## PHARMACOLOGY

### Inhibition of Ascites Tumour Growth by the Trypanocide, Ethidium Bromide, in Combination with Azaserine

ETHIDIUM bromide (3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide) has been used to treat trypanosome infections of cattle in Africa for some time[1,2]. (This chemical was kindly supplied by Dr. M. R. Gurd, Boots Pure Drug Co., Ltd., Nottingham. Its alternative name is 2,7-diamino-9-phenyl-10-ethyl-phenanthridinium bromide.) Recently we have examined the biochemical effects of this compound on Ehrlich ascites tumour cells, and have found that it inhibits completely the incorporation of preformed purines (adenine, guanine and hypoxanthine) into nucleic acids[3]. The possible application of this biochemical effect to cancer chemotherapy was immediately considered. It was believed, however, that ethidium bromide, if used alone, would at best be only moderately carcinostatic because it does not appreciably impair the *de novo* pathway of purine biosynthesis[3]. Because the antibiotic azaserine is well known to inhibit completely the *de novo* synthesis of purines, it was therefore believed that if these two compounds could be used in combination without undue host toxicity, the conditions for a complete concurrent blockade[4] of the purine nucleotide supply for nucleic acid synthesis could be effected. Because both drugs maintain their inhibitory effects for less than 24 hr.[3,5], for more nearly maximal effect it was felt that both should be administered more often than in the daily treatment schedule usually used in tests of carcinostatic efficacy.

Female Swiss mice, 26–28 gm., were inoculated intraperitoneally with approximately $1 \times 10^6$ Ehrlich ascites carcinoma cells (hypotetraploid line). Morning and evening drug treatments were begun the following day and continued for 6 days. The mice were weighed at the beginning and end of this period. Both drugs were administered in solution in isotonic saline, and control groups received saline injections. The experiments were terminated 50 days after tumour implantation, and the average survival times calculated. All animals alive at 50 days were considered to be 50-day survivors in calculating the average survival-time. The animals received food and water *ad lib*. Averaged results from two experiments are presented in Table 1.

Table 1. TREATMENT OF EHRLICH ASCITES TUMOURS WITH COMBINATIONS OF ETHIDIUM BROMIDE AND AZASERINE

| Dose (mgm./kgm.) Ethidium bromide | Azaserine | Average survival-time (days) | 50-day survivors/ total No. of animals | Average weight change (gm.) |
|---|---|---|---|---|
| 0 | 0 | 8·9 ± 1·9* | 0/14 | + 6·2 |
| 0 | 0·1 | 17·5 ± 1·8 | 0/14 | + 2·0 |
| 4 | 0 | 11·8 ± 3·8 | 0/14 | − 1·1 |
| 3 | 0 | 12·5 ± 3·4 | 0/14 | + 2·2 |
| 4 | 0·1 | 34·8 ± 8·3 (31·8)† | 7/14 | − 1·6 |
| 3 | 0·1 | 26·7 ± 9·7 (23·6)† | 6/14 | + 1·2 |

* Average deviation from the mean.
† Numbers in parentheses are the average survival-times of the animals which did not survive 50 days.

Ethidium bromide prolonged survival-time slightly if at all at a dose of 4 mgm./kgm., which was the highest dose which could be given on this treatment schedule without excessive weight loss or death[3]. The combination of azaserine with this dose of ethidium bromide, however, increased survival-time almost 400 per cent, which clearly indicates potentiative action. Of particular interest was the finding that half the mice so treated survived 50 days and were free of gross tumour growth at that time.

To our knowledge, phenanthridinium compounds have not previously been shown to have carcinostatic properties, alone or in combination with other drugs. It is hoped that the carcinostatic effects of ethidium bromide will be examined in more detail, and that closely related compounds will be examined for their biochemical and biological effects.

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### Inhibition of Release of Corticotrophin

MOST present concepts of the mechanism of stress activation of the adenohypophysis include the idea of neuro-endocrine 'releasing factors' which liberate a portion of the stored preformed corticotrophin[1]. In contrast to the depletion of corticotrophin demonstrated by Rochefort *et al.*[2] during prolonged stresses, we have not been able to demonstrate any depletion after stress of short duration during the period of marked elevation of circulating corticotrophin. In adrenalectomized animals, there is actually an elevation of adenohypophyseal corticotrophin content coincident with the period of peak blood-level of corticotrophin[3]. This has led us to consider that, in the pituitary, stimulation of biosynthesis of corticotrophin is a very important element in the response to acute stresses. For this reason, we felt that it might be of interest to determine whether a blockade of corticotrophin biosynthesis would have any effect on the subsequent ability to release this hormone. We examined the effect of ethionine pre-treatment upon the stress-induced secretion of corticotrophin in rats.

D,L-ethionine (25 mgm./c.c. in saline) was administered subcutaneously in doses of 50 mgm. to groups of 150 gm. female Wistar rats. At various time-intervals after this treatment, the rats were stressed by 1 min. of ether anæsthesia followed by sham unilateral adrenalectomy. At 2·5 min. following the onset of the stress, the rats were decapitated, bled, and the mixed trunk blood assayed for corticotrophin activity by measuring adrenal ascorbic acid depletion according to the method of Hodges and Vernikos[4]. Control groups were pre-treated with saline or with ethionine plus a small molar excess of methionine.

This experiment (Table 1) demonstrates clearly the inhibitory action of ethionine on the acute secretion of corticotrophin after stress. The inhibition is well established in as short a time as 1 hr. of pre-treatment,