The ratio CSF-B_{12}/dialysable-B_{12} in serum during the experiments is explicitly lower than the ratio CSF-B_{12}/serum-B_{12} in the control group.

Although only the concentration of dialysable vitamin B_{12} is brought into calculations in the treated group,[3] it is evident that equality is not reached since the ratio has not been as high as in the normal controls. (It has not been possible to demonstrate dialysable vitamin B_{12} in the normal rabbit-serum.)

The ratios show considerable variation but without any significant correlation to time as would have been expected. This may be due to variations in the age of the rabbits, since the blood-brain barrier is known to vary with age.[4]

From these experiments no conclusions can be drawn regarding the mechanism of the transport of vitamin B_{12} from blood to CSF, but the results demonstrate that vitamin B_{12} is transported from blood to CSF with relative ease, since, as already mentioned, the concentration of vitamin B_{12} is raised in the CSF 30 min after injection of the vitamin into the blood stream.

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5-Bromo-2′-deoxycytidine (BCDR)–I. Studies on metabolism *in vitro* and in mice

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HALOGENATED pyrimidine derivatives have been known for more than half a century, but investigations of their metabolic activity in mammalian systems have been conducted only recently. Although 5-iodo-2′-deoxyuridine (IUDR) markedly inhibited the growth of experimental neoplasms in mice,[1] the results obtained in man have been only moderately encouraging.[2] Whether this discrepancy may be explained by the more rapid catabolism of IUDR in man,[2, 3] as compared to the mouse,[4] is not yet clear. In both species there is rapid cleavage of IUDR to 5-iodouracil and dehalogenation to uracil.[3, 4]

Because halogenated derivatives of deoxyuridine can lower the threshold of mamalian cells to radiation-injury,[5, 6] the analogous derivatives of 2′-deoxycytidine have been investigated in the hope that these molecular species might have greater metabolic stability, a circumstance which could result in their more extensive incorporation into the acid-soluble nucleotide pools, as well as into deoxyribonucleic acid (DNA). The possible advantage of the use of the deoxycytidine derivatives as chemotherapeutic agents, because of their potentially greater resistance to metabolic degradation, has been discussed elsewhere.[7] Biochemical evidence suggests that the presence of the amino group, in lieu of oxygen in position 4 of the pyrimidine ring, should result in greater metabolic stability.[8, 9] These studies have shown that the enzyme, deoxycytidylate (dCMP) deaminase, present in rapidly growing mammalian tissues, e.g., neoplasms, embryonic tissues, regenerating liver, thymus, and bone marrow, is absent or is suppressed,[8, 9, 10] in most normal tissues. Thus, 5-bromo-2′-deoxycytidine (BCDR)[11] and 5-iodo-2′-deoxycytidine (ICDR)[12] might be expected to exhibit relatively selective action for tumour tissues, since many mouse, rat and human neoplasms are rich in dCMP deaminase. A previous note[13] has demonstrated that ^{3}H-BCDR is incorporated into the DNA of cells as 5-bromo-2′-deoxyuridine 5′-phosphate, replacing an equivalent amount of thymidylic acid, but it is not yet

certain whether phosphorylation precedes or follows deamination. However, the metabolic conversion of BCDR to deaminated derivatives has been observed both in a cell-free system and in intact neoplastic murine mast cells (P8I5Y).[13, 14]

The triphosphate of BCDR was first synthesized by Markham, as reported by Bessman *et al.*[15]; later, Chang and Welch[11] described an improved synthesis of BCDR, as well as the preparation of the ^{3}H-labeled compound. In an abstract by Cramer *et al.*[13] and in an oral report by Kriss *et al.*,[16] the latter describing the use of ^{82}Br-BCDR, BCDR was shown to be more stable metabolically in the mouse and rat than is 5-bromo-2′-deoxyuridine (BUDR).

The present report is concerned with studies of the metabolism of ^{3}H-BCDR.

Studies in vivo. Ten mice were placed individually in glass beakers and the urine was collected (for either a 4-hr or a 24-hr period) on filter paper protected by a wire screen. Following the administration to each mouse of a single injection of ^{3}H-BCDR (1 μmole; 6×10^{5} cpm), 5 mice were sacrificed after 4 hr and the remainder after 24 hr. The filter paper was eluted with water in a Waring blendor and the eluate was filtered; this extract, adjusted to pH 10·5, was passed through a Dowex-1 formate column (200–400 mesh, $30 \times 2{\cdot}5$ cm); the column was subjected to gradient elution with formic acid as was done previously in similar studies of IUDR.[4] Although the many compounds excreted have not yet been identified or quantitated, at least six major radioactive fractions were obtained in the 4-hr specimen of urine, and at least eight such fractions were present after 24 hr.

It has been reported that following the administration of either 6-azathymine,[17] IUDR,[4] or 5-bromouracil,[18] free uracil appears in the urine of mammalian species; these studies have shown that BCDR causes a similar result. The uracil excreted is derived from at least two sources: (1) the dehalogenation of 5-bromouracil derived from BCDR, and (2) the inhibition of the enzymic degradation of uracil (derived from normal metabolic precursors) in a manner comparable to that reported previously with 6-azathymine.[17, 19] The specific activity of the uracil excreted in the urine was 0·5 per cent of that of the ^{3}H-BCDR administered; in a previous experiment with ^{3}H-IUDR,[4] the specific activity of the uracil excreted in the urine was 3 per cent of that of the IUDR.

Studies in vitro. Findings related to the stability of BCDR to metabolic degradative attack *in vitro* are shown in Table 1. When BCDR was incubated with homogenates of adult mouse liver, as much as 87 per cent of the substrate was recovered intact after incubation for 1 hr; with slices of mouse

TABLE 1. INCUBATION OF BCDR WITH LIVER, BLOOD AND INTESTINAL MUCOSAL PREPARATIONS

Incubation time	BCDR recovered			
	Liver		Whole blood	Dialysed rabbit intestinal mucosa
min	Homogenate %	Slices %	%	%
3	93	—	—	—
10	95	—	—	—
60	87	94	—	—
120	—	—	—	97
180	—	75	—	—
480	—	—	97	—

The incubation medium with liver homogenate (final volume, 3·0 ml) consisted of: 100 μmoles of potassium phosphate buffer (pH 7·4), 120 μmoles of nicotinamide, 200 μmoles of KCl, 50 mg of liver homogenized in 0·25 M sucrose–0·002 *M* $CaCl_2$ and 3·2 μmoles of BCDR; with liver slices (final volume, 3·1 ml): 3·0 ml of Krebs III-phosphate buffer,[20] 70–80 mg of liver slices and 3·2 μmoles of BCDR; with blood: 3 ml of whole human blood with heparin and 4·8 μmoles of BCDR in 0·5 ml of water, gas phase was O_2:CO_2 (95:5); with intestinal mucosal enzyme (final volume, 3·0 ml): 1·5 ml of Krebs III-phosphate buffer, 100 μmoles of ATP, 4·8 μmoles of BCDR and dialysed enzyme preparation (scraped mucosal tissue, freeze-thawed four times, dialysed against water overnight, and the dialysand taken up in Krebs III-phosphate buffer). All incubations were performed at 37° with air as the gas phase, unless indicated otherwise. The incubations were terminated by the addition of perchloric acid (final concentration, 0·2 N) or TCA (final concentration, 5%) and the acid-soluble compounds were separated on Dowex-1 formate columns (1×25 cm) by gradient elution with formic acid.

liver, 94 per cent and 75 per cent of the added BCDR was recovered unchanged after incubation for 1 hr and 3 hr, respectively; 5-bromocytosine was not produced under these conditions. Of BCDR incubated for 6 hr with whole human blood, almost all was recovered unchanged. When BCDR was incubated with a dialysed preparation of either mouse or rabbit intestinal mucosa, rich in enzymic activity for the phosphorolysis of IUDR and BUDR, 97 per cent recovery of the substrate occurred.

DISCUSSION

On the basis of presently available information, the resistance of BCDR to catabolic influences within the intact mouse, as compared to that observed with either BUDR or IUDR, has been disappointing. Nevertheless, quantitative measurements of the sequence and products of biochemical transformations, responsible for this metabolic degradation, should be made with various tissues, including neoplasms, in order to determine whether intrinsic differences exist between BCDR and BUDR which could permit preferential utilization of the former by cells. Such studies, now in progress and reported on in part in the following communication, are concerned with the entrance of BUDR and BCDR into the acid-soluble fraction of cells, the incorporation of these compounds into DNA, and their effects on the threshold of cells to radiation injury.

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