tigens, hemocyanin and ferritin, in rats and mice. This effect occurs not only when antigen is administered in the chamber but also after insertion of a saline-filled chamber followed by intraperitoneal injection of the antigen. Chamber components were shown to be not responsible for the enhanced antibody production. The possible mechanism has been discussed in terms of a depot function and also in terms of the cellular response on and around the chambers. Also discussed were the implications of these findings on studies of antibody formation in diffusion chambers.

1. Algire, G. H., Borders, M. L., Evans, V. J., *J. Nat. Cancer Inst.*, 1958, v20, 1187.

2. Holub, M., Riha, I., *Mechanisms of Antibody Formation*, M. Holub, L. Jaroskova, eds., Academic Press, 1960, 30.

3. Fishman, M., *J. Exp. Med.*, 1961, v114, 837.

4. Boyden, S. V., *ibid.*, 1951, v93, 107.

5. Snedecor, G. W., *Statistical Methods*, Iowa State College Press, 1940.

6. Granick, S., *J. Biol. Chem.*, 1942, v146, 451.

7. Amos, D. B., *Transplantation of Cells and Tissues in Diffusion Chambers*, R. E. Billingham, W. K. Silver, eds., Wistar Inst., 1961, 69.

8. ———, *J. Nat. Cancer Inst.*, 1958, v21, 657.

Received July 30, 1962. P.S.E.B.M., 1962, v111.

Antimitotic Action of 5-(2'-Bromoacetamido)-Uracil on the Ehrlich Ascites Tumor.* (27894)

TADASHI A. OKADA AND EUGENE ROBERTS
Department of Biochemistry, City of Hope Medical Center, Duarte, Calif.

Since the first reports of the antimetabolic effects of 5-fluorouracil (5-FU)† by Heidelberger *et al.*(1,2), there has been considerable interest in this and other halogenated pyrimidines. The antimetabolic activity of these compounds has been attributed largely to their ability to interfere with the deoxyribonucleic acid synthesis of several lower organisms and transplanted tumors of animals. In related studies, Visser(6) and his co-workers tested 5-(2'-bromoacetamido)-uracil (BAU)† on wild and mutant strains of *Neurospora* and found that this compound inhibited growth of the wild type. In this paper are reported observations on the cytological effects of BAU on Ehrlich ascites tumor cells and the results are compared with those obtained with 5-fluorouracil.

Materials and methods. A mouse tumor, Ehrlich ascites tumor (hypotetraploid strain), was used to study the effects of BAU and 5-FU. The ascites tumor was transmitted serially in Swiss/HaICR mice weighing 18-20 g, fed on a stock diet of Purina chow and water. Intraperitoneal injections of freshly prepared aqueous solutions or suspensions of chemicals usually were begun on the fifth day after transplantation of the tumor, when the tumor was well established and active proliferation of the tumor cells was taking place in the peritoneal cavity. At various intervals, samples of tumor ascites were removed by peritoneal puncture, squashed with acetic dahlia, and examined cytologically. The numerical data were calculated on the basis of approximately 500 interphase and dividing cells for every sample.

Results. 1. *Effects of 5-(2'-bromoacetamido)-uracil (BAU).* Serial cytological studies were made of tumor cells obtained from mice after single injections of BAU on the fifth day after tumor transplantation. The following numbers of mice were employed at each dose level: 10 received 1 mg; 11, 2 mg; and 3, 3 mg. The life span of the animals receiving 1 and 2 mg was the same as that of the controls, 10-12 days after

* This work was supported in part by USPHS grant.

† The authors gratefully acknowledge the generosity of Dr. D. W. Visser, Univ. of Southern California, Los Angeles, who supplied the sample of BAU, and Dr. M. J. Shiffrin, Hoffmann-LaRoche, Inc., Nutley, N. J., who supplied the sample of 5-FU.

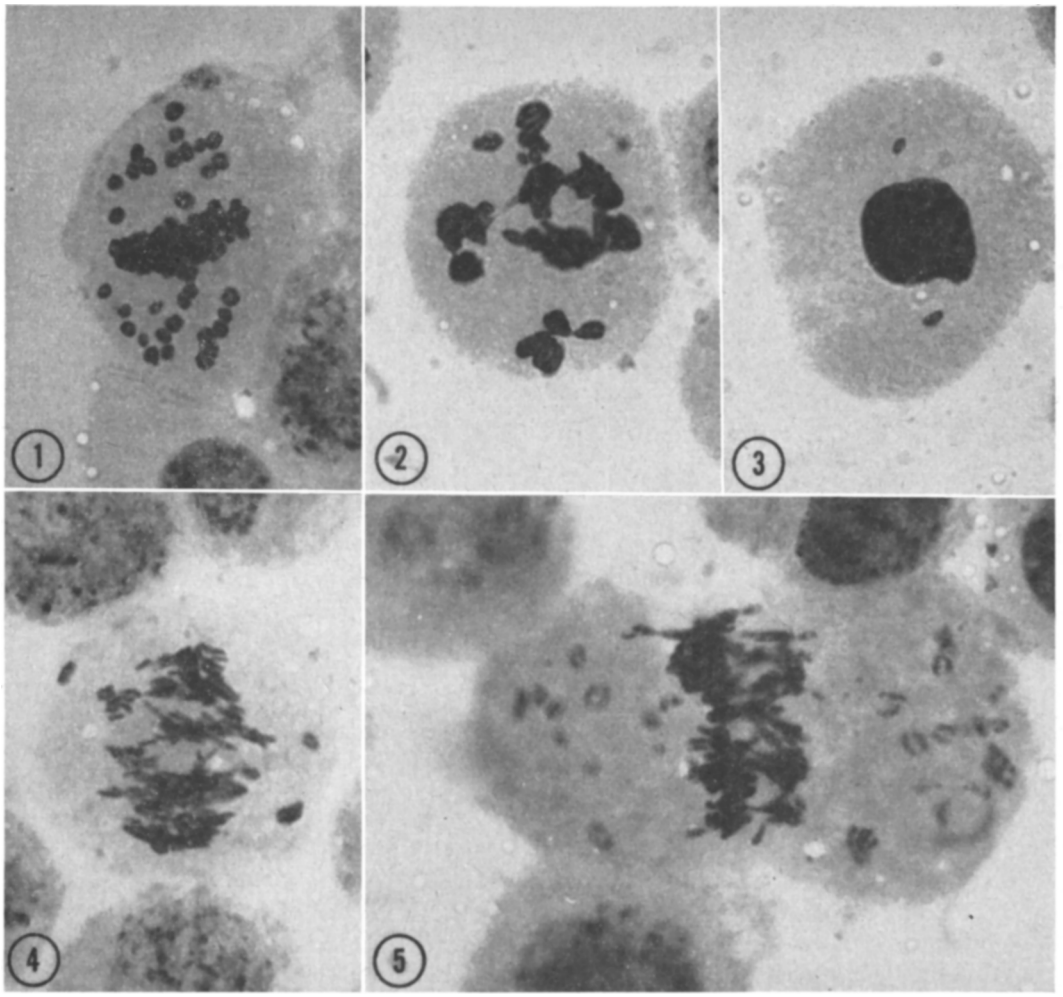

FIG. 1-5. Various types of abnormalities induced by inj of 2 mg of BAU as described in text. ×1170.

transplantation. The animals receiving the 3 mg dose died sooner, suggesting a toxic effect of the drug.

Results are tabulated in Table I. Within the first 2 hours after injection of BAU, only a few normal mitotic figures were still observable; most had disappeared, and numerous abnormal mitotic figures were present (Fig. 1-5). This condition usually persisted from 6 to 12 hours with 1 mg doses, and for longer periods at the higher dosage levels. Also larger numbers of abnormal mitotic figures were observed at higher doses. After the appearance of abnormal mitotic cells, tumor cells with pycnotic nuclei were observed in the same ascites fluid as early as

TABLE I. Mitotic Frequency of Ehrlich Ascites Tumor After Treatment with 5-(2'-Bromoacetamido)-Uracil.

Dose, mg	Mitotic cells (% of total)							
	At injection		3 hr		10 hr		24 hr	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
1	4.6	0	2.4	8.6	1.1	2.3	1.2	2.2
2	4.3	0	.6	3.8	.3	7.5	0	4.3
3	4.5	0	.3	2.8	.3	4.4	0	9.7

TABLE II. Mitotic Frequency of Ehrlich Ascites Tumor after Treatment with 5-Fluorouracil.

Time after injection (hr)	Mitotic cells (% of total)		
	3 mg	5 mg	10 mg
0	4.6	4.3	4.3
0.5	2.9	3.2	2.4
1	2.1	3.6	1.6
3	2.2	3.5	1.3
6	1.9	3.0	.4
12	3.0	3.0	—
24	3.0	2.3	3.0

10 hours, 6 hours and 3 hours after 1 mg, 2 mg, and 3 mg doses respectively.

The following description of sequential cytological changes is based upon observations made on cells from mice receiving single doses of 1 mg and 2 mg. In cells of the mouse which received 1 mg, a gradual decrease occurred in the number of normal-appearing dividing cells about 1 hour after injection. Disintegration of some tumor cells was observed at 3 hours and shortness and stickiness of the chromosomes was observed (Fig. 1, 2). A few chromosomes were scattered in the cytoplasm. Despite abnormalities, chromosomes in metaphase often proceeded to anaphase and telophase when they appeared to begin dividing in an irregular manner (Fig. 4, 5). Tripolar divisions were observed and often displacement was seen of several chromosomes from the main spindle region. Dividing cells from mice which received 2 mg showed visible damage 1 hour after injection. The most remarkable change at this dose level was the high degree of stickiness of the chromosomes, with 2 or 3 usually being scattered in the cytoplasm. At 6-24 hours, many metaphase figures contained chromosomes strongly contracted into balls or masses (Fig. 3). In the same ascites, tumor cells with pycnotic nuclei were observed, and such cells increased in number with time.

2. *Effects of 5-fluorouracil (5-FU).* A single intraperitoneal injection of 5-FU was made into each tumor-bearing mouse. The number of mice used at each dose level was: 2 received 3 mg; 4, 5 mg; and 4, 10 mg. The life span of the treated animals was not prolonged, and remained the same as that of the controls.

Results are summarized in Table II. In all groups, within 30 minutes to 6 hours after injection mitotic frequency in the tumor ascites decreased.

In addition to a lessening of mitotic activity, the number of tumor cells per unit volume of ascites fluid dropped sharply 3 hours after the injection, although in the 24-hour samples the number gradually increased again. Within 24 hours after injection of the drug, the amount of ascites still was somewhat reduced, but by 48 hours, the tumor had begun to regrow, and many tumor cells were in active division. During the entire experimental period no abnormal mitotic cells were seen in the ascitic fluid.

From these results it can be concluded that the inhibitory effect of 5-FU on tumor growth is not correlated with easily observable cytological damage. This drug exerts no readily demonstrable injurious influence on the cytoplasm of the interphase and mitotic cells, or on the chromosomes of mitotic cells.

3. *BAU combined with other chemotherapeutic agents.* In an attempt to produce synergistic therapeutic effects in tumor-bearing animals without increasing toxicity, a number of experiments were performed in which the following compounds in varying amounts of radiation were administered simultaneously with 2 mg of BAU: 3 mg of maleuric acid (MA)(4); 2 and 2.5 mg of β-hydroxyethyl N-carbamylmaleamate (βOH)(5); and 200 r of localized X-ray.

BAU with MA or βOH: In addition to the expected chromosomal abnormalities produced by BAU, severe distortion of cytoplasm of tumor cells due to blebbing was observed 1 hour after simultaneous injections of MA or βOH with BAU. Results of these treatments showed that BAU combined with MA and βOH was more destructive at a cytological level than these substances alone, although there was no evidence that the life span was prolonged. *BAU with X-ray:* This treatment produced the same effect as treatment with BAU alone.

4. *Protective action of glutathione (GSH) and cysteine.* When 25 mg of GSH (pH, 7.0) was injected intraperitoneally into tu-

mor-bearing mice simultaneously with 2 mg of BAU, the chromosomal abnormalities usually produced by BAU were not seen. Only a few abnormal mitotic cells were observed in the ascites within the first 6 hours after the injection, and none thereafter. In these cells, the abnormalities were much less severe than those produced by BAU alone. Occasionally 2 or 3 chromosomes were scattered in the cytoplasm while the rest retained their normal appearance and arrangement on the metaphase plate. An experiment was then performed in which 25 mg of GSH was injected 10 minutes after administration of 2 mg of BAU, and 30 minutes after. Average results for 3 animals with each treatment showed that under these conditions GSH did not protect the cells from damage by BAU. Similar experiments were carried out employing cysteine (25 mg). No abnormal mitotic cells appeared after simultaneous injection of the latter with BAU. Cysteine injected 10 minutes after administration of BAU offered no protection against BAU.

Discussion. In the doses employed BAU appeared to produce a variety of abnormalities in mitotic tumor cells, causing a decrease in mitotic rate from 3 to 24 hours after injection, but apparently not completely inhibiting all cells from entering division. The extent of morphological abnormality produced in the mitotic cells increased with increasing dose of BAU.

BAU acts against proliferating cells. When BAU was administered, cells already in division, as well as those which entered division while the drug was still active, showed the typical cytological effects described. Cells in various stages of interphase also may be affected by BAU, but the effects may not become apparent until the cell enters division. However, these abnormalities of chromosomes are prevented when GSH or cysteine are injected simultaneously with BAU.

According to Heidelberger *et al.*(1,2) and Linder(3), 5-FU prevents the growth of transplanted tumors by interfering with DNA synthesis in tumor cells without producing any morphological damage. Furthermore, both thymidine and uridine reversed the effects of 5-FU but not those of BAU. Therefore, it seems clear that the effect of BAU on tumor cells is different from that of 5-FU. The work reported herein suggests that BAU may form free radicals in aqueous solution and that this compound may act as an alkylating agent or as an initiator of free radical chains. The type of cytological damage observed and the protection against this damage afforded by GSH and cysteine are compatible with the latter suggestion. It is interesting in this connection that glutathione also prevented the damaging effects of maleuric acid(4).

Summary. 1. A single intraperitoneal injection of 5-(2'-bromoacetamido)-uracil (BAU) into mice bearing the Ehrlich ascites tumor produced marked mitotic abnormalities which persisted for long periods and resulted in appearance of tumor cells with pycnotic nuclei. 2. This chromosomal damage could be prevented by injecting glutathione or cysteine simultaneously with BAU. 3. It was suggested that BAU might act as an alkylating agent *via* free radical formation. 4. Administration of a single dose of 5-fluorouracil temporarily decreased the concentration of ascites cells in ascites fluid and also the mitotic frequency, but no morphological alterations were observed in the tumor cells. 5. It was concluded that the mechanism of action of the above two 5-substituted uracils on Ehrlich ascites cells was different.

1. Heidelberger, C., Chaudhuri, N. K., Danneberg, P., Mooren, D., Griesbach, L., Duschinsky, R. J., Pleven, E., Scheiner, J., *Nature,* 1957, v179, 663.

2. Heidelberger, C., Griesbach, L., Montag, B. J., Mooren D., Cruz, O., Schnitzer, R. J., Grunberg, E., *Cancer Research,* 1958, v18, 305.

3. Lindner, A., *ibid.,* 1959, v19, 189.

4. Okada, T. A., Roberts, E., *ibid.,* 1960, v20, 1154.

5. ———, *Proc. Am. Assn. Cancer Research,* 1961, v3, 256.

6. Visser, D. W., *Antimetabolite and Cancer, A.A.A.S.,* Washington, D. C., 1955, v47.

Received July 30, 1962. P.S.E.B.M., 1962, v111.