per cent of the radioactivity of the first sample taken 1 minute after injection of the BUdR-Br\(^{82}\). The percentage recovery declined rapidly during the first 10 minutes and thereafter at a slower rate.

Chart 3 shows the Br\(^{82}\) and C\(^{14}\) activity of blood samples as determined by the liquid scintillation counter. An early dip and a slight rebound elevation in the Br\(^{82}\) curve, and a fall in the C\(^{14}\) curve in a manner similar to that observed in the previous experiment, were noted (cf. Chart 1). According to the information obtained from resin treatment of the corresponding PCA-soluble aliquot (cf. Chart 2), the Br\(^{82}\) activity, retainable or not retainable by resin, of each blood sample was calculated. During the first 5 minutes after injection a rapid rise in Br\(^{82}\) activity retainable by resin occurred, which thereafter approximated but was slightly less than the total Br\(^{82}\) activity of the blood samples. The elutable Br\(^{82}\) activity fell rapidly for the first 10 minutes and more slowly thereafter. The rate of fall of elutable Br\(^{82}\) activity corresponded closely to that observed for C\(^{14}\) activity.

Chart 2.—Elutable Br\(^{82}\) activity of PCA-soluble blood fractions after treatment with anion exchange resin, following intravenous injection of BUdR-Br\(^{82}\). The radioactivity of the eluate samples is expressed as a percentage of that activity present before resin treatment.

Chart 3.—Observed blood C\(^{14}\) and Br\(^{82}\) activities, and corresponding calculated Br\(^{82}\) activity retainable and not retainable by anion exchange resin, after intravenous administration of TdR-C\(^{14}\) and BUdR-Br\(^{82}\).
Plasma samples and the PCA-soluble fractions of blood samples obtained at various intervals from 2 to 60 minutes after the intravenous injection of doses of BUdR-Br\textsuperscript{82} of higher radioactivity (100–200 μc.) were subjected to descending paper chromatography, with the use of solvent systems of butanol:water:ammonia and aqueous isopropanol:HCl\textsuperscript{-}, and standards of BUdR, bromouracil, BCdR, bromocytosine, and bromide-Br\textsuperscript{82}. Two minutes after injection of BUdR-Br\textsuperscript{82} the plasma contained BUdR-Br\textsuperscript{82}, bromide-Br\textsuperscript{82}, and bromouracil-Br\textsuperscript{82} in the approximate proportions 9:2:1, respectively. At 4 minutes post-injection, BUdR-Br\textsuperscript{82} and bromouracil-Br\textsuperscript{82} activities had declined to about 29 and 14 per cent of the total plasma radioactivity, respectively. Bromouracil-Br\textsuperscript{82} was not detected in the samples obtained 6 minutes or more after administration of the labeled BUdR. The bromide-Br\textsuperscript{82} component of the blood samples was removable by treatment of the sample with exchange resin. The Br\textsuperscript{82} activity retained by the resin can be considered, therefore, to represent bromide-Br\textsuperscript{82}, and elutable Br\textsuperscript{82} activity represents BUdR and bromouracil. The results shown in Chart 3 would thus indicate that a rapid degradation of part of the administered BUdR occurred. Cells being impermeable to bromide, the bromide-Br\textsuperscript{82} liberated would mix within the extracellular fluid (15). A rapid extracellular distribution of Br\textsuperscript{82} would account for the relative constancy of the values for the total and resin-retainable Br\textsuperscript{82} activity of the blood samples after 10 minutes.

The question arose whether the rate and/or extent of degradation of brominated deoxyribose-nucleosides is related to their chemical structure, specifically whether the presence of an amino group on the C-4 position might increase the stability of the molecule. An experiment was undertaken, therefore, to study the degradation of bromodeoxyctydine, BCdR, which has such an amino group. A mixture of BCdR-Br\textsuperscript{82} (30 μc., .47 μmole) and thymidine-C\textsuperscript{14} (10 μc., .64 μmole) was injected intravenously into a rat prepared for blood clearance study. Blood samples were taken at intervals up to 60 minutes and processed to determine Br\textsuperscript{82} activity in PCA-soluble fractions of the samples before and after resin treatment. Chart 4 shows that the percentage recovery of Br\textsuperscript{82} activity in the respective resin eluates after BCdR-Br\textsuperscript{82} injection decreased very slowly and did not reach a value below 70 per cent even after 60 minutes. After BUdR injection the recovery of activity fell to about 4 per cent during the same period (cf. Chart 2). Chart 5 shows the C\textsuperscript{14} and Br\textsuperscript{82} activities of the blood samples, expressed as a percentage of the administered activity in the circulation. The rate of disappearance of C\textsuperscript{14} activity from the blood was of a similar pattern as that observed in the previous experiments described above (cf. Charts 1, 3). However, the rate of disappearance of Br\textsuperscript{82} activity after the injection of BCdR-Br\textsuperscript{82} was different from that observed previously after the injection of BUdR-Br\textsuperscript{82}. Although
the initial values were in the same range, a con-
tinuous fall in total Br\textsuperscript{82} activity after BCdR-Br\textsuperscript{82} administration was noted, as contrasted to the
early dip and rebound elevation in Br\textsuperscript{82} activity observed with BUdR-Br\textsuperscript{82} (cf. Charts 1, 3). By the end of 60 minutes, only 8 per cent of the ad-
mnistered Br\textsuperscript{82} activity was in the circulation after BCdR injection, whereas about 20 per cent
remained circulating after the same period when
BUdR was given. The values for elutable Br\textsuperscript{82} activity were calculated from the values for total
Br\textsuperscript{82} activity and the percentage of Br\textsuperscript{82} activity
elutable from resin and are also shown in Chart 5.
These values are only slightly lower than those of
total Br\textsuperscript{82} activity. The clearance curve of elutable
Br\textsuperscript{82} differs from that of C\textsuperscript{14}, in contrast to the
data obtained when BUdR-Br\textsuperscript{82} and thymidine-C\textsuperscript{14}
were administered (Chart 3).

Plasm@a samples and the PCA-soluble fractions
of blood samples obtained at intervals of from 2 to
60 minutes after the intravenous or intraperitoneal
injection of doses of BCdR-Br\textsuperscript{82} of higher activity
(100 μc.) into other rats were subjected to descending
paper chromatography with the use of the
solvent systems and standards employed in the
parallel experiments with BUdR-Br\textsuperscript{82}. The samples
obtained within the first 2 and 5 minutes after intravenous and intraperitoneal injection, respecti-
vively, contained Br\textsuperscript{82} activity corresponding only
to BCdR. At progressively later time intervals increa-
sing amounts of Br\textsuperscript{82} activity were found in the
position corresponding to bromide. However, even at the end of 60 minutes the major proportion of
the total radioactivity was found to represent
BCdR. Bromocytosine or bromouracil radioactivity
was not detected. As would be expected, bromide-Br\textsuperscript{82} activity of the sample was removable
by resin treatment. Elutable Br\textsuperscript{82} activity was
found to represent BCdR. The clearance curve of
elutable Br\textsuperscript{82} shown in Chart 5, therefore, indi-
cates the clearance curve of BCdR.

**Distribution of Br\textsuperscript{82} activity in different tissues
after administration of labeled BUdR and BCdR.**—
One hour after the intravenous administration of an
equimolar amount of BUdR-Br\textsuperscript{82} and thymi-
dine-C\textsuperscript{14} to each of two rats (22 μmole with 2 μc.,
and 22 μmole with 1.4 μc., respectively), and after serial blood samples were taken (cf. Chart 1), the
rats were killed, and the content and radioactivity of DNA were determined in portions of varying
sizes of the organs. The Br\textsuperscript{82} and C\textsuperscript{14} activities in the
DNA of liver, kidney, spleen, testis, femoral
marrow, and of the mucosa of colon, intestine,
and stomach of each animal were expressed as per cent of the administered activity per mg. DNA
and are shown in Chart 6. It will be noted that the
activity of C\textsuperscript{14} and Br\textsuperscript{82} per mg. DNA varied con-
siderably in the various tissues. Each activity was
greatest in the colon and relatively high in intesti-
tine, stomach, bone marrow, and spleen. Relative-
ly little activity was found in testis, liver, and kid-
ney. With the exception of the kidney, the ratio of
C\textsuperscript{14}/Br\textsuperscript{82} concentrations in the DNA varied little
and had a mean value of 2.35 (Chart 6).

Another study was conducted to investigate the
distribution of radioactivity in the DNA of various
tissues of two rats 1 hour after the simultaneous
administration of BCdR-Br\textsuperscript{82}, TdR-C\textsuperscript{14}, and \( H^2 \)-
labeled deoxycytidine-5'-monophosphate (CdRP-
H\textsuperscript{3}), each in amount of .62 μmole, with 100 μc.,
4 μc., and 50 μc., respectively. The results in
Chart 7 show the C\textsuperscript{14}, Br\textsuperscript{82}, and \( H^2 \) activities in per
cent/mg DNA in the various organs. Br\textsuperscript{82}
concentration was highest in the marrow, less in the intesti-
tine, and, in contrast to that observed after
BUdR-Br\textsuperscript{82} administration, was relatively low in
all the other organs. With the exception of a low value for the spleen, the C\textsuperscript{14} concentrations of the
other organs were of the same order of magnitude
as in a previous experiment (cf. Chart 6). \( H^2 \) con-
centrations, like those of Br\textsuperscript{82}, were highest in the
marrow, somewhat lower in intestine, but, in con-
trast to Br\textsuperscript{82}, were also relatively high in the colon
and stomach. In contrast to the relative constancy of the ratios of C\textsuperscript{14}/Br\textsuperscript{82} concentrations observed
after thymidine-C\textsuperscript{14} and BUdR-Br\textsuperscript{82} injection (cf.
Chart 6), the calculated C\textsubscript{14}/Br\textsubscript{82} ratios in this experiment ranged between 1.1 (liver) and 28.6 (stomach). Furthermore, no consistent relationship was found between the C\textsubscript{14} and H\textsubscript{3} concentrations or between the H\textsubscript{3} and Br\textsubscript{82} concentrations of the different organs.

**Distribution of Br\textsubscript{82} after injection of BUdR-Br\textsubscript{82} to CSH mice.**—In another experiment, each of 42 CSH mice was given injections intraperitoneally of 2.7 \( \mu \)c. BUdR-Br\textsubscript{82}. One hour after injection, and at various intervals thereafter up to 171 hours, groups of four or five animals were killed, the organs excised, and the mean weight and mean radioactivity for each organ were calculated. The radioactivity, expressed as per cent of the activity of the respective organ 1 hour after injection, is shown in Chart 8. It will be noted that the concentration of radioactivity varied considerably in the different tissues. All organs showed a progressive loss of activity with time, the greatest loss occurring in the spleen and intestine.

**Role of the liver in degradation of BUdR.**—In order to evaluate the possible role of the liver in the in vivo degradation of BUdR, a clearance study was done in a rat in which a porto-lumbar vein shunt and ligation of the coeliac artery had been performed. Such a procedure, carried out according to the technic of Bernstein and Cheiker, abolishes the arterial and portal venous blood supply to the liver, and blood can reach the organ only by reflux through the hepatic vein (3). An intravenous injection of 25 \( \mu \)c. (1 \( \mu \)mole) BUdR-Br\textsubscript{82} was given, and, subsequently, serial blood samples were collected through a carotid cannula until the animal's death 27 minutes later. The PCA-soluble fraction of blood aliquots was counted before and after treatment with anion exchange resin. The radioactivity in the eluate was persistently greater than 60 per cent of the activity which was observed in the corresponding samples before resin treatment (Chart 9). The percentage of the intravenously administered activity recovered in the liver at the end of the experiment was less than one-tenth of that percentage which is found in the liver of control animals with an intact hepatic blood supply.\textsuperscript{a}

The data in the previous experiment suggested that the liver plays a major role in the degradation of BUdR. Another experiment was designed to test the capacity of an isolated liver to accomplish this degradation. With the apparatus and technic of Burton et al. (4), the freshly excised liver of a 310-gm. Long-Evans rat was continuously perfused with 30 ml. of heparinized blood obtained from several animals of the same strain. After 90 minutes of perfusion, BUdR-Br\textsubscript{82}, 60 \( \mu \)c. (1 \( \mu \)mole), in 2.7 ml. saline, was added to the perfusing blood. During the next 60 minutes serial 100 \( \lambda \) samples were taken from a reservoir, which collected blood

\textsuperscript{a} Authors' unpublished observations.
both perfusing and bypassing the liver. During the same period, the total bile produced by the liver was collected separately. The Br\(^{82}\) activity in aliquots of the PCA-soluble fraction of the blood samples varied considerably during the first 10 minutes, indicating that mixing of the administered BUdR with the blood was incomplete. Thereafter, the fluctuation of activity of the samples was small, suggesting that mixing was complete. The per cent of Br\(^{82}\) activity recovered in the eluates after resin treatment of the PCA-soluble fractions is illustrated in Chart 9. It can be seen that, during the first 10 minutes, while the BUdR and blood were incompletely mixed, 60–90 per cent of the activity was recoverable in the eluate. Thereafter, the percentage recovery decreased at a rate slightly less than that observed to occur in the intact animal (Chart 9). At the end of the experiment, the bile contained 1 per cent of the administered Br\(^{82}\) activity, of which 84 per cent was retained on the resin.

**Table 1**

**Recovery of Br\(^{82}\) Activity from Ascites Tumor Cells 18 Hours After Injection with KBr-Br\(^{82}\), BUdR-Br\(^{82}\), and BCDR-Br\(^{82}\)**

<table>
<thead>
<tr>
<th>Injected Compound</th>
<th>Dose ((\mu)mole)</th>
<th>Collected Cells (\times 10^8)</th>
<th>Activity, % of dose</th>
<th>Activity, % of dose, washed cells</th>
<th>Br, (\mu)mole/cell (\times 10^{-11})</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBr</td>
<td>0.4</td>
<td>2.8</td>
<td>7.9</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>BUdR</td>
<td>0.4</td>
<td>2.4</td>
<td>8.0</td>
<td>1.77</td>
<td>3.0</td>
</tr>
<tr>
<td>BCDR</td>
<td>0.4</td>
<td>2.0</td>
<td>7.4</td>
<td>2.30</td>
<td>4.6</td>
</tr>
</tbody>
</table>

**Cellular conservation of Br\(^{82}\) activity after administration of labeled BUdR and BCDR.**—To determine to what extent BUdR-Br\(^{82}\) and BCDR-Br\(^{82}\) incorporated into cells in vivo are conserved during successive multiplications, an experiment was designed with the use of ELD ascites tumor cells. Such cells have been shown to incorporate Br\(^{82}\) into DNA on incubation with BUdR-Br\(^{82}\) in vitro\(^19\), and their multiplication can be determined quantitatively in vivo\(^18\). An intra-peritoneal inoculum of \(20 \times 10^6\) ascites tumor cells was given to each of three C57BL mice. Three days later the animals were given injections intra-peritoneally of equimolar (0.4 \(\mu\)mole) and equipotent (2.56 \(\mu\)l) amounts of KBr-Br\(^{82}\), BUdR-Br\(^{82}\), and BCDR-Br\(^{82}\), respectively, dissolved in 0.8 ml saline. The peritoneal fluid of each animal was harvested quantitatively 18 hours later, and the peritoneal cavity rinsed several times with 0.9 per cent saline. The volume of collected ascitic fluid, together with corresponding rinsings of each animal, was measured, and the cell concentration was determined. An aliquot of the cell suspension was used for radioactivity measurements. Another aliquot was subsequently inoculated into new untreated hosts.

The total radioactivity of each of the cell suspensions was calculated from activity measurements in 2-ml portions. These aliquots were subsequently centrifuged, the supernatant discarded, the cells resuspended in 0.9 per cent saline and recentrifuged. The radioactivity of the washed cellular sediment was measured, and the radioactivity confined to the neoplastic cell population of the respective animals calculated (Table 1, column 5). From this value and the specific activity of the bromine, the intracellular concentration of brominated material was calculated (column 6). The concentration was about 50 per cent higher after BCDR treatment than after treatment with BUdR.

Groups of three previously untreated C57BL mice were given inoculations intraperitoneally of 2-ml aliquots (containing \(25 \times 10^6\) cells) of the suspension collected from the KBr-Br\(^{82}\), BUdR-Br\(^{82}\), and BCDR-Br\(^{82}\)-treated animals, respectively. At various intervals up to 90 hours after inoculation, the ascitic fluid of one animal in
each group was collected quantitatively. The concentration and the radioactivity of the sedimented cells were measured, and the total neoplastic cell population and its radioactivity calculated for each animal. The increase of the tumor cell population in the animals is shown as the number of doublings of the cells in the inoculum (Chart 10). The total cellular activity is expressed in percentage of the activity contained in the inoculated cells. It can be seen that the BUdR- and BCdR-treated cells collectively retained nearly all their label during three and four cell generations, respectively.

Effect of x-radiation on the multiplication of ELD ascites cells treated with BUdR.—A group of ten C57BL male mice were given inoculations intraperitoneally of $20 \times 10^6$ ELD ascites cells. Beginning on the 3d day after inoculation, at 6-hour intervals, each of five mice received twelve intraperitoneal injections of 0.5 mg. BUdR dissolved in 0.2 ml. of sterile saline. At the same intervals, the five remaining animals of the group were used as controls and given injections intraperitoneally of 0.2 ml. saline. Six days after the inoculation of tumor cells the animals were killed, and the ascitic fluid of BUdR-treated and control mice was collected and pooled in separate flasks. The cell concentration in both pools was adjusted to $20 \times 10^6$ cells per ml. by the addition of an appropriate amount of saline. The BUdR-treated and control materials, in 3 ml. of diluted ascitic fluid, were each irradiated with 150 r, 350 r, and 700 r, respectively. Subsequently, $2 \times 10^6$ unirradiated cells and cells irradiated with the different doses were inoculated intraperitoneally into respective groups, each consisting of sixteen C57BL male mice. At various intervals after inoculation the tumor cell number was determined quantitatively in pairs of mice taken from each group. The animals were chosen at random at intervals up to 8 days. After this period, only mice with abdominal distension were selected.

Chart 11 illustrates the in vivo growth curves. Unirradiated cells of either BUdR-treated or control material grew similarly. However, irradiation with 150 r and 350 r, respectively, inhibited the multiplication of BUdR-treated cells to a progressively greater extent than that of the controls. Irradiation with 700 r inhibited the progressive multiplication of both kinds of cells. Animals without abdominal distension totaled 31 mice in all groups by the 16th day. The distribution of the mice in the different groups is shown in Table 2. Animals failed to develop tumors in greater number after inoculation of BUdR-treated, x-radiated cells than did mice which received x-radiated control inocula. This difference
is especially noticeable between the groups which received 150 r or 300 r. The mice without apparent tumors, together with six previously untreated mice of the same strain, were then given inoculations of $20 \times 10^6$ ascites cells which were collected from mice used in the routine transplantation of ELD. Except for one mouse which was inoculated with BUdR-treated cells given 700 r, the reinoculated animals survived 14 days without any sign of tumor growth. The six previously untreated hosts succumbed to progressively growing tumors between the 9th and 14th day.

**DISCUSSION**

These results indicate that when bromodeoxyuridine (BUdR) is administered parenterally to the rat, the compound undergoes a dual fate. A large part of the compound undergoes a rapid degradation in the liver, whereas that which escapes degradation diffuses into cells, where it may be subsequently incorporated into DNA. The degradation of BUdR in vivo appears, at least in part, to involve the formation and subsequent debrromination of bromouracil. According to the amount of the bromide-Br$^{82}$ remaining in the circulation 1 hour after the administration of BUdR-Br$^{82}$, the extent of debrromination must be large and is estimated to be at least 80 per cent of the administered dose. That the liver plays a major role in the degradation of BUdR is not surprising. This organ is known to be responsible for the reductive catabolism of pyrimidines in animals (5, 10). Pohl et al. (922) have obtained evidence that, in the human, following administration of 5-bromouracil, there is extensive reduction of the pyrimidine ring with liberation of HBr and subsequent cleavage of the ring. Barrett and West (1) studied the in vivo dehalogenation of a number of halogenated compounds in the rat, and these authors postulated a metabolic reduction of the 5:6 double bond with the formation of dihydropyrimidines with the elimination of HBr, with or without ring hydrolysis. In the case of both thymine and uracil, the reductive steps require reduced triphosphopyridine nucleotide (TPNH) (5). Of considerable interest is the observation that, when the catabolic capacity of the liver was exceeded by giving injections of thymidine every hour for 5 hours, the thymidylate kinase activity of liver and kidney was greatly increased (16). This observation suggests the possibility that a similar series of injections of thymidine to an animal might augment the incorporation into DNA of a subsequent injection of BUdR.

The degradation of BUdR in vivo appears to be analogous to the degradation observed after administration of the iodinated thymidine analog, 5-iododeoxyuridine (I UdR). Within 24 hours following the parenteral administration of IUdR-I$^{131}$ (and KI to prevent thyroidal accumulation of I$^{131}$) to mice, rats, or humans, deiodination of the major portion of the dose, as determined by measurement of urinary excretion of iodide-I$^{131}$, has been reported by a number of investigators (9, 11, 13, 23). In contrast to iodide, bromide is eliminated from the body relatively slowly by urinary excretion, so that short-term measurement of the cumulative excretion of bromide would not be a reliable indicator of the extent of degradation of BUdR. A more accurate estimate of the quantity of BUdR degraded would depend only to a small extent upon the measurement of urinary bromide and to a large extent on the measurement of the total amount of bromide in the extracellular space (independently measured) after a sufficient time has elapsed for the anion to equilibrate in the body.

Owing to the extensive degradation, only a minor part of the intravenously administered BUdR dose is available for incorporation into the cells of various tissues. Once incorporated, BUdR was shown to be retained by the cells of the ELD ascites tumor in the course of four to five successive generations in vivo. Such a conservation of BUdR confirms the expectation that it has been incorporated into a metabolically stable cell constituent and is forwarded to daughter cells with cell division. In a previous study (927), with another ascites tumor labeled with adenine-8-C$^{14}$ or glycine-2-C$^{14}$, an inverse relationship was found between the total cell number and the specific activity of the DNA. The close parallelism between the growth curve and the dilution of the radioactive label indicates a high metabolic stabil-

---

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Inocula harvested from:</th>
<th>0 r</th>
<th>150 r</th>
<th>350 r</th>
<th>700 r</th>
<th>0 r</th>
<th>150 r</th>
<th>350 r</th>
<th>700 r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice without abdominal distension 16 days after inoculation</td>
<td>Saline-treated control mice</td>
<td>1</td>
<td>10</td>
<td>3</td>
<td>4</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ity of DNA, implying that no exchange of the DNA molecule takes place in resting cells and that each cell division is correlated with the formation of a new DNA set per cell, without elimination or partial replacement of the old.

If most of the administered BUdR is rapidly destroyed before it can reach a tissue anatomically remote from the site of its injection, the resulting concentration of BUdR in the DNA of such a tissue may be too low to render the cells hypersensitive to X-rays. Such an explanation may account for the failure to induce sensitization of a subcutaneously transplanted mouse tumor, Hepatoma 134, to X-ray by daily intraperitoneal BUdR doses of 200 mg/kg for 8 days (17), and the failure to induce increased sensitivity of mice to total-body irradiation (21).

When multiple doses of BUdR were given intraperitoneally over a 3-day period to mice bearing the ELD ascites tumor, it was possible to show the subsequent intraperitoneal growth of BUdR-treated cells treated with 350 r was inhibited to a greater extent than that of saline-treated control cells. This indicates that increased radiosensitivity of ascites tumor cells could be achieved if a sufficient local concentration of BUdR was repeatedly made available to the neoplastic cells over a sufficiently long interval. The mice which did not develop tumors after the transplantation of irradiated cells were proved to be refractory also against viable cells at a subsequent challenge. The development of such resistance is in conformity with previous observations on experimental systems in which, as in ours, immunogenetic differences are bound to exist between tumor and host (24, 25). In such tumor-host combinations, irradiated cells bolster the homograft reaction (26), the strength of which can be superimposed upon the inherent radiosensitivity of the graft. Regression itself is, therefore, a rather unreliable criterion of the radiosensitivity in such a case. The radiosensitivity of a homotransplanted tumor may be better reflected by the quantitative assessment of the multiplication of irradiated cells during the first few (1–10) days following transplantation, before host resistance develops.

The extensive degradation, before any sufficient cellular incorporation can occur, presents a major obstacle in using BUdR for radiosensitization in vivo. Our results indicate that the debromination of bromodeoxyxycytidine (BCdR) proceeds at a comparatively slower rate than that of BUdR. A considerable advantage of BCdR over BUdR as a radiosensitizing agent in vivo may be anticipated from this finding and from the following observations: (a) after intravenous injection of BCdR-Br\(^+\), the radioactive label is detected in the DNA of a variety of tissues; (b) BCdR is incorporated, in the form of 5-bromo-2'-deoxyuridylic acid (UdRP), into the DNA of neoplastic cells in place of an equivalent amount of thymidine (7); (c) ascites tumor cells incubated with BCdR-Br\(^+\) incorporated Br\(^+\) in larger amounts than did ascites cells incubated with an equimolar amount of BUdR-Br\(^+\) (Table 1); (d) the Br\(^+\) incorporated by ascites cells after incubation with BCdR-Br\(^+\) was conserved by the cell population during at least four cell generations in a way similar to the cellular retention of the label after BUdR-Br\(^+\) incorporation (Fig. 10); (e) BCdR is as effective as BUdR in inducing radiosensitization in hamster cells in vitro.\(^8\)

After the simultaneous injection of equimolar amounts of thymidine-C\(^+\) and BUdR-Br\(^+\), a rather consistent ratio with a mean value of 2.35 was observed in the C\(^+\)/Br\(^+\) concentrations in the DNA of different tissues which indicated, in conformity with observations in vitro, a preference for thymidine to BUdR incorporation (19). No consistent relationship was found between the concentration of the radioactive labels in the DNA of different organs when thymidine-C\(^+\) and BCdR-Br\(^+\) were administered in equimolar amounts. In this case the C\(^+\)/Br\(^+\) ratios varied widely between 1 and 23. If deamination of BCdR to BUdR, reported by Cramer et al. (7), is a necessary step for incorporation into DNA, the inconsistent C\(^+\)/Br\(^+\) relationship may be interpreted as indicating the existence of considerable differences in the particular deaminase activity of different organs. Accordingly, the relatively large Br\(^+\) concentration observed in the DNA of bone marrow may be the result of a particularly high deaminase activity in this tissue. Such a conclusion is supported by observations reported by Maley and Maley (20) that the bone marrow and thymus are the sole tissues of the adult rat which have an appreciable content of deaminase capable of converting deoxyxycytidic acid (CdRP) to deoxyuridic acid (UdRP). The disproportionately large H\(^+\) concentration in bone marrow DNA after CdRP-H\(^+\) administration may be also explained by the deaminase activity of this organ, if one considers two metabolic pathways for the introduction of the label. After phosphorylation CdRP-H\(^+\) may be incorporated as cytosine-H\(^+\) (6, 12). In the presence of an appropriate deaminase, an additional amount of CdRP-H\(^+\) may be deaminated to UdRP-H\(^+\), which is subsequently converted to thymidic acid and incorporated as thymidine-H\(^+\) (80). Deoxyxycytidic deam-
inase has also been found in relatively large amounts in embryonic and neoplastic tissue (80). It will be important, therefore, to investigate the ability of malignant tissue to metabolize and utilize BCdR, since the selective concentration of this compound or its metabolic products in certain tissues may be of special practical importance in radiotherapy.

REFERENCES


The Distribution and Fate of Bromodeoxyuridine and Bromodeoxycytidine in the Mouse and Rat

Joseph P. Kriss, László Révész, Lucie Tung, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/22/2/254

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.