significant increase in liver to serum AIB ratios ($P > 0.05$); however, a significant decrease in muscle: serum AIB ($P < 0.001$) occurred.

Whether the uptake of α-amino*iso*butyric acid serves as a satisfactory model for the transport of all amino acids is unknown. It has recently been observed that its distribution is similar to that of glycine.[6] In any event, we have described a very early estrogen effect on the accumulation of this α-amino acid which corresponds in time with changes in distribution and metabolic fate of natural amino acids. Although this effect of estrogen is most pronounced in the uterus, there is a perceptible shift in the distribution of AIB in muscle in a direction opposite to that observed for the uterus.

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Potentiation of the carcinostatic action of azauridine by chloramphenicol*

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CHLORAMPHENICOL, a well-established antibiotic agent in the chemotherapy of bacterial infections, has also been shown to depress hematopoiesis in man.[1, 2]

This toxic effect upon bone marrow function led to further investigation of the possible utility of chloramphenicol as a carcinostatic agent in the treatment of experimental[3, 4, 5] and human neoplasms.[6] Although ineffective as a carcinostatic agent *in vivo*, chloramphenicol inhibits the growth of mammalian cells in culture.[7]

Observations made during the course of treatment with 6-azauridine (the ribonucleoside of 6-azauracil)[8] of three patients with leukemia,† suggested that potentiation of its action by chloramphenicol might have occurred when the antibiotic agent was given for the treatment of intercurrent bacterial infections. Preliminary studies of combinations of these compounds in the chemotherapy of an experimental neoplasm in mice have confirmed this impression and the results are presented in this communication.

METHODS

Male DBF_1 hybrid mice, 6–8 weeks old and weighing 19–24 g, were used in all experiments. All animals were fed powdered Purina Lab Chow, with and without chloramphenicol‡ added in concentrations ranging from 0·5 to 2·0 per cent. Azauridine§ was added to the drinking water of

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appropriate groups of animals in concentrations ranging from 0·15 to 0·5 mg per ml and was available *ad libitum*. Transmission of the L5178Y leukemia was made by removing, from a freshly sacrificed donor mouse, 1 ml of ascites fluid and immediately transferring this to a test tube containing 9 ml of culture medium;[9] of this dilution of ascitic fluid, 0·1 ml, containing 1·5–3·0 × 10^6 viable leukemia cells, was injected subcutaneously into the right flank of each mouse, in order to produce a solid neoplasm. In all the experiments the recipient mice were inoculated with the leukemic cells within 15 min after the withdrawal of the latter from the donor mouse. Treatment with azauridine and chloramphenicol was always begun 24 hr after the subcutaneous inoculation of the neoplastic cells into individually weighed mice. On the day following the final dosage of either azauridine or chloramphenicol, or of both agents, each mouse was weighed, sacrificed, and its tumor was removed and weighed. The average weight of the tumors in each treated group was compared with that of the controls, and the results were recorded in terms of percentage inhibition of neoplastic growth.

For experiments *in vitro*, L5178Y cells were grown in culture, as described by Fischer *et al.*[10], in the presence or absence of various concentrations of chloramphenicol.

RESULTS AND DISCUSSION

In the studies of the effect of chloramphenicol upon the growth of the L5178Y cells *in vitro*, concentrations of the antibiotic agent of 10–100 μg per ml of tissue culture medium inhibited the rate of cellular reproduction; the concentration required for 50 per cent inhibition of growth was 10μg per ml of tissue culture medium.

In contrast to these results with chloramphenicol in culture, even high doses of the antibiotic agent, when tested for activity with respect to the inhibition of the growth of the lymphoblasts growing as a solid tumor in mice, caused only moderate carcinostasis (Table 1). However, in combination with azauridine, chloramphenicol, in doses which by themselves caused no inhibition of growth, potentiated the carcinostatic effect of azauridine (Table 1). Achromycin, an antibiotic with a similar spectrum of antibacterial activity to that of chloramphenicol, failed to increase significantly the inhibition of tumor growth obtained with azauridine alone (Table 1).

TABLE 1. EFFECTS OF AZAURIDINE, CHLORAMPHENICOL AND ACHROMYCIN ON THE GROWTH OF LYMPHONAS (L5178Y) IN MICE

Treatment*	Change in body weight, g	Mortality	Average tumor weight, mg (± s.e.)	Imhibition of tumor weight per cent
Controls	+ 2·0	0/10	363 ± 17·7	
CAP, 0·5%	− 0·5	0/10	326 ± 24·7	10
CAP, 1·0%	− 2·0	0/10	200 ± 23·8	45
Achromycin, 1·0%	+ 0·5	0/10	276 ± 24·4	25
AzUR, 0·25 mg/ml	− 0·5	0/10	137 ± 18·8	62
AzUR, 0·25 mg/ml, + achromycin, 1·0%	− 3·0	0/10	121 ± 16·2	66
AzUR, 0·25 mg/ml, + CAP, 0·5%	− 4·0	0/10	58 ± 8·7	84†
Controls	+ 2·0	0/20	242 ± 18·6	
CAP, 0·5%	+ 1·1	0/20	277 ± 20·2	0
AzUR, 0·15 mg/ml	+ 0·7	0/20	174 ± 16·1	29
AzUR, 0·15 mg/ml, + CAP, 0·5%	− 1·4	0/20	79 ± 10·2	67‡

* For chloramphenicol (CAP) and achromycin, the concentrations given refer to the percentage of the antibiotic agent in the diet, for azauridine (AzUR), the concentrations given refer to those administered in the drinking water.

† AzUR vs. AzUR + CAP: $P = 0{\cdot}001$.

‡ AzUR vs. AzUR + CAP: $P < 0{\cdot}001$.

The mechanism by which chloramphenicol potentiates the effects of azauridine has not yet been established. It is known that azauridine, after its intracellular enzymic conversion to azauridine 5′-phosphate, acts as a competitive inhibitor of orotidylic acid decarboxylase, in this manner interfering with the biosynthesis of pyrimidines *de novo*.[11, 12] Evidence has been presented that, in bacteria, chloramphenicol may exert an inhibitory effect primarily upon protein synthesis;[13, 14] however, it has not been shown that this antibiotic agent can produce a similar effect in mammalian cells. On the other hand, as demonstrated in this laboratory[15] and elsewhere by Fallon *et al.*,* the rate of conversion of orotic acid, via orotidylic acid, to uridine nucleotides by isolated leukemic cells from patients treated with azauridine is generally inhibited at first; subsequently, however, the magnitude of these conversions in increased to levels close to or above those found in cells obtained before treatment with azauridine. This increased enzymic activity may reflect the presence in the cells of higher levels of the enzyme orotidylic acid decarboxylase.[16] If these assumptions are correct, chloramphenicol may suppress this attempt on the part of the cells to compensate for the inhibition by azauridine 5′-phosphate of the decarboxylation of orotidylic acid, through the formation of additional orotidylic acid decarboxylase, and perhaps of enzymes concerned with earlier steps in the pathway of pyrimidine biosynthesis *de novo*.

Regardless of the mechanism involved, the potentiation of the carcinostatic effect of azauridine by chloramphenicol suggests that controlled studies should be carried out with combinations of these two drugs in the treatment of human neoplastic disease. Although the data obtained in mice with this combination show that chloramphenicol increases the toxicity of azauridine for the host, the situation may not be comparable to that which obtains in man, since treatment with azauridine of approximately 50 patients has not disclosed any appreciable toxicity, while in many cases it has favorably influenced the course of certain types of acute leukemia.† In fact, azauridine is a unique agent in man, since even massive doses have failed thus far to inhibit normal hematopoiesis,† a situation in marked contrast to that which obtains with other anti-cancer agents, or, indeed, with azauridine in such mammalian species as the dog.[17]

In conclusion, evidence has been presented to indicate that chloramphenicol potentiates the carcinostatic effect of azauridine upon L5178Y cells, when grown as lymphomas in mice, as well as the toxicity of azauridine for this host. A possible mechanism by which this potentiation is achieved has been discussed, and the use of chloramphenicol in combination with azauridine in the treatment of neoplastic disease in man is suggested. Although the increased potency of the combination of azauridine and chloramphenicol engenders greater host-toxicity in mice, selectivity of action might be obtained in man, since azauridine by itself has evoked no significant toxicity in leukemic patients in whom the drug has caused unequivocal evidence of objective improvement.

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† These studies, carried out by groups at Yale, the National Cancer Institute, and the Roswell Park Memorial Institute, have been summarized, in part[17, 18] and full papers describing these clinical investigations are in preparation.

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