Induction of synchronous cell division in mammalian tumors is an intriguing possibility. X-ray and numerous tumoricidal agents depend, for their cytotoxic action, upon mechanisms associated with cell division. Any procedure which would result in an increased number of cells dividing might increase susceptibility of the tumor to such agents over that to be expected with randomly dividing cells.

Several investigations have shown, in lower forms of life, that some degree of mitotic synchrony can be induced by cyclic temperature fluctuations and by chemical means, as reviewed by Prescott (5). Division synchrony in HeLa cells in tissue culture was observed by Newton and Wildy (4) with a single change in temperature. The use of hyperthermia in the adjunctive treatment of tumors has been reviewed by Selawry et at. (7). The effects of heat on cells in tissue culture have been studied by Selawry (8). More recently, Crile (3) has studied the effects of single heat exposures in experimental mouse tumors, with and without x-ray treatment, and has observed apparent augmentation effects.

It was the purpose of this investigation to evaluate the effect of cyclic thermal stimuli on the Walker 256 rat carcinoma as an extension of our previous studies (2) and specifically to determine the effect of intermittent hyperthermia upon mitotic activity of this tumor. The experimental observations to be reported show that synchrony can be induced in this mammalian tumor.

MATERIALS AND METHODS

Adult Sprague-Dawley rats of the Holtzmann strain, weighing approximately 200 gm., were used. The experimental tumor was the rat Walker 256 carcinoma obtained from the University of Rochester 4 years ago. The tumor has been transplanted bi-weekly by trocar and has not changed strikingly in either its growth characteristics or histologic appearance during this time. Two weeks after transplantation into the ventral subcutaneous tissue rats were placed in individual cages and given free access to food and water. The method of intermittent hyperthermia was similar to that described by Brett and Schloerb (2). Over each cage, at a distance of 60 cm., was mounted an infrared lamp (250 watts). The lamps could be turned on at selected intervals for predetermined periods by a program timer.

The experimental objective was to measure the incidence of tumor mitoses hourly after cessation of intermittent hyperthermia for varying intervals. Groups of rats with transplanted tumors were exposed to intermittent hyperthermia for periods of 15 minutes' duration at intervals of 3, 6, or 12 hours. At intervals after cessation of the heat two rats from each group were sacrificed by ether inhalation, and the tumors were weighed and examined histologically after hematoxylin and eosin staining. A total of 4000 cells were counted on each slide, and the mitotic index was calculated as described by Widner et al. (9). Each slide was prepared as an unknown so that its place in the experimental sequence was not known until after all counting had been completed. Rectal temperatures were taken with a thermistor probe in one or two rats in each experimental series. It had been determined previously, with a needle thermistor, that the rectal temperature represented the actual tumor temperature under these experimental conditions.

RESULTS

The effect of this form of infrared heat exposure upon the temperatures of the rats is shown in Chart 1, which represents average data from all the experiments. Ele-
of intermittent hyperthermia for 15 minutes every 12 hours was studied in nine rats, with a total of fourteen heat exposures. After cessation of the heat the mitotic index, determined at intervals, again revealed a peak at 6 hours as shown in Chart 4. The experimental recurrence of the 6-hour peak of mitotic activity prompted further experimental evaluation of this time interval. Every 6 hours nine rats were exposed to infrared heating for 15 minutes, with a total of 28 heat exposures, and in another study eight rats were similarly exposed for the same number of heat exposures. At intervals up to 18 hours after cessation of the heat mitotic indices were measured, with results shown in Chart 5. The peak of mitotic activity at 6 hours was again confirmed. A lesser but significant peak was also observed at 12 hours, and a suggestion of an even smaller peak of mitotic activity was seen at 18 hours. It is also obvious (Chart 5) that in six normal rats intestinal mucosa (jejunum) did not share this cyclic response to intermittent heat. This suggests that the effect may be limited to neoplastic tissue.

To examine the effect of hyperthermia of this frequency
mitotic time was found to be 11.0 hours. This value is in good agreement with the findings of Widner and co-workers of 11.4 hours for the Walker tumor, according to different experimental methods.

**DISCUSSION**

These experimental observations establish the fact that mitotic synchrony can be induced at intervals of 6 hours in the Walker 256 carcinoma by intermittent heat exposure at 3-, 6-, or 12-hour intervals. It is not yet known whether cyclic thermal stimuli, at functions of time other than multiples of 3 hours, would produce similar findings.

Achievement of mitotic synchrony in this mammalian tumor confirms experimental observations made in lower forms of life by others. Scherbaum et al. (6) have achieved 85 per cent synchrony in Tetrahymena by cyclic shifting of the incubation temperature between 29°C. and 34°C. This temperature cycling actually prevented further cellular division but not cell growth, the average cell size increasing 3-4 times. DNA, RNA, and protein synthesis persisted. In this synchronous system a striking separation between growth and division was experimentally induced. Division synchrony in HeLa cells was observed by Newton and Wildy (4) with a single shift in temperature from 4°C. to 37°C. DNA synthesis was delayed for 14 hours at 37°C., and cell divisions began in a few hours after the DNA synthesis period.

Barner and Cohen (1) obtained synchronized cell division in a mutant thymine-dependent strain of *Escherichia coli* by withholding thymine for 30 minutes, with resultant cessation of DNA synthesis and cell division. Addition of thymine at the end of this time was followed by DNA synthesis, and all cells divided 10-15 minutes later. Protein and RNA synthesis occurred in the absence of DNA synthesis. Irreversible cell damage occurred if growth and division processes were separated beyond the period equal to one generation. Many studies indicate that DNA synthesis does not occur during nuclear division itself. It is also apparent that DNA synthesis is usually completed well before nuclear division, that DNA synthesis is essential for nuclear division, but that DNA does not, itself, stimulate division. Our previous report (2) indicated that intermittent hyperthermia, by the method described here, with 133-236 heating episodes, resulted in decreased incidence of tumor "takes" and increased survival time in rats when intermittent heating was continued for prolonged periods. In contrast to this observation, only sixteen heating episodes (comparable to the numbers in the present study) resulted in decreased incidence of tumor "takes" and increased survival time in rats when intermittent heating was continued for prolonged periods. In contrast to this observation, only sixteen heating episodes (comparable to the numbers in the present study) resulted in apparent stimulation of tumor growth. The documentary evidence of cyclic increases in tumor mitoses, afforded by the present study, is consistent with, and may explain, this augmentation effect observed previously.

These experimental observations, although incomplete, appear to be of interest because of the implications which they may have as an adjunct to radiation or chemotherapy of malignant tumors.

**REFERENCES**

Induction of Mitotic Synchrony by Intermittent Hyperthermia in the Walker 256 Rat Carcinoma

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